International

C. elegans Conference

June 21 – 24, 2021

Abstract Book

GSA GENETICS G3 Genes | Genomes | Genetics

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9 **Rewiring quality control in *C.elegans* meiosis using a new chemically-induced proximity system** *Chenshu Liu*[1,2], Abby F. Dernburg[1,2] 1) University of California, Berkeley, CA; 2) Howard Hughes Medical Institute, Chevy Chase, MD.

During the extended meiotic prophase, homologous chromosomes must pair, culminating with the assembly of synaptonemal complex (SC) along their lengths (synapsis). Synapsis is essential for crossover formation between homologs, and thus for their accurate segregation. In *C.elegans*, defects in synapsis are monitored by a quality control program called the synapsis checkpoint, as one or more pairs of unsynapsed chromosomes lead to a cell cycle delay and can eventually trigger apoptosis. Previous work from our lab revealed that the synapsis checkpoint requires the presence of unsynapsed pairing centers (PCs), special regions on each chromosome that promote homolog pairing and synapsis. However, how cells detect defects in SC assembly remains unknown. In *C.elegans*, the Polo-like kinase PLK-2 shows dynamic subnuclear localization during meiotic prophase: it is first recruited to PCs during early prophase, and following synapsis it relocalizes to the SC. This suggests that the localization of PLK-2 might be part of the signal that triggers the synapsis checkpoint.

To test this idea, we deployed a new chemically-induced proximity (CIP) system that we engineered by modifying a core component of the auxin-inducible degradation system. We engineered mutations into the F-box protein TIR1 to prevent it from interacting with other ubiquitin ligase components. By fusing one protein to this TIR1 sequence and another to a “degron” peptide, we can induce proximity between the two tagged proteins using the small molecule indole acetic acid (auxin). With this system, we successfully targeted PLK-2 to specific chromosomal/nuclear structures. We found that ectopic targeting of PLK-2 to X-chromosomal PCs following synapsis was sufficient to induce apoptosis. Importantly, such induced apoptosis did not require HUS-1, an essential component of the DNA damage checkpoint, but was abrogated by mutation of PCH-2, an essential component of the synapsis checkpoint. By combining this CIP system with various meiotic mutants, I will also discuss about how PLK-2 coordinates with other meiotic kinases and the nucleoskeleton during meiotic quality control and cell cycle progression. Together, we have developed a simple, versatile CIP system and leveraged it to better understand the mechanisms underlying meiotic progression and quality control. This CIP system will enable a wide variety of new experiments in *C. elegans* and other model organisms.

10 **R-loop-induced irreparable DNA damage in *C. elegans* meiosis**

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Most DNA-RNA hybrids are formed naturally during transcription and are composed of a nascent RNA strand hybridized to DNA as part of R-loops. The accumulation of these structures in S-phase can result in replication-transcription conflict, an outcome which can lead to the formation of double strand breaks (DSBs). While R-loops’ role in mitotically dividing cells has been characterized, there are only a handful of studies describing the effect of R-loops in meiosis and these studies present a complex picture of the outcome of R-loop formation on germ cells. Here we show that DSBs formed by R-loops trigger an altered cellular response to DNA damage.

RNase H is an enzyme responsible for degradation of the RNA strand in DNA-RNA hybrids and plays an essential role in preventing this outcome and its deleterious consequences. Using null mutants for the two *Caenorhabditis elegans* genes encoding for RNase H1 and RNase H2 (hereby *rnh* mutants), our studies explore the effects of replication stress-induced DNA-RNA hybrid accumulation on meiosis. As expected, *rnh*∫mutants exhibit an increase in R-loop formation. Consequently, an elevation of DSBs in germline nuclei is evidenced by the accumulation of RAD-51 foci. Despite no repair mechanism abrogation, *rnh* mutants fail to repair all DSBs generated, leading to a fragmentation of chromosomes in diakinesis oocytes. By

combining our double mutant with a *spo-11* null mutation, we show that although replicative defects are the main contributor to the phenotype, R-loops formed in meiosis are likely contributors as well. We present evidence that while *rnh* mutants accumulate DNA-RNA hybrids and subsequent DSBs may signal a degree of checkpoint activation in mitosis, some damaged nuclei prevail past the checkpoint, enter into meiosis, and remain unrepaired throughout. Moreover, we find no evidence of an increase in apoptosis, which indicates that DNA damage generated by R-loops remain undetected by an apoptotic checkpoint. This data altogether points to DSBs initiated by R-loops representing an irreparable type of DNA damage that evades cellular machineries designed for damage recognition.

11 Multiple levels of regulation ensure robust cell cycle exit during *C. elegans* vulva formation

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Regulated cell cycle withdrawal is crucial for cell differentiation and tissue homeostasis, with impaired cell cycle exit leading to hyperplasia, a hallmark of cancer. The reproducible pattern of cell proliferation and differentiation in *C. elegans* provides an excellent model to study the regulatory pathways that control cell cycle arrest. Using tissue-specific gene knockout combined with lineage tracing, we explored whether known cell cycle inhibitors and tumor suppressor genes regulate cell cycle exit of the epidermal vulva precursor cells (VPCs). Lineage-specific knockout of *cki-1*, the main *C. elegans* CIP/KIP cell-cycle inhibitor, resulted in a surprisingly weak extra vulval cell division phenotype. We found that a second CIP/KIP inhibitor, *cki-2,* becomes transcriptionally upregulated in these *cki-1* knockout animals. Simultaneous knockout of both CKIs substantially increased the number of extra vulva cells. In addition to *cki-2*, we discovered that *cep-1* p53 and *daf-3* Smad4 contribute to proper cell cycle arrest of *cki-1* knockout vulval cells. Genetic analyses support a model in which these transcriptional regulators act upstream of *cki-2* to promote *cki-2* expression, which is critical only when *cki-1* is absent. Furthermore, a forward genetic screen identified the HECT-domain ubiquitin ligase UBR-5 as another factor contributing to the timely VPC cell division arrest. The *ubr-5* overproliferation phenotype was markedly enhanced when combined with *cki-1* knockout, indicating that UBR-5 may act in parallel to CKI-1. Notably, all tested combinations of negative cell-cycle regulator knockout/knockdown resulted in limited increases in vulval cell numbers. Our data support that multiple redundant levels of regulation ensure remarkably robust control of cell cycle withdrawal of *C. elegans* vulval cells.

12 The Ran pathway uniquely regulates cytokinesis in cells with different fates in the early *C. elegans* embryo

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Cytokinesis occurs through the ingression of a contractile ring that cleaves the daughter cells. This process is tightly controlled to prevent fate changes or aneuploidy, and the core cytokinesis machinery is highly conserved among metazoans. The central dogma is that spindle-dependent pathways regulate ring assembly, but studies have shown that spindle-independent pathways do so as well. In human cells, we found that active Ran is a chromatin-associated cue that forms an inverse gradient with importins that can control protein function near the cortex. We found that importin-binding is required for the cortical localization and function of anillin, a ring scaffold. To study how requirements for different cytokinesis pathways vary with cell type, we studied cytokinesis in the differently fated AB and P_1 cells of the early embryo. We found that AB and P_1 cells have different assembly kinetics supported by different levels and cortical patterning of equatorial non-muscle myosin II (NMY-2). The ring assembles rapidly in AB cells, which has higher midplane levels and cortical patches of NMY-2. In contrast, the levels of NMY-2 are lower and there are no/few patches in P_1 cells, which have slower ring assembly. Depleting polarity regulators (PAR-1, -3 or -6) equalized assembly kinetics, indicating that kinetics depend on cell fate. By generating stable tetraploid strains, we show that differences in kinetics also depend on cell size. Diploid AB-sized tetraploid P1 cells had faster ring assembly supported by higher levels of NMY-2. However, ring assembly was slower in tetraploid vs. diploid AB cells, which had very high levels of NMY-2. This suggests that ideal NMY-2 levels support ring assembly, which is hindered when levels are too low or high, and that P_1 cells operate close to a minimum threshold. Next, we determined if the chromatin pathway controls differences in AB and P_1 cell ring assembly. We found that depleting the RanGEF (RAN-3/RCC1) caused ring assembly to occur equally and rapidly in both AB and P_1 cells, which may be due to increased importin-regulation of cortical proteins. In support of this, the faster kinetics were suppressed by co-depletion of ECT-2 (RhoGEF). Interestingly, ANI-1 (anillin) suppressed kinetics in AB cells but not P_1, suggesting that the chromatin pathway functions differently in these cell types. Collectively, our results show that there are different pathway requirements in AB and P_1 cells that supports differences in cytokinesis.

13 DNA repair is altered during *C. elegans* germline aging

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Reproductive aging leads to a decline in oocyte quality and female fertility. Previous studies in multiple organisms have found altered expression of DNA repair genes during oocyte aging, however it is largely unknown whether DNA repair is defective in aged oocytes. To determine whether DNA break formation and repair changes in oocytes upon aging, we used *C. elegans* to examine DNA repair in aged germlines of both wild-type hermaphrodites, which continuously produce new oocytes, and *fog-2* mutant females, which can hold and age their oocytes in specific stages of meiotic prophase I. Our results found that RAD-51 foci, which mark DNA double-strand breaks (DSBs), were elevated in both aged wild-type and *fog-2* germlines, indicating that oocytes accumulate DSBs in old germlines regardless of oocyte age. We further determined that the elevated DSBs in aged germlines were SPO-11-dependent, indicating that age either increases SPO-11 activity or delays DSB repair. To assess efficiency of meiotic DSB repair upon aging, we introduced exogenous DSBs via irradiation to both young and old germlines and examined the kinetics of DSB repair following irradiation. Our results indicate that old germlines maintained a higher number of DSBs in a subset of nuclei following irradiation, supporting a model in which DSB repair is less efficient upon aging. To determine how DSB repair is regulated during oocyte aging, we examined mutants deficient in the E2 ubiquitin ligase variant UEV-2, which is upregulated in mutants with an extended reproductive lifespan and is suggested to function in DSB repair. Our analyses indicate that both young and aged *uev-2* mutant germlines exhibit elevated but similar levels of RAD-51 foci, implicating UEV-2 as a key regulator of DSB repair during germline aging. Taken together, our work reveals defects associated with germline aging and identifies novel players ensuring efficient DSB repair in young oocytes.

14 Deciphering the mechanism of mitotic spindle orientation in *Caenorhabditis elegans* germline stem cells

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Tissue-resident stem cells contribute to tissue development, homeostasis and repair in response to signals from a specialized microenvironment termed the niche. We are using *Caenorhabditis elegans* germline stem cells (GSCs), which are distinctly accessible for intravital imaging, as a model to elucidate how the interactions between stem cells, their niche and their tissue of residence regulate their division *in situ*. The orientation of cell division is controlled by the mitotic spindle and determines the position, and potentially the fate, of GSCs within the germline. However, the mechanisms that regulate mitotic spindle orientation in GSCs are not well known. Deciphering these mechanisms requires live-imaging of GSCs under physiological conditions. We have carried out a systematic investigation into the technical factors that impact GSC physiology during live imaging and have determined an optimized protocol for monitoring GSC divisions under minimally disruptive conditions. To permit large-scale analysis of spindle dynamics during GSC mitosis, we constructed CentTracker, an automated analysis tool, based on tracking and pairing of centrosomes, which allows a variety of mitotic parameters, including mitotic spindle orientation, to be rapidly assessed, and which is adaptable to other cell types in *C. elegans* and in other organisms. Analysis of a large dataset of dividing GSCs, indicated that GSCs have a strong spindle orientation bias towards the gonadal axis, that persists throughout mitosis, but do not exhibit spindle dynamics consistent with asymmetric cell division. We hypothesize that underlying cell or tissue anisotropies contribute to GSC spindle orientation and we are using CentTracker to identify and characterize these regulators. This work will contribute to our understanding of how GSCs are positioned within the germline during cell division, which may have implications for both GSC self-renewal and germline tissue organization.

15 Characterising single-stranded telomere binding proteins in *C. elegans*

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Telomeres are nucleoprotein complexes that protect the ends of linear chromosomes. Loss of telomere capping activates the DNA damage response, normally resulting in senescence or apoptosis. *C. elegans* telomeres are unusual in that they end in C-rich single-stranded DNA overhangs as well as the more common G-rich single-stranded DNA overhangs. Distinct proteins, namely POT-1 and POT-2, bind these different overhangs. However, neither of these proteins are essential, which suggests that there may be other telomeric single-stranded DNA binding proteins in worms. We characterise POT-3 as a single OB-fold containing protein that specifically binds the G-rich telomere strand with remarkable selectivity and affinity. We map its minimal DNA recognition sequence to a 6nt GCTTAG sequence. Strikingly, POT-3 and POT-2 bind precisely the same minimal nucleotide sequence but POT-3 has higher selectivity when the GCTTAG recognition sequence is at the extreme 3' hydroxyl end. We believe that POT-3's ability to cap the terminal end of the G-overhang mediates a unique telomeric function.

16 Using the *C. elegans* zygote to study principles of actin cytoskeleton self-organization

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The dynamic actin cytoskeleton is continually remodeled during organism development, assembling and disassembling functionally diverse filamentous (F-) actin networks at precisely the right time and place. A major question is how a cell can organize and maintain multiple F-actin networks with diverse architectures and dynamics from a common pool of actin and actin-binding proteins (ABPs) within a single cytoplasm. We use the *C. elegans* zygote to study this question *in vivo*, and purified *C. elegans* proteins to reconstitute cytoskeletal dynamics *in vitro*. We hypothesize that a series of self-organization mechanisms facilitates the differential recruitment and activation of ABPs that determine actin filament architectures and dynamics of different F-actin networks. A key determinant of F-actin dynamics and architecture is the length of filaments within a network. A powerful regulator of actin filament length is the ABP capping protein (CP), which binds the fast-growing barbed ends of actin filaments to prevent polymerization and depolymerization. Using both 'bulk' and single-molecule/ filament assays,we have biochemically characterized the dynamics of *C. elegans* ceCP on actin filament barbed ends *in vitro*. We find that ceCP has a high affinity for actin filament barbed ends and a slow off-rate relative to the lifetime of an actin filament in a *C. elegans* zygote. To characterize the biological activity of ceCP, we compared this in vitro data to the dynamics of ceCP in the *C. elegans* zygote using a powerful *in vivo* single-molecule approach. We found that the lifetime of ceCP single molecules on barbed ends in a zygote is much shorter than *in vitro*, suggesting the presence of regulatory mechanisms that act on ceCP in the zygote. We also characterize ceCP perturbation phenotypes at both the whole-network and single-filament level, and determine how ceCP regulates the balance of actin assembly and contributes to the self-sorting of other ABPs to different F-actin networks. Upon depletion of ceCP in a zygote, assembly of some F-actin networks is increased, while others are diminished, indicating a role for ceCP in the proper distribution of actin amongst networks. Additionally, we are characterizing other ABPs using similar combined *in vitro*/*in vivo* approaches, with the ultimate goal of using the *C. elegans* zygote to determine the minimal components for self-organization of multiple distinct F-actin networks within a common cytoplasm.

17 Identification of factors regulating the localization of a microtubule regulator EFA-6

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The EFA6 (Exchange Factor for Arf6) family is conserved from yeast to human, originally named after the Sec7 homology domain that has GEF activity specific to ARF6 GTPases. Functional studies have shown that EFA6 family proteins can also regulate neuronal microtubule (MT) dynamics, involving a conserved 18 aa motif embedded in an intrinsic disordered region in the EFA6 N-terminus (O'rourke et al., *Nat. Cell Biol.*, 2010). EFA6 proteins additionally contain a pleckstrin homology (PH) domain and a coiled-coil domain that enable their association with plasma membrane and actin cytoskeleton. In *C. elegans,* both expression level and localization of EFA-6 are critical for its function in neurons (Chen et al., *Neuron*, 2011; Chen et al., *Elife*, 2015). However, endogenous expression of EFA-6 remains unknown. To dissect mechanisms regulating EFA-6, we first generated a GFP knock-in to tag endogenous EFA-6. Wild-type GFP::EFA-6 is found in multiple tissues, including neurons, pharynx, epidermal cells and germline. We then carried out a visual genetic screen and isolated several classes of mutants that affect GFP::EFA-6 localization in various tissues. Among these we focused on the ones showing EFA-6 mis-localization in touch receptor neurons (TRNs) or adjacent epidermal cells. Combining whole-genome sequencing and genetic mapping, we identified several components of the specialized extracellular matrix (ECM) for TRNs, known as the mantle, which has been well studied for its role in touch sensitivity. The mantle consists of MEC-1, MEC-5, MEC-9, and HIM-4 (Emtage et al., *Neuron*, 2004). MEC-1 is essential for accumulation of the collagen MEC-5 and other ECM components. MEC-5 and MEC-9 do not affect the general structure of mantle and attachment of the neurons to the epithelia by themselves. The hemicentin HIM-4 functions separately from the MEC ECM proteins, and is needed for TRN attachment but not touch sensitivity. Our results suggest that *mec-1, mec-5,* and *him-4* have similar effects on EFA-6 localization and may act in a common pathway. We also find that disruption of the TRN specific tubulins MEC-7 and MEC-12 affect EFA-6 localization. We are currently investigating how ECM interacts with MTs to regulate EFA-6 localization and function in neurons and other tissues.

19 Nerve ring reconstructions reveal principles of brain organization across larval development

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Animal nervous system organization relies on features at every scale, from nano-level localization of synapses, through neuronal morphologies, to the high-level stereotyped connections between different regions of the brain. Previously, we developed methodologies for determining and characterizing multi-scale brain features from reconstructed serial sectioned electron micrographs (EM) of the L4 and adult nerve rings[1]. To obtain a development timeline of the nerve ring, we now integrate our analysis with results that we obtain from new EM datasets for the L1, L2, L3 and adult[2]. We find that membrane contacts between neurites are well described by a conserved core, embedded in a sea of variable contacts. We present a parsimonious model that consistently predicts that about 28-33% of conserved membrane contacts are actively targeted for synapse formation with high precision (≈93%), while the significant variability across datasets is accounted for by a non-negligible basal synaptic contact rate (≈20-30%), across all these developmental stages. Thus, while the numbers of membrane and synaptic contacts increases with age, our model predicts that the tendency to make synaptic contacts remains relatively constant. By clustering membrane contacts on each of the animals, we found a nerve ring organization of five spatial neighborhoods that supports a similarly modular information processing synaptic circuit. Extending this analysis over development, we find that the structural and synaptic modularity of the nerve ring is robust across all developmental stages, indicating that the macro-structure of the nerve ring is rooted in embryonic development (see accompanying abstract for a postulated mapping between this structure of the nerve ring and collective cell behaviors in the embryo[3]). We further present a developing brain map (a complete single cell resolution synaptic map of the *C. elegans* nerve ring) and use it to highlight key features of the post-embryonic development of the nerve ring. Our network analysis of the brain map points to a combination of individuality and robustness of brain organization that likely scale to larger nervous systems.

1. Brittin et al., (2021) Nature. https://doi.org/10.1038/s41586-021-03284-x

2. Witvliet et al. (2020), biorxiv. https://doi.org/10.1101/2020.04.30.066209

3. Brittin et al. (2021). Multicellular rosettes organize neuropil formation. [*C. elegans* conference abstract]

20 A computational approach linking neuron-specific gene expression with connectivity

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There is strong prior evidence for genetic encoding of synaptogenesis, axon guidance, and synaptic pruning in neural circuits. Despite these foundational observations, the transcriptional codes that drive neural connectivity have not been elucidated. The *C. elegans* nervous system is a particularly useful model for studying the interplay between genetics and connectivity since its wiring diagram is highly stereotyped and uniquely well-defined by electron microscopy. Furthermore, recent evidence in *C. elegans* has suggested that a unique combination of transcription factors specifies each of the 118 neuron classes[1]. Motivated by evidence for the stereotypy of neural circuits and for the genetic encoding of neural identity, we introduce a novel statistical technique, termed Network Differential Gene expression analysis (nDGE), to test the hypotheses that neuron-specific gene expression dictates connectivity. Specifically, we test the hypothesis that pre-synaptic neural identity is defined by a «key» gene combination whose post-synaptic targets are determined by a «lock» gene combination.

For our approach, we utilize neuron-specific gene expression profiles from the CeNGEN project[2] to investigate transcriptional codes for connectivity in the nerve ring[3]. We hypothesize that the expression of specific cell adhesion molecules (CAM) among synaptically-connected neurons drives synaptic maintenance in the mature nervous system. We posit that CAMs mediating synaptic stability would be more highly expressed in synaptically-connected neurons than in adjacent neurons with membrane contacts but no synapses. Thus, for each neuron, we compare the expression of all possible combinations of pairs of CAMs in the neuron and its synaptic partners relative to the neuron and its non-synaptic adjacent neurons. Two independent comparisons are generated, one for presynaptic neurons and a second result for postsynaptic neurons. Our nDGE analysis reveals that specific combinations of CAMs are correlated with connectivity in different subsets of neurons and thus provides a uniquely comprehensive road map for investigating the genetic blueprint for the nerve ring wiring diagram.

Open source software of Network Differential Gene Expression (nDGE) is publicly available at https://github.com/cengenproject/connectivity_analysis along with a vignette showcasing the CAM results.

1. Reilly, M. B., Cros, C., Varol, E., Yemini, E., & Hobert, O. (2020). Unique homeobox codes delineate all the neuron classes of C. elegans. Nature, 584(7822), 595-601.

2. Taylor, S. R., Santpere, G., Weinreb, A., Barrett, A., Reilly, M. B., Xu, C. Varol, E., ... & Miller, D. M. (2020). Molecular topography of an entire nervous system. bioRxiv.

3. Cook, S. J.,... & Emmons, S. W. (2019). Whole-animal connectomes of both Caenorhabditis elegans sexes. *Nature*, *571*(7763), 63-71.

21 The HSPG Syndecan is a core organizer of cholinergic synapses in *C. elegans*

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The extracellular matrix has emerged as an active component of chemical synapses regulating synaptic formation, maintenance and homeostasis. The heparan sulfate proteoglycan syndecans are known to regulate cellular and axonal migration in the brain. They are also enriched at synapses, but their synaptic functions remain more elusive. Here we show that SDN-1/Syndecan is a key organizer of the neuromuscular junctions (NMJs) and is the core component that clusters the homomeric α7-like nicotinic receptor ACR-16 at cholinergic NMJs. SDN-1 is concentrated at neuromuscular junctions (NMJs) by the neurally-secreted synaptic organizer MADD-4/Ce-Punctin. Punctin is secreted by cholinergic and GABAergic motoneurons into the synaptic cleft and triggers appropriate postsynaptic differentiation. We now show that Punctin has at least two parallel functions at cholinergic NMJs. First, it localizes the syndecan produced by muscle cells at postsynaptic sites. Second, it concentrates and likely activates the netrin receptor UNC-40/DCC. Those cooperatively recruit the FRM-3/FARP and LIN-2/CASK through direct interaction with the PDZ domain binding site of SDN-1 and the C-terminal P3 domain of UNC-40, respectively. The FERM-FA domain of FRM-3/FARP also engages direct interaction with SDN-1, likely with its submembrane C1 domain. The resulting CASK/FARP/Syndecan complex localizes N-AChRs at cholinergic NMJs through physical interactions. Interestingly, we were able to relocalize N-AChRs at GABAergic NMJs using a chimera containing the extracellular domain of the NLG-1/Neuroligin and the intracellular domain of SDN-1. Therefore, SDN-1 stands at the core of the cholinergic synapse organization by bridging the extracellular synaptic determinants to the intracellular synaptic scaffold that controls the postsynaptic receptor content. Surprisingly, the molecular mechanisms that control α7-like nicotinic receptors localization and dynamics are still largely unknown. Because all the components identified in *C. elegans* are evolutionarily conserved and expressed in mammalian neurons, our results provide a new framework to test if syndecan regulates the localization of nicotinic receptors in the mammalian brain.

22 Mapping the neuropeptidergic connectome of *Caenorhabditis elegans*

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The synaptically-wired neuronal circuitry is modulated by monoamines and neuropeptides, which act mostly through extrasynaptic volume transmission. This modulation is critical to nervous system function, yet little is known about the structure and function of extrasynaptic signaling networks at a whole-organism level.

To this end, we used the recently published single neuron gene expression from the CeNGEN database along with deorphanization data for neuropeptide-activated G-protein coupled receptors (GPCRs) (see Jan Watteyne *et al*. abstract for this meeting) to generate a draft connectome of neuropeptide signaling networks in *C. elegans*. We based our network on single-cell neuronal expression patterns of 93 neuropeptide-receptor couples. In our baseline network edges were formed when the sending neuron expressed a given neuropeptide, the receiving neuron expressed the cognate receptor, and both neurons extended overlapping processes in the same neuropil. We also generated an unrestricted network with no spatial restriction on edge formation which allowed for potential long-range signaling. Since all 302 neurons of the adult hermaphrodite express at least one neuropeptide precursor gene and nearly all express at least one neuropeptide GPCR, both the baseline

(with 31866 edges) and the unrestricted (with 54267 edges) neuropeptide networks were extremely dense compared to the synaptic connectome (with 2284 edges).

In addition to its high density, the neuropeptide connectome differs in significant ways from the synaptic and monoamine signaling networks. For example, whereas the synaptic network consists of a small (11 neurons) core of high-degree hubs and a low-degree periphery, the neuropeptides network is more decentralised with a great number (103 neurons) of very high-degree nodes that form an interconnected rich club. Moreover, in contrast to the monoamine network, which shows very low reciprocity, the neuropeptide connectome shows higher than expected reciprocity, even though the networks formed by individual ligand-receptor couples are not. Finally, although the premotor neurons of the synaptic rich club have high neuropeptide degree, several of the most important nodes in the neuropeptide network are little-studied neurons that may be specialised for peptidergic neuromodulation. In the future, functional studies of these neurons and their role in behaviour may provide new insight into the control of behavioral states.

23 The molecular atlas of *C. elegans* glia across sex and age

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Interactions between neurons and glia are essential for all normal nervous system functions. *C. elegans* glia have similar types of function as mammalian glia and are now established as a powerful genetic model to study glial biology. The adult hermaphrodite nervous system contains 302 neurons and 56 glia, with at least some of the glia shown to profoundly impact associated neuron shape and associated animal behaviors. Adult males have an additional 36 glia and 89 neurons, many of which control male mating-related behaviors.

A major limitation in studying *C. elegans* glia functions is the lack of cell-type specific markers for each glial subtype in the adult worm, and a global comparative assessment of glial functional and molecular heterogeneity. Further, each *C. elegans* glia is born of an invariant developmental lineage and makes invariant neuron-contact. This not only makes the nematode a powerful model to study glia-neuron interactions, but also suggests a unique advantage of this model to examine glia at single-cell and molecular detail.

Addressing this gap, we present our single nuclear RNAseq (snRNAseq) studies on adult *C. elegans* glia across the entire nervous system. We have done these studies in three settings, (1) young adult hermaphrodites, (2) young adult males, and (3) aged hermaphrodites. These data will be compiled and shared as a searchable three-dimensional online atlas of adult glial gene expression as a community resource. Our datasets allow us to now create a gene expression and marker atlas of glia at single cell resolution. Our preliminary data reveal 30 clusters, hinting already at interesting biological insights into glial heterogeneity.

Further, studies in our lab and others have found that multiple glial cues within a single glia, the amphid sheath (AMsh), regulate neuron morphology and sensory behaviors, with age-related effects. Therefore, in addition to describing glia-specific and glial subclass markers, we are currently validating our datasets functionally in similar assays, including neural aging. The aim of our studies is to expand our understanding of how different glia execute these regulatory neural functions across cell-type, sex and animal aging.

24 Insulin-like signaling regulates left/right asymmetric synaptic connection

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Lateral specialization of the central nervous system is a well-established feature across species, yet the underlying mechanism through which functional asymmetry arises is largely unknown. EM reconstruction of the *C. elegans* connectome found that ASE to AWC synapses were stronger on the left than the right. To corroborate and elucidate this asymmetric connection, we generated a reporter strain labelling ASE->AWC connections using *in vivo* Biotin Labelling of Intercellular Contact (iBLINC). While we observed the same left-sided bias of this connection, we also discovered that the asymmetric fates of the ASEs are a necessary but not sufficient factor in establishing this left-side bias. Furthermore, we found that *ins-6/insulin-like* is involved in the establishment of this asymmetric connection. Using a fosmid-based *ins-6::GFP* reporter, we observed that *ins-6* expression in ASJ also exhibits left-sided bias. The asymmetry of ASE to AWC iBLINC signal is abolished in cell-specific knock-out animals of *ins-6* in ASJ but not in ASI. Moreover, genetically removing *ins-6* in ASJL by use of *tbx-37p::Cre* reversed the asymmetry of the

ASE to AWC connection. Meanwhile, removing the putative *ins-6* receptor *daf-2* in ASEL but not ASER symmetrized the ASE to AWC connection. Finally, we observed that mutation on an antagonistic insulin, *ins*-22, partially suppressed the phenotype of *ins-6* mutants. These results taken together suggest that the left-side bias of ASE to AWC connection is controlled by insulin signaling, where asymmetrically expressed insulin-like molecules from ASJs act locally to regulate connectivity of the ASE>AWC synaptic connection. We aim to further investigate the effect of insulin signaling on the plasticity of ASE to AWC connection and general synapse dynamics. We also aim to characterize the previously unreported asymmetric gene expression in ASJ

25 Sexually-dimorphic responses to noxious stimuli in *C. elegans* result from differences in interneuron connectivity rather than in sensory processing

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Sexually reproducing animals display sexually dimorphic behaviors, geared towards reproductive success. Are there differences in the way the two sexes interpret and respond to the same aversive input?

To address this question we analyzed the worm's avoidance responses to hazardous conditions. *C. elegans* generates an escape response to aversive stimuli by integrating sensory information from the polymodal nociceptive ASH head neurons and tail neurons, and conveying it to the main reversal interneuron AVA. The recent full mapping of the male connectome (Cook *et al.* 2019) suggests that the sex-shared neurons in the avoidance circuit are dimorphically connected, e.g. ASH to AVA connection is predicted to exist only in hermaphrodites. We measured the response of both sexes to the aversive stimuli SDS and glycerol using a behavioral tail-drop assay. We found that the two sexes exhibit dose-dependent sexually dimorphic responses to the aversive stimuli – across multiple nociceptive modalities, hermaphrodites exhibited a lower pain threshold than males.

The behavioral differences and the suggested anatomical maps prompted us to functionally deconstruct the avoidance circuit. To examine potential sexual dimorphism at the sensory level, we compared ASH receptor expression levels (OCR-2, OSM-9, OSM10, QUI-1, ODR-3, GPA-3), ASH glutamatergic secretion by imaging the pHluorin sensor, and neuronal activation by calcium imaging in both sexes. We found that the ASH sensory neuron is non-dimorphic for all these parameters and responds similarly in the two sexes. Furthermore, we activated ASH optogenetically, thus bypassing the sensory input level, and found that hermaphrodites responded with a reversal at a lower LED intensity compared to males, in agreement with the tail-drop assay. Lastly, imaging of the downstream AVA interneuron revealed a stronger and longer response to the stimulus in hermaphrodites compared to males, further pointing to the connectivity and interneuron levels as the key sources for dimorphism in the circuit.

Together, our results suggest that dimorphic responses to noxious cues arise due to neuronal circuit dimorphism downstream of sensory processing. We hypothesize that differences in circuit connectivity, rather than sensory perception per se, allow for sex-specific behavioral adaptation.

26 Age-related decline of neuronal function is linked to a loss of inhibitory signaling in *C. elegans*

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Neuronal function declines with age resulting in behavioral deficits. How this age-related decline is related to changes in neuronal connectivity and circuit function on the cellular level is largely unknown. Here we employ multi-neuronal fluorescence imaging in *C. elegans* to measure changes in neuronal activity and connectivity with age across the animal's nervous system. Employing transgenic expression of the fluorescent calcium indicator GCaMP6s in specific neurons, we identify changes in the activity of individual neurons and circuits linked to locomotory behaviors that are modulated by age. We further perform multi-neuronal imaging, employing light sheet microscopy to capture neuronal activity at single-cell resolution across the organism's entire head region. We identify substantial age-related alterations in the activity dynamics of neurons as well as changes in connectivity and functional organization. As the animals age, we observe a loss of system-wide organization and a corresponding shift in individual neuron activity toward higher frequencies. We also observe a specific loss of anti-correlative (i.e. inhibitory) signaling between neurons, resulting in an overall shift in the excitatory-inhibitory (E/I) balance of the system. In support of this, we find that application of the $GABA_A$ agonist muscimol diminishes certain aspects of nervous system decline in aged animals. Interestingly, age-related effects are recapitulated in young animals bearing a gain-of-function mutation within the *unc-2* gene, encoding a pre-synaptic voltage-gated calcium channel. Conversely, aging effects are

greatly diminished by loss-of-function mutations in either *unc-2* or in *ced-4*. *Ced-4* is a key mediator of the conserved apoptotic cell-death pathway and is known to play a role in synaptic elimination across multiple species. During development, UNC-2/CaV2 activity is known to trigger the removal of inhibitory GABAergic synapses in motor neurons through a CED-4 dependent pathway. Our results suggest that a similar process is occurring as the animals age, resulting in a loss of inhibitory signaling and disruption of the system dynamics. Through comprehensive multi-neuronal imaging, we are able to measure the progressive breakdown of neuronal activity and system dynamics with age and begin to identify the cellular processes and changes in synaptic signaling that contribute to this decline.

27 The DEG/ENaC ion channel DEL-4 maintains neuronal ionstasis and promotes neuronal survival under stress

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Physiological stress is sensed and processed by neurons, which initiate systemic stress responses. At the cellular level, ion homeostasis is of utmost importance for neuronal function, both for the maintenance of resting potential, and for the creation and propagation of action potentials. Indeed, imbalance in neuronal sodium homeostasis has been linked with many pathologies of the nervous system. However, the effects of stress on neuronal sodium homeostasis, excitability and survival are not understood. We find that DEL-4, an ENaC/DEG family member, which forms proton-inactivated homomeric sodium channels, is differentially regulated under stress to trigger appropriate cellular stress responses and motor adaptation. DEL-4 exhibits neuronal, non-synaptic localization and modulates the characteristics of *Caenorhabditis elegans* locomotory behavior. Heat stress and starvation alter DEL-4 expression, which in turn modulates the expression and activity of key stress response transcription factors, leading to increased autophagy and triggering of the ER stress response. Notably, similar to heat stress and starvation, DEL-4 deficiency causes hyperpolarization of dopaminergic neurons and impacts neurotransmission in dopaminergic and motor neurons. Utilizing two humanized models of Parkinson's and Alzheimer's disease in *C. elegans*, we show that DEL-4 promotes neuronal survival in the context of these proteinopathies. Our findings provide insight on the molecular mechanisms via which sodium channels uphold neuronal function and promote adaptation upon stress.

29 End-of-life targeted auxin-mediated degradation of DAF-2 Insulin/IGF-1 receptor promotes longevity free from growth-related pathologies

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Preferably, lifespan-extending therapies should work when applied late in life without causing undesired pathologies. However, identifying lifespan-extending interventions that are effective late in life and which avoid undesired secondary pathologies remains elusive. Reducing Insulin/IGF-1 signaling (IIS) increases lifespan across species, but the effects of reduced IIS interventions in extreme geriatric ages remains unknown. Using the nematode *C. elegans*, we engineered the conditional depletion of the DAF-2/insulin/IGF-1 transmembrane receptor using an auxin-inducible degradation (AID) system that allows for the temporal and spatial reduction in DAF-2 protein levels at time points after which interventions such as RNAi may lose efficacy. Using this system, we found that AID-mediated depletion of DAF-2 protein efficiently extends animal lifespan. Depletion of DAF-2 during early adulthood resulted in multiple adverse phenotypes, including growth retardation, germline shrinkage, egg-retention, and reducing offspring. By contrast, however, AID-mediated depletion of DAF-2 specifically in the intestine resulted in an extension of lifespan without these deleterious effects. Importantly, AID-mediated depletion of DAF-2 protein in animals past their median lifespan allowed for an extension of lifespan without affecting growth or behavioral capacity. Thus, both late-in-life targeting and tissue-specific targeting of IIS minimize the deleterious effects typically seen with interventions that reduced IIS, suggesting potential therapeutic methods by which longevity and healthspan can be increased in even geriatric populations.

30 piRNA pathway-mediated Hedgehog signaling encodes a germline-to-soma pro-aging signal

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The reproductive system regulates the aging of the soma. Removal of the germline extends somatic lifespan through conserved pathways including Insulin, mTOR, and steroid signaling, while germline hyperactivity cuts lifespan short through mechanisms that are still elusive. Here, we show that mating-induced germline hyperactivity leads to the downregulation of piRNAs, which

in turn release silencing on their targets, two Hedgehog-like ligand encoding genes. Germline-originating Hedgehog signaling is perceived by the Patched-related family receptors in the soma, ultimately causing somatic collapse and early death. Our results reveal an unconventional role of the piRNA pathway and Hedgehog signaling in longevity regulation, and suggest that Hedgehog signaling controlled by the tunable piRNA pathway encodes the previous unknown germline-to-soma pro-aging signal.

31 A single-cell expression atlas of *C. elegans* adulthood uncovers new aging trajectories

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Key aspects of aging biology remain mysterious. Does every tissue age at the same rate and in the same way? In this work, we applied the most recent advances in single-cell RNA sequencing in combination with a dissociation protocol to isolate worm cells in suspension (Zhang, Banerjee, and Kuhn, Plos One, 2011). Our goal was to address this question analyzing changes in gene expression regulation in every tissue across adulthood.

Our final dataset contains 59,376 cells quantifying 24,297 genes at 6 ages ranging from day 1 of adulthood to day 15. We identified 211 unique expression states and matched these states to the majority of known cell types using systematic annotation. The quality of our cell identity annotation was validated by comparison to existing scRNAseq data and by using transgenic reporters *in vivo*.

In young worms, we could assign functions to a few cell types whose expression was never accessible before. For instance, we observed that uv1 and uv3 vulva cells as well as GLR cell transcriptomes fell into neuronal categories. Moreover, we established a complete list of marker genes and candidate promoters for every cell type. We then analyzed the activities of 212 transcription factors (TFs) in every cell type, confirming some previously known TF activities (including *elt-7, ces-1*) and uncovering new ones (such as *hlh-15*, *lin-32*, *klf-2, vab-15*).

The comparison across aging revealed a gene expression program that changes uniformly in the vast majority of cell types. This program includes genes previously found by bulk expression profiling (mitochondrial respiration, small heat shock proteins, vitellogenin and collagen). It also includes new transcription factors that appear to be functionally relevant. Interestingly, some critical GO-term gene-sets, like translation, nucleolus and proteasome have opposite aging trajectories among different tissues, suggesting that these tissues may age in fundamentally different ways. Our analysis also suggested that some tissues age faster than others, substantiated *in vivo* in preliminary experiments.

All our data will be made publicly accessible online in a portal released upon publication.

32 A *daf-18/PTEN* variant uncouples longevity from impaired fitness *via* differentially calibrating the activities of DAF-16 and SKN-1

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Insulin/IGF-1 signaling (IIS) modulates various physiological aspects, such as longevity, metabolism, development, reproduction, and behavior in multiple organisms. In *Caenorhabditis elegans*, mutations in *daf-2*/insulin/IGF-1 receptor dramatically increase lifespan and resistance against various biotic and abiotic stresses. However, *daf-2* mutations generally impair fitness, including motility, feeding, development, and reproduction. Whether these pleiotropic effects of *daf-2* mutations can be dissociated from longevity at specific steps in IIS pathway remains poorly understood. Here we show that a specific hypomorphic allele of *daf-18*/PTEN phosphatase retained longevity and enhanced pathogen resistance in *daf-2(e1370)* mutants without apparent fitness defects. Through an unbiased large-scale mutagenesis screen, we identified a missense mutation in *daf-18* [*daf-18(yh1)*], which suppressed constitutive dauer formation in *daf-2(e1370)* mutants with small impacts on resistance against *Pseudomonas aeruginosa*, PA14. We found that *daf-18(yh1)* maintained the long lifespan, enhanced immunity, and improved the reduced motility in *daf-2(e1370)* mutants. In contrast, *daf-18(nr2037)*, a strong loss-of-function *daf-18* mutant allele affected all these phenotypes indiscriminately. Notably, we showed that *daf-18(yh1)* substantially reduced the lipid phosphatase activities of DAF-18/PTEN *in vivo* and *in vitro*, while retaining a partial protein phosphatase activity *in vitro*. Thus, the remaining protein phosphatase activity of DAF-18 in *daf-18(yh1)* appears to allow *daf-2* mutants to maintain longevity and health. Next, by performing unbiased RNA-sequencing analysis, we found that *daf-18(yh1)* and *daf-18(nr2037)*

differentially affected the activities of two longevity-promoting transcription factors, DAF-16/FOXO3A and SKN-1/NRF2. Specifically, *daf-18(yh1)* retained the DAF-16 activity while suppressing the hyper-activation of SKN-1 in *daf-2(e1370)* mutants. We further showed that DAF-16 was required for the longevity and improved motility in *daf-2(e1370); daf-18(yh1)* animals, whereas hyper-activation of SKN-1 was detrimental to longevity and fitness. These data suggest that a proper calibration of DAF-16 and SKN-1 activities by modulating a single component in IIS, DAF-18 promotes healthy aging in animals with reduced IIS. Our study raises an intriguing possibility that DAF-18 serves as a therapeutic target for promoting healthy longevity without fitness defects.

33 Intergenerational adaptations to stress are evolutionarily conserved, stress specific, and have deleterious trade-offs

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Despite reports of parental exposure to stress promoting adaptations in progeny in diverse organisms, there remains considerable debate over the ecological significance and evolutionary conservation of these multigenerational effects. Here, we investigate four independent examples of intergenerational adaptations to stress in *C. elegans* – bacterial infection, microsporidia infection, osmotic stress and starvation – across four different *Caenorhabditis* species. We found that all four intergenerational adaptations to stress are conserved in at least one other species, that the responses and evolutionary conservation patterns are stress specific, and that intergenerational adaptive effects have deleterious trade-offs in mismatched environments. By profiling the intergenerational and transgenerational effects of different stresses on gene expression across species, we identified 3,174 genes that exhibited intergenerational changes in expression in multiple species in response to stress. Furthermore we found that an inversion in the expression of certain stress response genes required for intergenerational adaptations, from increased expression in the offspring of stressed parents to decreased expression in the offspring of stressed parents, correlates with an inversion of an adaptive response to infection in *C. elegans* and *C. kamaaina* to a deleterious intergenerational effect in *C. briggsae*. By contrast, we did not observe any conserved transgenerational changes in gene expression in response to stress, suggesting that the intergenerational effects of stress on offspring gene expression are not maintained transgenerationally. Our results demonstrate that intergenerational responses to stress play a substantial, evolutionarily conserved, and largely reversible role in regulating animal physiology.

34 *C. elegans* provide milk for their young

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Aging in *C. elegans* is unusual in terms of the severity and early onset of senescent pathology, particularly affecting organs involved in reproduction (1-3). For example, in post-reproductive hermaphrodites, intestinal biomass is converted into yolk leading to intestinal atrophy and yolk steatosis (3). While such post-reproductive yolk production has long been viewed as futile (1,4), we wondered if it could somehow promotes fitness.

We report that sperm-depleted hermaphrodites vent copious amounts of yolk through the vulva. This yolk can be consumed by larvae and promote their growth. This implies that post-reproductive mothers can continue to contibute to reproductive fitness by converting their biomass into a substance that serves a similar function to milk. This suggests that gut atrophy is a cost of a form of primitive lactation (also seen in certain insects). Moreover, unfertilized oocytes function as vectors carrying yolk to larvae. This type of massive reproductive effort involving biomass repurposing that causes organ degeneration is characteristic of semelparous organisms (i.e. that exhibit only a single reproductive episode) ranging from monocarpic plants to Pacific salmon, where it frequently leads to rapid death (reproductive death).

We also observed yolk venting in hermaphrodites of other *Caenorhabditis* species and in *Pristionchus pacificus* hermaphrodites, but not in females of either genus. Moreover, females do not exhibit gut atrophy or steatosis and are longer lived, suggesting absence of reproductive death. Furthermore, across species germline ablation strongly increases lifespan in nematode hermaphrodites but not females; similarly, in diverse animal and plant species blocking sexual maturation e.g. by gonadectomy frequently causes large increases in lifespan in organisms that undergo reproductive death, but not in those that don't. Insulin/IGF-1 signaling, which accelerates *C. elegans* aging, also promotes yolk production and gut atrophy (1,3). These results suggest that *C. elegans* hermaphrodites exhibit reproductive death, suppression of which increases lifespan. If correct, this has significant implications in terms of what one can learn about human aging from *C. elegans.*

(1) *Nature* 2002 419: 808. (2) *Genetics* 2002 161: 1101. (3) *Curr. Biol*. 2018 28: 2544. (4) BMC Physiol. 2011 11: 11.

35 The DRM complex functions as master regulator of somatic DNA repair capacities

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The repair of DNA damage is crucial to ensure organismal health. However, the response to DNA damage differs 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 very low mutation rates compared to somatic cells. In addition, replicating cells can use high-fidelity repair mechanisms such as homologous recombination as well as Single Strand Annealing, Microhomology End-Joining and long-patch Base Excision 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 DRM/DREAM complex is a highly conserved transcriptional repressor of cell cycle genes that promotes quiescence. Using *C. elegans*, we have found that DRM can bind and repress multiple genes involved in the DNA damage response. A deficiency in the DRM complex leads to a gene expression signature with a significant component of DNA repair genes upregulated in the soma that resembles a germline-like expression pattern. DRM complex deficient mutants show a remarkable resistance to and improved repair of various DNA-damage types, both during development and aging. We propose that the DRM complex represses all major DNA repair pathways in somatic tissues, thus limiting their resistance to genotoxic stress. The DRM mutants' resistance to various DNA damage types indicates that this complex functions as a master regulator of somatic DNA repair capacity.

36 A robotic system for automated manipulation of *C. elegans* on agar media

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The time and labor required for worm picking is a major bottleneck for many *C. elegans* experiments, especially those requiring a large number of strains. Many genetic screens and genetic manipulations would benefit greatly from an automated method for worm pushing. We developed a robot capable of transferring worms between agar plates using movements similar to those used for manual worm picking. The robot contains a motorized 3D stage that positions a wire loop pick mounted on a robotic arm to manipulate worms on an array of standard plates. Capacitive touch sensing is used to monitor contact between the pick and the agar substrate and provide feedback for the fine movements needed for picking. We constructed a dual-magnification fluorescence and bright field microscope capable of identifying developmental, morphological, or fluorescence based phenotypes of individual worms at high resolution while simultaneously imaging the entire plate at low resolution. We developed software to identify, classify, and track worms using a combination of machine vision methods, including motion detection, adaptive thresholding, and a convolutional neural network trained to recognize worms. In a test of its fluorescence-based sorting capabilities, the robot accurately identified, phenotyped, picked, and transferred worms to other plates at a rate of about 3 animals per minute. We are developing a high-level scripting language that will enable the robot to autonomously perform multi-step procedures, such as integrating extrachromosal arrays, performing genetic crosses, generating clonal populations of mutagenized worms, and other tasks. Automation of worm manipulation will both increase researchers' productivity and enable experiments that are impractical using standard methods.

37 Transcriptomic analyses of hermaphrodite responses to the male pheromone

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Social signals, including sex pheromones, play important roles in the decision-making of animals. These decisions range from short-term behavioral choices to binary, costly commitments. For example, our recent work established that *C. elegans* hermaphrodites select germline investment allocation strategies based on external stimuli, including food abundance and the presence of potential mating partners, signaled by the male ascaroside pheromone, ascr#10. This decision can only be made during a window of several hours in early adulthood. We seek to understand how this pheromone is sensed and integrated with other inputs by the nervous system, and its impact on hermaphrodite physiology. We used transcriptomics

as an unbiased way to test several hypotheses regarding ascr#10 effects on hermaphrodites. First, prior work suggested that pre-reproductive adult hermaphrodites do not respond to this male pheromone. Instead, we found that these young adults show a substantial transcriptional signature that resembles the one seen in pheromone-responsive adults. We interpret this result as an indication that the lack of overt behavioral and physiological responses in younger worms may be due to targeted response modulation, not the complete absence of relevant receptor(s) or signal transduction components. Second, a specific serotonergic circuit, that is engaged upon the onset of egg laying, licenses behavioral and physiological responses to ascr#10. Transcriptomic responses to the pheromone in mutants that disrupt activity of this circuit identified ways in which serotonin modulates hermaphrodites responses to the presence of males and couples physiology to reproductive status. Finally, a comprehensive analysis of genes differentially expressed in response to ascr#10, revealed several physiological processes, and their regulators, that are likely responsible for the beneficial effects of this male pheromone on the hermaphrodite germline and the detrimental effects on organismal longevity.

39 Transcription rates in the early embryo

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Rapid changes in gene expression are necessary for cells to respond to environmental, immunological or developmental signals. As a critical first step, high transcription synthesis rates are required during these responses. In the rapidly dividing *C. elegans* embryo, where cell cycles are as short as 15 minutes, temporal constraints on transcription are particularly severe. Early cell fate specification genes have to be transcribed at very high rates to accumulate to threshold levels within short developmental time windows. Studies in other labs have shown that reducing the normally high expression levels of some endodermal regulators results in aberrant intestinal fate specification. We hypothesize that high rates of transcription could be required for the fate decisions in several additional early cells. We estimated transcription rates in the early embryo by analyzing single cell RNA sequencing data and found that maximal rates vary across cell types, lineages, and embryonic stages. We have validated some of these by single molecule RNA fluorescence in situ hybridization (smFISH) and identified features of rapidly transcribed genes such as short gene length, shorter and fewer introns, and lack of trans-splicing. We found several motifs enriched in the core promoters of high-rate genes including the initiator element (inr), sites for the SP1 transcription factor, and binding sites for lineage-specific regulators. Using the high-rate mesoderm specification gene *ceh-51* as a test case, we measured the effect of these promoter elements on transcription rates using CRISPR-mediated promoter mutagenesis and smFISH. Our analysis shows significant redundancies built into the *ceh-51* core promoter with modest contributions to rate from each motif, suggesting that control of transcription rates is surprisingly robust. We are currently designing experiments to test for a direct impact of transcription rate on cell fate. Ultimately, we hope to understand how transcription parameters such as bursting are influenced by rate control and how this contributes to gene expression precision underlying the invariant *C. elegans* embryonic lineage.

40 Translation dependency of *erm-1* mRNA localization to the cell cortex in the early *C. elegans* embryo

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mRNA transport is an integral step in gene regulatory control. Understanding this process in the context of development is essential to determine how spatially regulated protein production impacts cell differentiation. Post-transcriptional control is especially important in embryos when *de novo* transcription is paused. We have identified maternal mRNAs with cell-specific and subcellular patterning in the early embryos of *Caenorhabditis elegans*. In particular, *erm-1 (Ezrin/Radixin/Moesin)* mRNA is asymmetrically enriched in anterior blastomeres and localizes to the cell cortex where the ERM-1 protein is also enriched. ERM-1 is a membrane-actin linker essential for apical membrane morphogenesis and is critical for intestine and excretory canal formation and function. How *erm-1* mRNA arrives at the cell cortex and how mRNA localization interplays with translation regulation remain unclear.

Broadly, mRNA can localize subcellularly in either translation-independent or dependent manners. In the translation-independent model, localization is driven by an mRNA sequence feature often found within the 3'UTR. In the translation-dependent model, a peptide signal or domain within the protein directs transport. Prior studies from our lab have demonstrated the 3'UTR of *erm-1* is not sufficient for mRNA localization. Here, recoding the nucleotide sequence of *erm-1* while preserving its amino acid sequence did not alter *erm-1* mRNA cortical localization suggesting the information directing *erm-1* mRNA localization resides in the protein. Further, we employed multiple methods of translation inhibition, through

small molecule inhibitors, heat stress, or genetic depletion of translational machinery. By all three measures, localization of *erm-1* mRNA was impaired upon translational disruption illustrating that *erm-1* mRNA localization is translation-dependent.

ERM-1 contains an N-terminal PIP2-binding FERM domain important for ERM-1 protein function. To test whether FERM domain containing transcripts generally to the cell cortex in the early embryo, we computationally identified 14 early embryonic genes containing FERM or a similar domain and we are assessing their mRNA localization patterns. The first transcript screened, *frm-7,* does indeed have cortical localization. Future studies will seek to identify cytoskeletal and motor dependencies of ERM-1 localization, whether ERM-1 translation is paused during transport, and whether future rounds of translation occur at the cortex.

41 A genome-wide analysis of developmentally regulated alternative splicing across *C. elegans* tissues

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Alternative splicing plays a major role in establishing tissue-specific transcriptomes and contributing to tissue identity and function. Our lab has previously identified hundreds of isoforms that exhibit distinct splicing patterns across broad tissue types in the *C. elegans* L4 stage. Echoing observations from comparative analyses of splicing regulation in vertebrates, our data also demonstrates that tissue-regulated alternative exons are more likely to be frame-preserving and are enriched in specific *cis*-regulatory motifs when compared with constitutively spliced exons. Second, regulated exons are more often shorter than constitutive exons, but are flanked by longer intron sequences. Intriguingly, our analysis has also identified examples of highly conserved alternatively spliced microexons less than 27 nucleotides in length. Finally, alternatively spliced exons also overlap less frequently with conserved protein domains than constitutively spliced exons but overlap more frequently with intrinsically disordered regions, which are emerging as important peptide sequences controlling the localization,

In this study, we have expanded our analysis of tissue-regulated splicing across developmental stages, augmenting our L4 data with embryonic and adult transcriptomes from various tissues. Our results have identified several hundred splice variants regulated in a temporal manner in specific tissues. Interestingly, a significant number of temporally-regulated splicing events have already adopted their adult-stage splicing pattern by late embryogenesis, suggesting early developmental patterning of splicing during cell differentiation to terminal states. Using this data, we are isolating putative RNA binding proteins that direct the splicing of co-regulated genes and examining the functional differences that arise in these protein isoforms to contribute to tissue development. Collectively, our results indicate an important and rich layer of spatio-temporal gene regulation at the level of alternative splicing in *C. elegans*.

42 Principles of mRNA Cleavage and Polyadenylation in *C. elegans*

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One of the most fundamental questions in RNA biology is how transcriptional termination is executed in eukaryotes, and how the location of the cleavage reaction influences mRNA stability and its expression levels. The mechanism of this process is important because determines the length of the 3' Untranslated Regions (3'UTRs), which are defined as the sequences located between the STOP codon and the polyA tail of mature mRNAs. 3'UTRs are targeted by a variety of regulatory factors, including miRNAs and RNA Binding Proteins (RBPs).

Here, we have used a genomic approach to map and study 3'UTR data from 1,094 transcriptome datasets downloaded from the public SRA repository at the NCBI. These datasets correspond to the entire collection of *C. elegans* transcriptomes stored in this repository from 2015 to 2018, which allowed us to map 3'UTRs with an unprecedented ultra-deep coverage of several magnitudes (the average coverage at the mRNA cleavage site is close to 220X). Given the amount of data used in this study, to our knowledge this is the most comprehensive and high-resolution analysis of 3'UTRs in a living organism performed anywhere to date. We have assigned novel 3›UTR isoforms to ~1,000 protein coding genes, refined and updated 3'UTR boundaries for ~23,000 3'UTR isoforms, and performed a detailed comparative genomic analysis of the *C. elegans* cleavage and polyadenylation complex (CPC) performing *in vivo* studies to probe principles of mRNA cleavage and polyadenylation. We found that the CPC in *C. elegans* is conserved to its human counterpart, with most of the functional domains and critical amino acids preserved. While most of the 3'UTRs possess a known Polyadenylation signal element (PAS) localized around -19 nt from the cleavage site (AAUAAA), non-canonical PAS 3'UTRs possess a less stringent requirement but preserve the chemical nature

of the element which is RRYRRR. The majority of *C. elegans* 3'UTRs terminate with a terminal Adenosine nucleotide, which we speculate is included by the RNA polymerase II during the transcription step, since. This Adenosine nucleotide is required for proper cleavage since its removal impacts the location of the cleavage site *in vivo*.

43 Critical contribution of 3' non-seed base pairing to the *in vivo* function of the evolutionarily conserved *let-7a* microRNA.

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MicroRNAs (miRNA) are endogenous regulatory non-coding RNA that exist in all multi-cellular organisms. It is well established that base pairing to the miRNA seed region (g2-g8 from the 5' end) is essential for targeting. However, the *in vivo* biological function of 3' non-seed region (g9-g22) are less well understood, especially for evolutionarily conserved miRNAs such as *let-7a,* whose entire sequence is deeply conserved among bilaterians. Here we report the first systematic investigation of the biological functions and targeting principles of the 3' non-seed nucleotides of miRNA *let-7a* as an example of evolutionarily conserved miRNAs. We used CRISPR/Cas9 genome engineering in *C. elegans* to show that the 3' non-seed sequence of *let-7a* determines the specific and essential *in vivo* function that distinguish *let-7a* from its family paralogs. We also found that the identity of each single nucleotide at g11-g16 (referred to as the "critical non-seed region") is essential for the *in vivo* function of *let-7a.* We confirmed that the conserved NHL protein *lin-41/TRIM71* is a major functional target that requires the critical non-seed pairing of *let-7a*. Furthermore, by phenotypic assays and ribosome profiling, we found that *let-7a* has additional *in vivo* targets that require pairing in the critical non-seed region, including heterochronic genes *daf-12* and *hbl-1.* We show that the repression mechanisms for these targets can include either translational inhibition, mRNA destabilization or both. Outside the critical non-seed region, we found that nucleotides at g17-g22 are less critical for *let-7a* function; however, we found that g17-g22 pairing can contribute to full function for sites with mismatches in the g11-g16 region. Strikingly, despite the involvement in compensating for imperfect seed pairing, the 3' non-seed pairing of *let-7a* is nevertheless necessarily required even in the context of perfect seed complementarity, at least for full repressing efficacy of *lin-41*. Our research provided phenotypic and molecular evidence that for certain miRNA and targets, exemplified by the evolutionarily conserved *let-7a::lin-41* interactions, the specific configuration of the 3' non-seed base-pairing critically influence miRNA function *in vivo.*

44 *In vivo* DNA Topology and Transcriptional Regulation in *Caenorhabditis elegans*

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DNA is an antiparallel double helix; its transcription requires separating the two strands. This creates supercoils, underwound and overwound duplex DNA, which accumulate behind and in front of the transcription bubble, respectively. Overwound supercoils are detrimental to helix opening and transcription bubble formation, as they increase the energy necessary to separate strands. *In vivo*, these structures are resolved by the action of topoisomerases which release supercoils from DNA. Conversely, the action of condensins, molecular machines creating loops in chromatin, has been shown *in vitro* to increase supercoiling. The interplay between supercoiling, transcription and condensin action could be a powerful regulator of gene expression.

To understand this interplay, we mapped supercoiling genome-wide *in vivo* in L3 larvae using biotinylated 4,5,8-trimethylpsoralen (bTMP). bTMP preferentially intercalates in negatively supercoiled DNA and is crosslinked to it using UV illumination. Using biotin pulldown, we can then enrich for negatively supercoiled DNA before high-throughput sequencing. Our results show highly reproducible supercoiling profiles. At chromosome scale, we did not observe a significant difference in supercoiling between chromosomes. In contrast, telomeric thirds of autosomes (perinuclear heterochromatic B domains) show a lower bTMP enrichment than at central domains (euchromatic A domains), in agreement with higher transcription levels in euchromatin. At the gene level, bTMP enrichment shows two peaks, one 5' of the transcription start site (TSS) and one at the transcription end site (TES). This suggests the accumulation of negative supercoils in the promoter region of genes and at the end of the transcription units. In agreement with a transcriptional cause, the bTMP enrichment profile along the genes correlates with gene expression levels.

In hermaphrodite animals, a variant of condensin I called condensin I^{DC}, is part of the dosage compensation complex which downregulates gene expression of X-linked genes. Condensins I/I^{DC} purified from nematodes can induce the formation of

supercoils *in vitro*. *In vivo,* we observe differential bTMP enrichment at X-linked TSS, coinciding with the location of the condensin I^{DC}. This suggests that supercoiling may link the X-specific transcriptional repression and condensin loading.

45 Spliceosomal component PRP-40 regulates alternative splicing of microexons.

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Alternative splicing plays an important role in cellular diversity and is executed by a specialized splicing machinery, the spliceosome. Splicing of very short "microexons" (≤ 51 nucleotides) is a mechanistic challenge for the spliceosome due to their short length. Therefore, our understanding of microexon splicing has remained poor. By performing a forward genetic screen for regulators of UNC-16/JIP3 alternative splicing, we have identified novel regulators of cassette exon splicing. One candidate, *prp-40* (***Pr***e-mRNA ***P***rocessing Factor), encodes an essential component of the U1 snRNP of the spliceosomal complex. We show that an interaction between *prp*-40 and *exc-7*/ELAV, a RNA binding protein (RBP)-encoding gene, governs the splicing of the cassette exon. In addition, through transcriptomic analysis of *prp-40* loss-of-function mutants, we determined that *prp-40* affects alternative splicing, but not constitutive spicing. Loss of *prp-40* had the strongest effect on microexons. In the absence of *prp-40*, nearly all microexons were reduced to undetectable levels, indicating that *prp-40* is a central regulator of microexon splicing. Furthermore, gene ontology analysis of genes with altered splicing profiles showed an enrichment of components of the neuronal synaptic signaling machinery, indicating a potential role for *prp-40* in neurological disorders. Subsequent analyses in pursuit of the underlying mechanism revealed that *prp-40*-mediated microexon splicing is influenced by the length of introns flanking the microexon and by the length of the microexon itself. Shortening of the upstream intron or lengthening of the microexon leads to a *prp-40*-independent inclusion of the microexons, establishing that *prp-40* regulates microexon splicing in a length-dependent manner. Finally, in order to understand if the function of *prp-40* is evolutionarily conserved, we performed a knockdown of PRPF40A in a mouse neuroblastoma cell line. Indeed, we found that the splicing of a set of microexons was perturbed, whereas that of normal-sized exons remained largely unaffected. Thus, prp-40 performs an evolutionarily conserved function in alternative splicing.

46 A nutrient-dependent epigenetic priming mechanism by the pioneer factor BLMP-1 modulates transactional output to control gene dosage during temporal patterning *in C. elegans*.

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Gere regulatory networks in multicellular eukaryotes operate in a dosage dependent manner. Defects in managing transcriptional output of key regulatory genes, even by a subtle factor of two, can induce catastrophic effects during development. This is especially true in the gene regulatory networks that control stage-specific patterns of gene expression. Recently, we demonstrated that the conserved pioneer factor BLMP-1 modulates the transcriptional output of many cyclically expressed target genes during *C. elegans* development; including several heterochronic microRNAs that program temporal patterning. At the molecular level, BLMP-1 is constitutively bound to its target genes where it functions to increase chromatin accessibility and promote robust transcriptional output. To determine which features of cyclical transcription BLMP-1 modulates (i.e., burst amplitude, duration or frequency), we have combined a novel microfluidics-based imaging platform with an MS2/MCP-GFP based RNA-localization approach to continuously measure transcriptional dynamics of heterochronic microRNA genes in developing lava. These efforts have revealed an unanticipated coordination of precise transcriptional patterns in somatic cell-types across the animal. Specifically, *lin-4* transcriptional induction occurs in diverse cell types for 45 min to 1 hour at specific phases of each molting cycle. In addition to in intriguing spatial relationship to firing patterns of specific cell types, we will discuss how mutations in blmp-1 modulate transcriptional output and also demonstrate how this chromatin remodeling-based mechanism counteracts the activity of the C. elegans Period ortholog, LIN-42, to modulate transcriptional dosage in different environments.

47 Cytoplasmic polyadenylation by TENT-5 regulates the innate immune response in worms

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Non-canonical poly(A) polymerases (ncPAPs) belong to the family of the terminal nucleotidyltransferases (TENTs) and are implicated in a range of physiological processes which they regulate on the post-transcriptional level. In *Caenorhabditis elegans*, cytoplasmic polyadenylation has been mostly studied in the context of gametogenesis, in which ncPAP GLD-2 elongates the poly(A) tails of many germline mRNAs, thereby promoting their stability. However, cytoplasmic polyadenylation may play a much broader role than previously anticipated. In worms, innate immunity is the main mechanism of host defense against pathogens, and hence it is tightly regulated at both the transcriptional and post-transcriptional levels. Although the role of post-transcriptional regulation of gene expression in innate immunity is well-appreciated, the particular role of cytoplasmic polyadenylation in this process has never been addressed. We show that *C. elegans* protein TENT-5 (PQN-44) is an active cytoplasmic ncPAP. Global transcriptome and proteome analyses revealed that TENT-5 is involved in the regulation of genes influencing the interaction between *C. elegans* and bacteria, including many genes encoding secreted proteins with antimicrobial activity. The poly(A) tail profiling by direct RNA sequencing on Nanopores showed that TENT-5 polyadenylates and stabilizes mRNAs with signal peptide-encoding sequences, that are translated at the endoplasmic reticulum. This aligns with TENT-5 localization in the intestine, which in worms serves as one of the major surfaces of host-pathogen interaction, and TENT-5 enrichment at the endoplasmic reticulum. Furthermore, loss of *tent-5* leads to higher susceptibility of mutant worms to infection with pathogenic bacteria. We propose that TENT-5 regulates the stability and modulates the expression of mRNAs that encode secreted proteins that ensures an energy-effective and rapid response to infection. Importantly, the role of TENT-5 in innate immunity is evolutionarily conserved since murine macrophages devoid of TENT5A and TENT5C ncPAPs also exhibit defects in polyadenylation of numerous mRNAs some of which are of genes orthologous to *C. elegans* TENT-5 targets. Taken together, our data reveal that cytoplasmic polyadenylation of mRNA encoding innate immunity effector proteins by TENT5 is a previously unknown and essential component of the post-transcriptional regulation of innate immunity.

49 Impaired peroxisomal import triggers a peroxisomal retrograde signaling

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Retrograde signaling pathways between different cellular organelles (such as mitochondria or ER) and the nucleus have been identified over the years. These retrograde signaling pathways have been shown to activate the expression of organelle-specific quality control proteins, in order to maintain the organelles' function in response to organelle-specific stress. Until now, the existence of a retrograde signaling pathway for other organelles such as peroxisomes had remained elusive. Using the nematode *Caenorhabditis elegans*, we identified for the first time such signaling for peroxisomes. Specifically, we showed that peroxisomal import stress caused by the knock-down of *prx-5,* the homolog of the human peroxisomal matrix import receptor *PEX5,* triggers a peroxisomal retrograde signaling (PRS). We show that the PRS is dependent on the transcription factor NHR-49, the homolog of human PPARα and its co-factor MDT-15, the homolog of human Mediator MED15. Lipidomic analysis revealed that *prx-5* knock-down causes increased levels of triacylglycerols with longer acyl chains, which are normally broken-down by peroxisomal β-oxidation. The presence of excess long chain fatty acids could represent the signal that activates the PRS. Consistent with this hypothesis, we found that directly perturbing peroxisomal β-oxidation is sufficient to activate the PRS. Using transcriptomic/proteomic approaches we found that peroxisomal import stress also induces the up-regulation of peroxisomal lipid metabolism, which could represent a mechanism to cope with the reduced β-oxidation. Additionally, proteins involved in the immune response are up-regulated, and we found that the PRS is activated upon infection with *Pseudomonas aeruginosa*. The PRS thus may fulfill two functions, maintaining the homeostasis of lipid metabolism and acts as a potential surveillance mechanism to protect against pathogens

50 Deciphering the ciliary extracellular vesicle (EV) proteome

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Extracellular vesicles (EVs) are emerging as a universal means of cell-to-cell communication and hold great potential in diagnostics and regenerative therapies. However, the EV field lacks a fundamental understanding of biogenesis, cargo content,

signaling, and target interactions. EVs that are transmitted by cilia represent a particular challenge due to small volume of the organelle. Here, we used our established *C. elegans* system to determine the composition and explore the function of ciliary EVs. We took advantage of the fact that *C. elegans* releases ciliary EVs from 21 male-specific neurons and 6 core IL2 neurons into environment and thus provides a great platform for discovery of evolutionarily conserved ciliary EV cargo. To collect ciliary EVs we developed a biochemical enrichment procedure based on buoyant density centrifugation and high-resolution fractionation. Using fluorescent-tagged EV cargo PKD-2::GFP and super-resolution microscopy we tracked ciliary EVs in the collected fractions and identified two populations of PKD-2 carrying EVs that differ in their densities. Proteomic analysis of the PKD-2 EV-enriched fractions revealed 2,888 proteins of *C. elegans* EVome that likely originate from multiple tissues. Top candidates were validated via generation of transgenic or CRISPR reporters and visualization of EV release using super-resolution microscopy. This strategy revealed that the male reproductive system is a major source of non-ciliary EVs. To extract ciliary EV cargoes, we integrated our dataset with published transcriptomic data. We identified new ciliary EV cargo involved in nucleotide binding and RNA interference, suggesting that environmentally-released ciliary EVs may also carry nucleic acids. Our work serves as a springboard for discoveries in the EV field and will help shed light on the contribution of ciliary EVs to the pathophysiology of abnormal EV signaling, including ciliopathies, cancer, and neurodegenerative diseases.

51 Ectosome uptake by glia sculpts *Caenorhabditis elegans* sensory cilia

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The sensory cilia of *C. elegans* protrude from sensory neurons. Cilia are sensory organelles crowded with the membrane receptors and signalling machinery detecting external cues. The composition of cilia is tightly regulated by the IntraFlagellar Transport (IFT) machinery. Regulation of traffic in and out of the cilia by IFT is thought to be the main path controlling ciliary receptors.

In our work, we show that packing and disposal of ciliary receptors in extracellular vesicles budding from the cilia (=ectosomes) complement their retrieval by IFT. In-vivo live recordings show ectosomes formed at the cilia base and/or at the cilia tip of a subset of *C. elegans* sensory neurons. Ectosomes budding from cilia tip are environmentally released, while ectosomes produced from the cilia base are concomitantly phagocytosed by the associated glial cells. We identified 2 proteins of the Tetraspanin family as ciliary ectosome markers to explore ciliary ectosome biogenesis, export and capture by the associated glia. In addition, we show that at least three endogenous ciliary membrane receptors expressed in 2 different neurons are packed in ciliary ectosomes and exported to their associated glial cells.

Mutations in the BBSome (*bbs-8*) and cilia dynein motor (*che-3*) genes, responsible for IFT retrieval of ciliary receptors, increase the export of the salt-sensing receptor GCY-22 from ASER neurons via ectosomes, preventing GCY-22 accumulation in ASER cilia. Surprisingly, the *daf-19* ciliogenesis mutant, which results in absence of cilia but does not prevent trafficking of GCY-22 to the distal dendrite, still packs and exports GCY-22 in ectosomes budding from ASER distal dendrite. We conclude that local accumulation of GCY-22 by cell trafficking -rather than presence of a cilia- triggers ectosome biogenesis.

The cilia base of most neurons is embedded in supporting glial cells, and ectosomes formed at the cilia base are readily taken up by these glial cells. We show that glial phagocytic activity is involved in ectosome uptake by glia. However, these ectosomes are still produced in absence of glia, suggesting ectosomes are intrinsically produced by the ciliated neurons. Interestingly, disturbing glia phagocytosis by *ced-10* mutations or by expression of a dominant negative Dynamin leads to abnormal shape of the ciliary base and defects in associated behaviours. We suggest that a neuron-glia coordination exists to remove from the cilia surface the ectosomes packed with receptors in order to maintain proper cilia function.

52 A three-step activation of autoinhibited RME-8 controls recycling and degradative activities on the endosome

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Upon endocytosis transmembrane cargo can be recycled for re-use or degraded via the Lysosome. Physically segregated microdomains on the sorting endosome direct transmembrane cargo to the appropriate route, and are influenced by stress and nutritional status of the cell. Keeping these microdomains physically distinct yet flexible is important for their functions and likely key to responding to changes in nutritional status.

RME-8, an Hsc70 co-chaperone is an important endosomal regulator that maintains segregated microdomains and balances recycling and degradative activities on the sorting endosome. In the absence of RME-8 microdomains converge and cargo is

missorted. Mutations in RME-8 are also associated with neurodegenerative disease in Humans such Parkinson's disease and essential tremors. As the activity of Hsp70 is specified by its cognate DNAJ protein, it has been proposed that RME-8 directs the uncoating activity of Hsp70 to limit the degradative domain on endosomes to promote recycling activities.

While RME-8 represents an important endosomal regulator balancing recycling and degradative activities, how RME-8 itself is regulated has been unclear. Given the ancient origins of the DNAJ/DNAK pairing we analyzed the evolution of the RME-8 sequence to provide insight into important functional domains. We found RME-8 and its IWN and DNAJ domains to be remarkably conserved throughout Eukarya with notable absences in Fungi and Gymnosperms. Moreover, we identified new potential domains of interest. To better understand the mechanism of RME-8's function in light of the RME-8/SNX-1 interaction and potential autoinhibition, we performed structure-function analysis of RME-8. We found that the region between the lipid binding domain and the DNAJ domain is important for RME-8 endosomal recruitment from the cytoplasm, while the C-terminal IWN3 and IWN4 domains are required for RME-8 microdomain positioning. Using a directed evolution approach, we define an intramolecular IWN4/DNAJ-domain interaction that informs RME-8's interaction with SNX-1 and its uncoating activity toward ESCRT-0. We present a model whereby SNX-1 controls the oligomerization status of RME-8, its position on the endosome, and productive exposure of the DNAJ domain for uncoating reactions in both the recycling and degradative sorting domains.

53 Super microscopy reveals zinc dependent morphological changes of intestinal gut granules and localization of zinc transporters in *C. elegans*

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Zinc plays many important roles in all organisms and its function is involved in a predicted 10% of the human proteome. The presence of zinc in both toxic and deficient amounts can lead to human disease. Thus, strict regulation of cellular zinc levels is critical for prevention of disease.. In *C. elegans*, zinc transporters CDF-2 and ZIPT-2.3 regulate zinc trafficking within gut granules. *cdf-2* and *zipt-2.3* mRNA and protein expression are regulated by zinc; in excess levels cdf-2 expression increases, while *zipt-2.3* decreases. Deficient levels cause increase expression of zipt-2.3 and decreased expression of *cdf-2*.

Our research goal was to uncover how CDF-2 and ZIPT-2.3 mediate zinc storage/mobilization, and how gut granules are capable of accommodating this activity. To detect the localization of these transporters, we used super resolution microscopy to obtain images of individual gut granules in zinc replete, deficient, and excess conditions. Animals co- expressing CDF-2::GFP and ZIPT-2.3::mCherry were synchronized and exposed to LysoTracker to stain gut granules and FluoZin-3 to detect zinc in replete, deficient, or excess conditions for 16 hours.

Images revealed that gut granules adopt unique bi-lobed morphology with specific compartments across all zinc conditions; a docking compartment containing CDF-2, but not ZIPT-2.3, and an acidified compartment and a zinc region that is surrounded by a membrane containing CDF-2 and ZIPT-2.3.

These results show that the granules create a complex architecture to respond to fluctuating zinc levels in the intestine. In addition, our findings show conformational changes prevent futile zinc cycling within granules.

54 Imaging of native transcription and transcriptional dynamics in vivo using a tagged Argonaute protein

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We present a novel approach to follow native transcription, with fluorescence microscopy, in live *C. elegans.* By using the fluorescently tagged nuclear Argonaute NRDE-3, programmed by exposure to dsRNA from available RNAi libraries to bind to nascent transcripts of the gene of interest, we demonstrate transcript labelling of multiple genes, at the transcription site and in the cytoplasm. This approach does not require genetic manipulation, and can be easily adapted to a gene of interest by relying on whole-genome dsRNA libraries. We apply this method to image the transcriptional dynamics of several genes: transcription factors *ttx-3* in embryos and *hlh-1* in larvae, and chaperone *hsp-4* in larvae.

55 Loss of a conserved protease can suppress molting defects

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Molting is essential for the growth and life cycle of ecdysozoa, a large group of animals that includes arthropods and nematodes. During *C. elegans* molting, a new cuticle is synthesized underneath the old cuticle, which is eventually perforated and discarded. Protease activity is thought to be required for the partial degradation and release of the old cuticle, as loss of specific proteases can lead to molting defects. Interestingly, peptide protease inhibitors are also necessary for nematode molting. This has led to the model that the precisely timed activation and repression of proteases is essential for normal molting. In this study we show that clathrin-mediated endocytosis (CME) promotes downregulation of the matrix metalloprotease ADM-2/ADAM9/12/19/33, a conserved member of the ADAM (a disintegrin and metalloprotease) family of transmembrane metallopeptidases. In previous work we have shown that NEKL-2/NEK8/9 and NEKL-3/NEK6/7, members of the highly conserved family of NIMA-related kinases, are required for normal molting in *C. elegans*. Moreover, defective molting in *nekl* mutants is due to defects in CME, and partial restoration of CME by mutations in the AP2 clathrin-adapter complex, also restores normal molting. Through a non-biased genetic screen, we found that loss of ADM-2 function can suppress molting defects in *nekl* mutants. Unlike mutations in genes associated with AP2, however, loss of ADM-2 does not correct *nekl* trafficking defects, indicating that suppression by *adm-2* occurs through a novel mechanism. Endogenously tagged ADM-2 is expressed in the epidermis and colocalization studies indicate that ADM-2 is trafficked via CME to the lysosome. Furthermore, loss of NEKL function leads to increased expression of ADM-2 at the epidermal plasma membrane. Importantly, CRISPR-derived mutations targeting the extracellular protease and disintegrin domains of ADM-2 strongly suppress molting defects in *nekl* mutants. Unlike AP2 mutations, however, loss of ADM-2 does not suppress null or strong loss-of-function *nekl* mutants, indicating that a failure to endocytose additional cargo, such as steroid hormone precursors required for molting, also contributes to *nekl* molting defects. Our findings lead to the model that excess ADM-2 activity at the plasma membrane impedes normal molting and that NEKL-mediated CME acts to restrict the activity of proteases during the molting process.

56 PAR polarity proteins buffer against epithelial assaults to create a continuous and functional intestinal lumen

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The ability of epithelia to protect and line our organs and bodies requires continued epithelial integrity through assaults like cell division and growth. Epithelia are made of adherent cells that polarize along an apicobasal axis and act as selective barriers; the apical surfaces of cells are collectively oriented toward the lumen or exterior and are separated from basolateral domains by junctional complexes that adhere neighboring epithelial cells. Tissue integrity, or correct and continued cell polarity and adhesion, is essential for epithelial function, but during development, epithelia face assaults on their integrity, such as cells dividing or resizing their apical surfaces. The developing *C. elegans* intestine provides a simple *in vivo* model to study how epithelia overcome these assaults. Intestinal cells polarize with apical surfaces facing a central midline, the future lumen. Four cells divide again, and all cells elongate to build a continuous apical surface and lumen and produce a functional intestinal tube. To understand what happens to polarized features during epithelial cell division, we live imaged cytoskeletal, polarity, and junctional proteins in mitotic intestinal cells. We observed that ACT-5/actin, apical PAR proteins, and the junctional proteins HMR-1/E-cadherin and DLG-1/Discs large remained localized during mitosis; in contrast, apical microtubules and associated proteins were transiently lost during mitosis and returned to the apical surface following mitotic exit. This loss of apical microtubules appeared to be coupled to the building of the microtubule-based mitotic spindle, suggesting a functional switch between these structures. Based on our localization findings, we hypothesized that PAR proteins actively maintain apical identity and continuity during epithelial assaults, directing the return of apical microtubules after mitosis and expanding the apical surface during elongation. Using intestine-specific depletion of PAR-6/Par6 and PKC-3/aPKC, we found that apical microtubule organization was indeed disrupted following mitosis and that gaps in apical and junctional proteins formed between cells both following mitosis and over time as intestinal cells elongated. When we examined the resulting PAR-depleted larval intestines, we found that gaps in apical and junctional proteins were present and correlated with luminal constrictions. The consequences of these intestinal defects were larvae that arrested and died with edematous intestines that failed to pass food. These experiments reveal a role for PAR proteins in maintaining apical and junctional continuity through mitosis and elongation, a critical feature of epithelial integrity across organisms. NIH NIGMS K99

57 DAPC and Wnt pathways pattern distinct planar-polarized membrane domains in *C. elegans* muscles

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The Dystrophin-Associated Protein Complex (DAPC) is a major actor of the relationship between cells and their extracellular matrix (ECM). Because this complex can physically connect the actin cytoskeleton and ECM proteins, it is generally assumed

that its main function is to ensure Cell cytoskeleton-ECM cohesiveness along muscle sarcolemma. This view is still prevalent to explain the pathogenesis of Duchenne Muscular Dystrophy and other muscle diseases resulting from DAPC disruption.

Almost all DAPC components are conserved in *C. elegans*, however their precise subcellular localization has not been systematically investigated so far. Therefore, we generated fluorescently-labeled Dystrophin/DYS-1, Dystroglycan/DGN-1, Sarcoglycan/SGCA-1/SGCB-1/SGN-1, Syntrophin/STN-1/STN-2 and Dystrobrevin/DYB-1 knockin strains. First, contrary to previous reports but consistent with single-cell RNAseq data, we found that DGN-1 is expressed in body wall muscle cells and colocalizes with DYS-1. Next, we discovered that DAPC components are enriched at the surface of muscle cells in two topologically-distinct membrane compartments, situated at opposite ends of the cell. Furthermore, diffraction-limited microscopy analyses suggested that DAPCs with different compositions exist within a cell, highlighting a further level of unsuspected molecular complexity and heterogeneity. Finally, by investigating the subcellular localization of muscle-expressed ion channels, we found that some were recruited to even more restricted sub-compartments, likely conferring them distinct electrophysiological identities.

The polarized distribution of membrane proteins at the cellular and tissue scale, and along the antero-posterior axis of the worm, hinted at the involvement of planar cell polarity pathways. Using a candidate gene approach, we found that mutants in DSH-1/Disheveled, the receptor ROR/CAM-1 and the Wnt ligand/EGL-20 altered the asymmetric localization of DAPC components and ion channels, rendering their pattern symmetrical along the anteroposterior axis of individual muscle cells.

Taken together these data reveal an entirely unsuspected complexity of the surface of *C. elegans* body wall muscle cells. Our results challenge the simplistic view of a purely sarcolemmal DAPC and suggest that this conserved protein complex may be at the heart of cellular mechanisms that precisely define subcellular membrane domains allowing specific functions to be compartmentalized within a single cell.

59 Forgetting generates a novel brain state that can reactivate memory

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Animals generate adaptive behaviors to the changing environment. For example, when some cues are associated with dangers, animals form an aversive memory of the cues. If the association is not consolidated or further strengthened, the memory dissipates over time and animals forget. However, it is not understood whether the forgetting process is the reverse of the learning process and whether the brain state returns to the naive state after forgetting. Here, we address these questions by analyzing the forgetting of pathogenic bacteria-induced aversive memory in adult *C. elegans*. We find that forgetting forms a novel brain state that is different from the naïve and the learned states at the levels of behavior, neuronal circuity and gene expression. Interestingly, although worms after forgetting respond to the pathogenic bacteria similarly to naïve worms, the aversive memory can be acutely reactivated. To understand the circuity and molecular mechanisms of forgetting, we performed behavioral assay and *in vivo* calcium imaging in combination with genetic perturbations. We find that the interneuron RIA, which is important for the learning of pathogenic bacteria, also plays a key role in forgetting. Two receptors, which are enriched in the pre- and post-synaptic regions of RIA respectively, antagonistically regulate the speed of forgetting. Thus, our study for the first time systematically shows that forgetting produces a novel state that can be rapidly induced to generate the learned behavior. Our findings on the neuronal mechanisms of forgetting not only better our understanding of the cognitive function of the brain, but also shed light on potential defects associated with forgetting-related mental disorders.

60 Sleep is required for odor exposure to consolidate memory and remodel olfactory synapses.

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Sleep is not only an essential function of humans but remains conserved across metazoans. Here we demonstrate that wild-type *C. elegans* sleep after repeated odor trainings This provides us with a platform to dissect how sleep affects memory at a synaptic resolution. We identified that sleep immediately after training is required for the animal to retain a long-term memory of the odor. We found that if animals do not sleep in the first two hours after training, memory is not consolidated. After identifying the neurons that are required for the memory, we show that the sensory-interneuron connections within

the circuit are downscaled after sleep. Therefore, we found a time-specific requirement of sleep that modulates synaptic downscaling to preserve memory. Conversely, lack of sleep post-training erases the long-term memory and destabilizes the synaptic downscaling, indicating that modulating the amount of sleep is sufficient to modulate memory. These results make *C. elegans* an excellent tool to ask what molecular mechanisms, cell biological processes and circuit level reorganizations are engaged during sleep to promote memory. This understanding will provide insights into the functions of sleep that contributes to our health and well-being.

61 Arrestin-mediated Desensitization Enables Olfactory Discrimination in *C. elegans*

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In the mammalian olfactory system, crosstalk among diverse olfactory signals is minimized through labelled line coding: individual neurons express one or few olfactory receptors among those encoded in the genome. The nematode worm *C. elegans* expresses over 1,000 putative olfactory receptors, all of which are 7-transmembrane G-protein coupled receptors, but has only approximately 32 chemosensory cells to express them in. With signal transduction through these receptors appearing to depend on a small set of approximately 5, partially redundant downstream G-protein alpha subunits, this presents a profound problem in signal transduction: how can olfactory cues activating one olfactory receptor be distinguished from those activating a different olfactory receptor on the same neuron? We find that olfactory discrimination in *C. elegans* is mediated by arrestin-dependent desensitization. Genetic, pharmacological and behavioural lines of evidence support a model in which olfactory cues present at high concentrations result in selective desensitization of their corresponding olfactory receptors, allowing for behavioural responses to other, less concentrated olfactory cues sensed by the same neuron. Our findings suggest that this process allows *C. elegans* to maximize responsiveness to rare or novel odors, allowing for behaviorally appropriate responses to olfactory stimuli despite the large number of olfactory receptors signaling in single cells. This represents a fundamentally different solution to the problem of olfactory discrimination than that evolved in mammals, allowing for economic use of an extremely limited number of sensory neurons.

62 Distinct neural circuits establish similar chemosensory behaviors across life stages in *C. elegans*

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Although similar behaviors across species are often established by different neural mechanisms, it is largely assumed that within a species, similar behaviors are established by the same neural mechanism. As a result, the possibility of divergent neural pathways shaping similar behaviors within a species has been largely unexplored. Here, we challenge this assumption by elucidating the neural basis of carbon dioxide (CO_2) response in *C. elegans* across life stages. We previously showed that well-fed adults are repelled by CO_2, while starved adults are attracted to CO_2; this behavioral shift is achieved through modulation of the response properties of two interneurons in the CO_2 microcircuit, RIG and AIY. Like starved adults, developmentally arrested dauer larvae are attracted to CO_2 despite significant differences in their internal physiology. Using a combination of *in vivo* calcium imaging and behavioral analyses, we show that dauers utilize a different circuit mechanism to establish the same behavioral state as that of starved adults. Notably, the AIB and AVE interneurons promote CO_2 attraction in dauers but not adults, whereas RIG promotes opposite responses to CO_2 across the two life stages. Moreover, AIY promotes CO_2 attraction in starved adults but does not modulate CO_2 response in dauers. Our results also highlight the role of dauer-specific changes in electrical synaptic connectivity in shaping interneuron activity in the CO_2 microcircuit. In addition, we show that distinct sets of neuropeptides modulate CO_2 attraction in dauers vs. adults, and identify a novel role for the insulin signaling pathway in mediating CO_2 attraction specifically in dauers. Together, our results demonstrate that distinct intra-species circuit mechanisms can establish similar behavioral states across life stages, highlighting an unexpected complexity to chemosensory processing.

63 Experience-dependent gene expression changes across a defined neural circuit in *C. elegans*

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Neurons adapt to sensory experience through mechanisms that couple neural activity to gene expression changes. To what extent this mechanism alters behavioral programs, and how activity-dependent changes are coordinated across a neural circuit, is unclear. In *C. elegans* prolonged exposure to low oxygen (O_2) levels reprograms O_2-escape behavior, sculpts

responses to other sensory cues and alters stimulus-evoked Ca^{2+} responses. To characterize the molecular changes that underpin this plasticity, we use cell-type-specific RNA sequencing to profile separate components of the O_2-circuit in animals grown at either high (21%) or low (7%) O_2. In each profiled component, we identify hundreds of experience-regulated genes, including neuropeptides, neuropeptide receptors, transcription factors and gap junctions. We show that experience-dependent regulation of these genes is contingent on O_2-evoked activity within the O_2-circuit. Across the circuit, transcriptional programs initiated by low O_2 are mostly different between O_2-sensing neurons and downstream interneurons. Despite these differences, we identify a large number of neuropeptide genes that show activity-regulated gene expression in both neural classes including short uncharacterised peptides which are likely to be secreted. Blocking peptidergic signalling from the RMG interneurons prevents animals to fully reprogram their O_2-escape behavior. By comparison, blocking synaptic communication or peptidergic signalling in O_2-sensing neurons has relatively little effect on the experience-dependent plasticity of O_2-responses. These findings suggest a model where information about O_2 experience is broadcasted from interneurons using neuropeptides to reprogram downstream circuits and behavior. This mechanism could help shed light on how activity-dependent changes are coordinated across neural circuits.

64 Mechanosensitive Piezo Channel, PEZO-1, regulates food deglutition in *C. elegans*.

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The PIEZO ion channel is an evolutionarily conserved mechanosensitive channel (Coste et al., 2010). Mammalian genomes encode two PIEZO genes, *Piezo1* and *Piezo2*, of which functions have been shown to be involved in mechanosensation (Woo et al., 2014, Nonomura et al., 2017, Li et al., 2014, Rode et al., 2017). *C. elegans* genome has a single PIEZO gene, *pezo-1*, which encodes 14 isoforms (Bai et al., 2020). The molecular function of PEZO-1 in *C. elegans* has yet to be fully determined. To examine *pezo-1* function, we grouped 14 isoforms depending on the mRNA length and observed their expression patterns. The promoter region of long isoforms is specifically expressed in the pharyngeal-intestinal valve, which is predicted to mediate food swallowing (Avery and Thomas, 1997). Next, to examine whether *pezo-1* has a role in food swallowing, we fed animals with OP50-sized GFP-microsphere and found that *pezo-1* mutant animals show excess accumulation of GFP-microsphere in the anterior part of the intestine lumen. Expression of long isoform PEZO-1 or mouse PIEZO1 under the control of valve cell-specific promoter restores the food swallowing defect of *pezo-1* mutant animals. We also observed that when GFP-microspheres are fully accumulated at the anterior part of the intestine, the pharynx is pulled posteriorly to push GFP-microspheres down into the posterior intestine. We named this a pharyngeal plunge. We next found that the pharyngeal-intestinal valve exhibits calcium transient during pharyngeal plunge and the optogenetic activation of valve cells induces the pharyngeal plunge. Moreover, elevated pressure in the anterior part of the intestinal lumen by microinjecting buffer solution causes pharyngeal plunge, not in *pezo-1* mutant but wild-type animals. Moreover, two gap junction genes, *inx-2* and *inx-20*, which have been shown to connect the valve cells to the pharyngeal muscles, appear to mediate pharyngeal plunge. Currently, we are investigating whether PEZO-1 is activated upon pressure by performing electrophysiology in a heterologous system. These results demonstrate that the *C. elegans* PIEZO channel regulates pharyngeal plunge and provides insights to understand the function of the mammalian PIEZO channel shown to be expressed in the esophagus.

65 Diverse sensory cues and internal state converge on AWA chemoreceptor expression to enhance sensitivity to food odors

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To behave appropriately in dynamic environments, animals must integrate external stimuli with their recent experience and internal state. For example, successful foraging necessitates prioritizing food-related cues when hungry. To investigate how sensory experience and internal state can lead to lasting changes in nervous system function, we examined how recent experience impacts neuronal gene expression in the context of feeding behaviors. First, we used a ribosome-tagging approach to profile whole-nervous system gene expression in fed versus fasted animals. This approach identified 203 neuronal genes that are upregulated by fasting. Among these genes, G-protein coupled chemoreceptors putatively expressed in the AWA sensory neuron were overrepresented. We generated CRISPR knock-in transcriptional reporters for several of these chemoreceptors and confirmed their fasting-dependent upregulation in AWA. Of these, *str-44* showed the most robust change in expression, so we performed in depth studies of its function and regulation. Strikingly, *str-44* expression is influenced

by a broad range of internal and external cues: the fed/fasted state of the animal, the presence of bacterial odors, and the presence of pathogens and additional stressors. Feeding state-dependent *str-44* expression depends on insulin and TGF-beta signaling, as it is disrupted in *daf-16*/FOXO and *daf-7*/TGF-beta mutants. Experiments with food odors and non-ingestible bacteria suggest that, independent of feeding state, volatile food odors also impact *str-44* expression. Chemosensory cues that influence *str-44* expression are detected by AWA itself as well as additional tax-4-expressing neurons. Osmotic stress, heat stress, and infection by pathogenic bacteria also suppress the increase in *str-44* expression during fasting, indicating that *str-44* expression represents an integration of states rather than strictly nutritional state. AWA calcium responses to bacterial food are potentiated in the fasted state relative to the fed state, and this potentiation is reduced by osmotic stress during fasting. Preliminary experiments suggest that *str-44* expression may be sufficient to confer sensitivity to putative food odors previously found to be detected by AWA with as of yet unidentified receptors. Based on these results, we suggest that environmental conditions that promote food-seeking behavior converge to regulate chemoreceptor expression in AWA, which in turn controls the animal's sensitivity to bacterial food.

66 A genetically linked gene pair determines organismal self-identity in predatory nematodes

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The ability to distinguish self and kin from foreign entities has been observed across the natural world and is associated with an immensely diverse spectrum of biological processes, including both cooperative and competitive behaviours. While behaviours associated with the identification of self and kin are commonplace, understanding the proximate mechanisms are challenging due to a lack of genetic, molecular and neurobiological techniques available in many of these organisms. Nematodes are a group of organisms without such limitations and have been used as a mainstay of organismal behavioural research. Further, the recently discovered self-recognition behaviours in the free-living nematode *Pristionchus pacificus* provides an ideal system to uncover the mechanism involved in unprecedented detail. *P. pacificus* are omnivorous nematodes capable of supplementing their bacterial diet by predating on the larvae of other nematodes. They also display cannibalistic behaviours towards conspecifics but do not cannibalise their own self-progeny. This self-recognition system is dependent on the small-peptide SELF-1*,* which contains a hypervariable region and is expressed in all hypodermal cells. While, s*elf-1* is undoubtably an important component in the self-signal, recent experiments exploring ecologically relevant con-specific interactions demonstrated it is not the only factor involved. To identify additional self-recognition elements, we explored genes closely linked to *self-1,* as self-recognition components are often clustered in small genetic intervals. Two genes were prioritised due to being lineage specific orphan genes like *self-1*. CRISPR/Cas9 induced mutations in either gene candidate displayed no self-recognition defect. However, a double mutant between *self-1* and one gene candidate displayed a self-recognition defect greater than *self-1* alone. This gene was named *self-2* and is the second gene in a two gene operon located adjacent to *self-1.* Subsequent analysis of *self-2* revealed it consists of 249 amino acids with a signal peptide and transmembrane domain. Ongoing experiments seek to clarify any interaction between *self-1* and *self-2* as well as any involvement from the additional operon gene. Furthermore, we are utilising surface-sensitive analytical mass spectrometry to determine any influence these mutants may have on cuticle surface coat composition and its effect on the self-recognition system.

67 Toward the understanding of molecular mechanism of electrical sensation and response

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Electricity is sensed by many animal species in addition to other stimuli, such as sound, light, and chemicals. Electricity is generally regarded to cause aversive responses, although it is also utilized and sensed for communication, navigation and/or prey detection not only by the well-known electric fishes but also by platypus, salamander and frog etc. The wide use of electricity as a type of environmental signal indicates the importance for animals to sense the world through electricity. However, compared to other environmental stimuli, the molecular mechanism(s) of sensing electricity is far less understood. The electrical sensor is known only in shark and skate, where the sensors are L-type voltage-gated calcium channels (VGCC) (Bellono et al. *Nature* 2017, 2018). By using a new behavioral paradigm, we are revealing the molecular mechanism of electricity sensation in worms. We first discovered that, when worms on food are stimulated by 32 V / 80 mA (4 Hz) AC, the worms' speed of migratory movement suddenly increases more than 2-fold, which persists for several minutes during electrical admission (ON response). Interestingly, a different response is observed when a stronger electric stimulus is applied: with 80 V / 200 mA (4 Hz), the speed does not increase on average during the stimulus but does increase immediately after the stimulus removal, which lasted for a few minutes (OFF response). This OFF response suggests that the speed increase is likely caused

not by direct and/or non-specific motor activation but by activation of a sensory mechanism, probably something like an alert system. Genetic candidate analysis demonstrated that *tax-2/4*, *osm-9;ocr-2*, *mec-4/10*, *cat-2*, and *tph-1* mutants showed wild type-like response, indicating that the conventional sensory mechanisms and the mechanisms for starvation-dependent speed increase are not required. Further, we found that, in addition to the above-mentioned L-type VGCC EGL-19, N-type VGCC UNC-2 is also involved, suggesting a new mechanism of electrical sensation. Currently, we are analyzing the neurons in which EGL-19 and/or UNC-2 function, the neurotransmitters required for the response, and the whole brain activities during the response (see abstracts by Wen). Combining findings from this series of experiments, we hope to elucidate the underlying molecular as well as neural mechanisms of electrical sensation and response which may be evolutionarily conserved across animal species.

69 A novel in vitro *Caenorhabiditis elegans* transcription system

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Caenorhabditis elegans is an excellent model organism for biological research, but its contributions to biochemical elucidation of eukaryotic transcription mechanisms have been limited. One of the biggest obstacles for C. elegans biochemical studies is the high difficulty of obtaining functionally active nuclear extract due to its thick surrounding cuticle. By employing Balch homogenization, we achieved effective disruption of larval and adult worms and obtained functionally active nuclear extract through subcellular fractionation. In vitro transcription reactions were successfully re-constituted using such nuclear extract. Furthermore, a PCR-based non-radioactive detection method was adapted into our system to either qualitatively or quantitatively detect transcription. Using this system to assess how pathogen infection affects *C. elegans* transcription revealed that *Pseudomonas aeruginosa* infection changes transcription activity in a promoter- or gene-specific manner. This in vitro system is useful for biochemically studying *C. elegans* transcription mechanisms and gene expression regulation. The effective preparation of functionally active nuclear extract in our system fills a technical gap in biochemical studies of *C. elegans* and will expand the usefulness of this model organism in addressing many biological questions beyond transcription.

70 The purine nucleoside phosphorylase *pnp-1* regulates epithelial cell resistance to infection in *C. elegans*

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The Intracellular Pathogen Response (IPR) was identified as a common host transcriptional response to diverse natural intracellular pathogens in *C. elegans*, including the Orsay virus, as well as Microsporidia, which cause the most commonly observed infection in wild-caught *C. elegans*. IPR genes in *C. elegans* are distinct from those induced by heat shock and other stressors, and several IPR genes encode predicted ubiquitin ligase components. We had previously identified *pals-22,* a gene of unknown biochemical function, as a repressor of the IPR. *pals-22* mutants have upregulated IPR gene expression, increased thermotolerance and pathogen resistance. Several components of a newly identified cullin-RING ubiquitin ligase mediate the increased thermotolerance of *pals-22* mutants.

To gain insight into how *C. elegans* regulates IPR gene expression and related phenotypes, we sought to identify additional regulators of the IPR. From a forward genetic screen, we identified *pnp-1* as a novel repressor of the IPR, with *pnp-1*mutants showing constitutive expression of IPR genes. *pnp-1* encodes the *C. elegans* ortholog of the vertebrate purine nucleoside phosphorylase (PNP), which functions in the purine salvage pathway. Interestingly, in humans mutations in PNP lead to immunodeficiency. We used metabolomics to show that *pnp-1* in *C. elegans* appears to function as a PNP, with mutants exhibiting higher levels of the nucleoside precursor and lower levels of the base product of the reaction catalyzed by PNP. *pnp-1*mutants have resistance to natural intracellular pathogens and *pnp-1* acts in intestinal epithelial cells to regulate defense. Interestingly, RNA-seq analysis revealed that *pnp-1* not only regulates the expression of IPR associated genes but also those induced by bacterial infection and *pnp-1* mutants display increased resistance to the extracellular bacterial pathogen *Pseudomonas aeruginosa*. Taken together, out work indicates that not only is *pnp-1* a new regulator of the IPR but that *pnp-1*, and host purine levels, may play a broader role in *C. elegans* immunity.

71 Hyperactive SKN-1 drives an innate immune response but inhibits the ability to learn pathogen avoidance

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SKN-1, the *C.elegans* ortholog of mammalian NRF2, is an essential transcription factor required for proper responses to oxidative stress and cellular homeostasis. We recently discovered that hyperactivation of SKN-1 activity leads to an age-dependent lipid dysregulation phenotype and a perceived pathogen infection. This lipid dysregulation phenotype can be recapitulated by environmental exposure of wild type worms to the pathogen *Pseudomonas aeruginosa* (PA14), indicating the interconnectedness between metabolism and immune response. The worm's ability to properly respond to pathogen is essential for its survival. Worms are initially attracted to PA14 but can learn to avoid the pathogen after some training. Surprisingly, when worms expressing hyperactivated SKN-1, which have an activated innate immune system, are exposed to PA14, they behave like naïve wild type worms and do not avoid the pathogen. Additionally, these worms cannot be trained to avoid PA14 like their wild type counterparts. This indicates that immune activation by hyperactive SKN-1 leads to the physiological consequences of pathogen exposure without the benefit of training and in fact inhibits the ability to learn avoidance. We report our investigation of the basis of learning impairment.

72 The *alg-1* gene is necessary for Orsay virus infection of *Caenorhabditis elegans*

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The discovery of Orsay virus, the first natural virus of *C. elegans*, has allowed us to better understand potentially evolutionary conserved virus-host interaction mechanisms. We developed a forward genetic screen to identify genes critical for Orsay virus infection. Specifically, we used a transcriptional GFP fusion reporter of a virus-responsive gene to isolate mutants unable to turn on GFP, and then used qRT-PCR to quantify defects in Orsay virus replication. Using this strategy, we previously identified multiple genes critical for Orsay infection, including *sid-3*, *viro-2*, *drl-1, nck-1* and *hipr-1*. Here, we focused on an independent mutant not previously characterized that had a <1000-fold reduction in orsay virus RNA relative to the unmutagenized control strain. Using a F2 bulk segregant strategy, an early stop codon mutation in the *alg-1* gene was identified as a candidate gene responsible for the mutant phenotype. The genetic rescue of such mutant with a fosmid containing the *alg-1* gene was sufficient to recover the susceptibility of the mutant to the parental levels of infection. Consistent with these results, an independent mutant allele in the *alg-1* gene, *gk214*, showed <1000-fold reduction in Orsay virus RNA compared to the wild type. The isolated *alg-1* mutant was able to induce GFP in response to the intracellular pathogen *Nematocida parisii*, suggesting that *alg-1* mutation leads to a virus specific phenotype. *alg-1* gene encodes an argonaute-like protein component of the microRNA pathway in *C. elegans* that influences lifespan, regulates gene expression, and is suggested to physically interact with RNA. Our results suggest that *C. elegans alg-1* gene plays an important role in promoting orsay virus infection. The observed pro-viral activity of *C. elegans alg-1* contrasts with the reported antiviral activity of its human ortholog AGO2.

73 Rotenone modulates the *Caenorhabditis elegans* immunometabolism and pathogen susceptibility

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Mitochondria are central players in host immunometabolism as they function not only as metabolic hubs but also as signaling platforms regulating innate immunity. Environmental exposures to mitochondrial toxicants occur widely and are increasingly frequent. Many pesticides target mitochondrial function, including the well-characterized complex I inhibitor, rotenone (Rot). Exposures to these mitotoxicants can pose a serious threat to organismal health and the onset of diseases by disrupting immunometabolic pathways. Our hypothesis is that Rot can disrupt *C. elegans* immunometabolism and consequently alter pathogen survival. *C. elegans* eggs were exposed to Rot (0.5 µM) or vehicle (Ctrl - 0.125 uM DMSO) in liquid and harvested once they reached the L4 larval stage (which was ~24h later for the Rot treatment, as it caused growth delay). Inhibition of mitochondrial respiration by Rot was confirmed by measuring the worm oxygen consumption rate. To explore pathways that were modulated by Rot, we performed a transcriptomic analysis and found 179 differentially expressed genes. WormCat analysis revealed that the two major broad enriched categories were stress response –which was mostly represented by pathogen response and detoxification genes– and metabolism –which was mostly represented by lipid and mitochondrial metabolism genes. Next, Ctrl and Rot-exposed worms were depurated for 48h, and further exposed to *Pseudomonas aeruginosa* (PA14), and *Salmonella enterica* (SL1344). Rot-exposed worms were more resistant to SL1344 but more susceptible to PA14. The mitochondrial unfolded protein response (mitoUPR) is a well-known immunometabolic pathway in *C. elegans* which links mitochondria and immunity and provides resistance to pathogen infection. Rot activated the mitoUPR pathway,

which was evidenced by increased *hsp-6*:GFP expression. Activation was also observed after 24h of exposure to PA14 and SL1344, however, Ctrl PA14-infected worms displayed lower *hsp-6*:GFP expression, and Rot rescued its expression only to the level of Ctrl OP50-raised worms. Thus, mitoUPR activation could be involved in the increased resistance to SL1344 but the level of activation in PA14 worms might not have been sufficient to promote resistance. By further exploring our transcriptomic dataset using WormExp and "module-weighted annotations" analysis tools, we identified genes that are known to confer resistance to PA14 that were downregulated by the Rot exposure, including HIF-dependent genes, which may underlie the increased susceptibility to PA14. Together, these results demonstrate that the mitotoxicant rotenone can modulate important pathways associated to the *C. elegans* immunometabolism and alter pathogen resistance.

74 NHR-49/PPAR-α and HLH-30/TFEB cooperate for *C. elegans* host defense via a flavin-containing monooxygenase

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During bacterial infection, the host is confronted with multiple overlapping signals that are integrated at the organismal level to produce defensive host responses. How multiple infection signals are sensed by the host and how they elicit the transcription of host defense genes is much less understood at the whole-animal level than at the cellular level. The model organism *Caenorhabditis elegans* is known to mount transcriptional defense responses against intestinal bacterial infections that elicit overlapping starvation and infection responses, but the regulation of such responses is not well understood. Direct comparison of *C. elegans* that were starved or infected with Gram-positive pathogen *Staphylococcus aureus* revealed a large infection-specific transcriptional signature. Both the starvation response and the infection-specific signature were largely dependent on the transcription factor, HLH-30/TFEB, highlighting its key role as a transcriptional integrator of organismal stress during infection. Interestingly, we identified six genes that were specifically induced during infection even in the absence of HLH-30/TFEB, potentially revealing an alternative transcriptional host response signaling pathway. The induction of two of the six genes, *fmo-2/FMO5* (encodes flavin-containing monooxygenase 2) and *K08C7.4* (an uncharacterized gene), was entirely dependent on nuclear hormone receptor, NHR-49/PPAR-α. NHR-49/PPAR-α was required non cell-autonomously for *fmo-2/FMO5* induction and host defense against *S. aureus*. Moreover, functional characterization of FMO-2/FMO5 suggested that its enzymatic activity is specifically required for host defense against *S. aureus*, revealing that FMO-2/FMO5 is a key host defense effector. Further, *fmo-2/FMO5* was specifically induced by Gram-positive pathogens and Gram-positive natural microbiota of *C. elegans*. These findings for the first time reveal an infection-specific host response to *S. aureus,* identify HLH-30/TFEB as its main regulator, reveal that FMOs are important innate immunity effectors in animals, and identify the mechanism of FMO regulation through NHR-49/PPAR-α in *C. elegans,* with important implications for innate host defense in higher organisms.

75 Nuclear hormone receptors mediate adaptive responses to the mold *Penicillium brevicompactum*

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Xenobiotic metabolizing enzymes (XMEs) play a major role in detoxification. Previous studies of XMEs in *C. elegans* have primarily revealed a role in the gut. By carrying out transcriptome analysis of amphid sheath (AMsh) glial cells, we have found many detoxifying enzymes to be expressed in glia, including cytochrome P450 oxygenases (CYPs), UDP-glucoronosyltransferases (UGTs) and glutathione-S-transferases (GSTs). Intriguingly, high expression levels of XMEs are also observed in the sustentacular glial cells of the mammalian olfactory epithelium, the analogous cell-type to AMsh glia, but the functions of these enzymes in olfactory tissues are poorly characterized.

We have found that expression of the cytochrome P450 gene *cyp-33C2*, in both glia and gut, is induced by exposure to *Penicillium brevicompactum*, a toxin-producing mold. *Penicillium* species are common laboratory contaminants, and they are also found naturally in environments that wild isolates of *C. elegans* inhabit, yet there have been surprisingly few studies exploring the interactions between *C. elegans* and *Penicillium* species. We have found that the nuclear hormone receptors *nhr-45* and *nhr-156* are required cell-autonomously for mold-dependent induction of *cyp-33C2*. *nhr-45* has been previously implicated in mitochondrial stress induced by the pathogen *Pseudomonas aeruginosa*. We have found that the mold *P. brevicompactum* causes mitochondrial stress in the gut and that this is greatly enhanced in *nhr-45* mutant animals that are unable to induce expression of detoxifying enzymes. This elevated mitochondrial stress is accompanied by severe developmental defects, which can be rescued by restoring *nhr-45* expression in the gut. These findings suggest that nuclear hormone receptor-dependent expression of detoxifying enzymes in the gut is required to clear mold toxins. We are currently exploring similar mechanisms in AMsh glia.

The nuclear hormone receptor family has undergone massive expansion and diversification in the *C. elegans* genome. It has been proposed that this expansion allows NHRs to act as receptors for varying environmental cues. Intriguingly, naturally occurring polymorphisms in the *nhr-156* gene in wild isolates of *C. elegans,* including the Hawaiian isolate CB4856*,* result in a reduced transcriptional response in AMsh glia upon mold exposure. Remarkably, using CRISPR to substitute the *nhr-156* locus from CB4856 in to the N2 background is sufficient to generate the observed phenotypic diversity. While the implications of these findings are still under investigation, they support the idea that diversification of nuclear hormone receptor sequences in *C. elegans* allows for adaptations to environmental conditions.

76 A parental transcriptional response to microsporidia infection induces inherited immunity in offspring

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Inherited immunity is an emerging field with important consequences for our understanding of health and evolution. Inherited immunity describes how infected parents can transfer immunity to offspring, promoting progeny survival in the face of infection. Critically, the mechanisms underlying inherited immunity are mostly unknown. Microsporidia are intracellular parasites that infect almost all animals, including humans; *Nematocida parisii* is a natural microsporidian pathogen of *C. elegans.* Here, we show that *N. parisii*-infected worms produce offspring that are resistant to microsporidia infection. We find that immunity is induced in a dose dependent manner and lasts for a single generation. Intergenerational immunity prevents host cell invasion by *N. parisii* and also enhances survival to the bacterial pathogen *Pseudomonas aeruginosa*. Further, we show that inherited immunity is triggered by the parental transcriptional response to infection, which can also be induced through maternal somatic depletion of negative regulators PALS-22 and the retinoblastoma protein ortholog LIN-35. We show that other biotic and abiotic stresses, such as viral infection and cadmium exposure, that induce a similar transcriptional response to microsporidia can also induce immunity in progeny. Our results demonstrate that distinct stimuli can induce inherited immunity to provide resistance against multiple classes of pathogens. These results show that activation of an innate immune response can provide protection against pathogens not only within a generation, but also in the next generation.

77 Regulation of DNA repair mechanism by NPR-8

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DNA replication and repair are two crucial biological processes that maintain genomic integiry and health of cells. However, very little is known about how these processes are regulated in response to pathogen infection. We have previously shown that NPR-8, a neuronal G protein-coupled receptor, functions in amphid sensory neurons to regulate *Caenorhabditis elegans* defense against *Pseudomonas aeruginosa* infection by controlling collagen expression and the dynamics of cuticle structure (Sellegounder *et al*., Science Advances 2019; 5: eaaw4717). In the current study, we analyzed protein expression in wild-type and *npr-8* mutant animals [*npr-8(ok1439)* null animals] using a mass spectrometry-based quantitative proteomics approach. In total, 1201 proteins were differentially expressed in *npr-8 (ok1439)* animals relative to wild-type animals exposed to *P. aeruginosa*, among which 74 and 134 proteins were upregulated and downregulated at least 1.5 fold, respectively. While Gene Ontology (GO) analysis of the upregulated genes did not yield any enriched GO terms, GO analysis of the downregulated genes identified 22 enriched biological processes and 8 enriched molecular functions, all of which are related to DNA metabolism. Twelve proteins with functions in DNA replication and repair contribute to these enrichments. Functional assays revealed that *P. aeruginosa*-infected *npr-8 (ok1439)* animals possess higher DNA double-strand break repair activity than infected wild-type control animals, indicating that NPR-8 suppresses DNA repair activity in wild-type animals in response to infection. How DNA replication activity is affected by the *npr-8* mutation and *P. aeruginosa* infection as well as whether DNA replication and repair are regulated by the NPR-8-expressing amphid sensory neurons are currently under investigation. Our study suggests that DNA replication and repair processes could be modulated by the nervous system in the defense response to pathogen infection.

79 Chromo domain proteins maintain germline immortality and restrict transgenerational RNAi inheritance

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Mechanisms that preserve germ cell fate and genome stability safeguard the germ cell lineage over generations, thus ensuring germline ‘immortality’. In *C. elegans*, germline immortality and transgenerational epigenetic inheritance (TEI) phenomena have shared genetic requirements. In particular, several genes with key roles in small RNA pathways and chromatin regulation

are required for maintaining fertility and for RNA interference (RNAi) inheritance, an established model for TEI. However, the mechanisms that limit the duration of RNAi inheritance are less well understood. We previously reported a loss of germline immortality upon deletion of *cec-3* and *cec-6*, two genes encoding chromo domain proteins that recognize heterochromatin-associated histone marks (H3K9 and H3K27 methylation). To understand how *cec-3* and *cec-6* contribute to germline immortality, we used several reporter and phenotypic assays for small RNA and chromatin regulation. Unexpectedly, we found that *cec-3* and *cec-6* together limit the transgenerational duration of RNAi inheritance. We also found that cec-3 and *cec-6* differentially affect repetitive transgene regulation and RNAi sensitivity in the germline versus the soma. In *cec-3;cec-6* mutant animals, we see an enhanced nuclear RNAi and a mildly reduced germline RNAi response. In addition, whereas a repetitive transgene was desilenced in the germline of *cec-6* mutant animals, it was ectopically silenced in the soma in both *cec-6* and *cec-3* mutant animals. This transgene silencing was partially dependent on the H3K9me2 methyltransferase *met-2*. We further observed changes in the morphology of perinuclear germ granules, which are organizing centers of RNA regulation. Together, our data suggest that *cec-3* and *cec-6* modulate small RNA-directed chromatin regulation to limit TEI and preserve germline immortality.

80 A novel sperm-specific compartment secures a cytoplasmic Argonaute protein for paternal epigenetic inheritance of small RNA-mediated gene silencing

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Germ cells possess specialized perinuclear, phase-separated compartments, also named condensates. Amongst others, they contain the mRNA surveillance machinery responsible for transposon silencing and fertility. In the nematode *Caenorhabditis elegans*, three condensates, P granules, Z granules and *Mutator* foci, are home to RNA interference-related pathways, driven by a highly diversified Argonaute sub-clade (WAGO) that mediates gene silencing. Intriguingly, it has been shown that WAGO-mediated gene silencing can be inherited via both oocyte and sperm. Especially the inheritance via sperm is remarkable, since significant amounts of cellular material, including Argonaute proteins, are expelled from maturing spermatids into so-called residual bodies. *How then does sperm-mediated inheritance of cytoplasmic RNAi work?* We genetically identify WAGO-3 as a major Argonaute protein required for the paternal inheritance of endogenous small RNAs. Just like other Argonaute proteins, like WAGO-1 and ALG-3, WAGO-3 localizes to P granules in naïve germ cells. During spermatogenesis, however, P granules disappear and WAGO-3, but not WAGO-1 and ALG-3, accumulates in a newly identified condensate, the PEI granule. In contrast to P granules, PEI granules remain stable during later stages of spermatogenesis. They are retained within maturing spermatids and selectively keep WAGO-3 from accumulating in the residual body. Using immunoprecipitation experiments followed by label-free quantitative mass spectrometry, we identified two uncharacterized proteins: PEI-1 and PEI-2. Both proteins are specifically expressed during spermatogenesis and we dissect their roles in PEI granule transport and function. Based on correlative light and electron microscopy (CLEM) and genetic studies, proper segregation of PEI granules in mature sperm is coupled, likely via S-palmitoylation, to the myosin-driven transport of membranous organelles. Our results identify a new sperm-specific condensate, which we call PEI granules. While not essential for spermatogenesis, PEI granules are required for paternal inheritance of small RNAs and we reveal a novel mechanism for the subcellular sorting of condensates through coupling to transport of membranous structures. *pei*-like genes are also found in human and often expressed in testis, suggesting that the here identified mechanism of subcellular transport of membraneless organelles may be more broadly conserved.

81 Regulation of transgenerational epigenetic H3K27me3 inheritance

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A crucial process in life is the ability of cells to pass on information to their descendants. This transmission can occur at several levels from genetics to epigenetics. Epigenetic inheritance refers to the heritable phenotypes affected by gene expression without altering the DNA sequence and occurs through various factors including histone modifications. Interruption of this transmission process can lead to severe developmental defects and fertility diseases.

Methylation of histone H3 at position 27 (H3K27me3) is an epigenetic mark that is associated with heterochromatin and repressed gene expression. Several studies have demonstrated that the genomic patterns of H3K27me3 can be inherited

from one generation to the next by depositing H3K27me3 histones and PRC2[1]. However, the limits of this inheritance are difficult to test because PRC2 mutants are maternal-effect sterile in *C. elegans*. We have recently shown that the expression of an H3.3K27M mutant acts in a dominant-negative manner and alters genomic H3K27me3 patterns and distribution of PRC2 and results in infertility phenotypes in *C. elegans*[2]. We used this strain to follow the trans-generational inheritance of the H3.3K27M-induced phenotypes. Strikingly, we observed that the altered patterning of H3K27me3 and the fertility defects are heritable for multiple generations after the H3.3K27M mutation is lost. These findings were also validated in a tetracycline-inducible system where we can abruptly switch on and off the H3.3K27M expression. We performed a targeted RNAi screen in our tetracycline-inducible system and detected several enhancer and suppressor modifiers of H3.3K27M defects such as chromatin remodeling complexes, the nuclear RNAi pathway, and histone writer and reader complexes.

Overall, our results revealed that the transmission of H3K27me3 is an epigenetically programmed event that can last for generations and that several biological pathways seem to be involved in the regulation of transmission of H3K27me3 patterns across generations.

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82 Interrogating the role of paternally contributed tRNA fragments in *C. elegans* fertilization and development

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Across organisms, males experiencing environmental stressors contribute non-genetic information to future generations. While sperm chromatin retains a small percentage of histones, the overwhelming exchange of histones for protamines in sperm paired with the erasure of DNA methylation after fertilization challenges the traditional epigenetic inheritance paradigms as mechanisms of intergenerational transmission. Prior work has demonstrated that modulating the diet of mammals has effects on the metabolism of offspring by altering the abundance of small RNAs in sperm, particularly the levels of highly abundant ~28-34 nt tRNA fragments (tRFs). After fertilization, the altered abundance of tRFs correlates with modified embryonic gene expression and development. While the potential role of tRFs as a carrier of epigenetic information in sperm is exciting, little is known about the molecular functions of tRFs. In mammals there are greater than 10 RnaseA and RnaseT2 enzymes with the potential to generate tRFs. Thus, redundancy of murine endonucleases makes it difficult to establish a tractable genetic model of tRF function.

Recently, we have identified abundant tRFs in *C. elegans* males which are specifically enriched in sperm. Fortunately, the *C. elegans* genome encodes only one RnaseT2 (*rnst-2*) and no RnaseA homologs, allowing for simple genetic manipulation of tRF abundance. We have generated several loss-of-function and catalytically dead alleles of *rnst-2* which all exhibit developmental and male fertility defects. Additionally, by endogenously tagging *rnst-2* we have found that it is specifically expressed in sperm and in the developing embryo. Finally, *rnst-2* regulates the levels of tRFs in male worms, establishing a model to determine if tRFs in sperm can transmit epigenetic information to progeny in *C. elegans*. To support these studies, we have developed a method for single embryo RNA-Sequencing in worms, permitting the quantitation of gene expression changes across the transcriptome from 2- and 8-cell embryos to determine the first molecular effects of modulating the levels of tRFs in sperm and early development.

83 Concentrates of histone methyltransferase MET-2 promotes gene silencing independent of its H3K9 methyltransferase catalytic activity

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Segregation of genomic regions into accessible euchromatin and inaccessible heterochromatin is essential for temporal and tissue-specific gene transcription. In *C. elegans*, the SETDB1 homolog MET-2 promotes heterochromatic silencing of satellite

repeats, transposable elements, and tissue-specific genes by di-methylating histone H3 lysine 9 (H3K9me2). Animals lacking *met-2* are viable but show defects a loss of fertility, developmental delay, and are short-lived. We previously demonstrated that MET-2's ability to preserve heterochromatin repression requires concentration in nuclear foci through physical interaction with the intrinsically disordered protein LIN-65. Here we show that MET-2 foci have a second, non-catalytic function that contributes to gene repression. Genetic ablation of *met-2* or dispersion of MET-2 foci with heat stress results in loss of silencing coincident with an increase in histone acetylation. Restoration of MET-2 foci deficient in methyltransferase activity prevents H3K9 and H3K27 hyperacetylation and mitigates gene derepression, infertility, and developmental delay. These results demonstrate that foci of a conserved heterochromatin histone methyltransferase are functionally relevant for gene silencing and can act in parallel with enzymatic activity.

84 Dissecting the functional genomic landscape of epidermal patterning in *C. elegans* using Targeted-DamID.

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Development is driven by complex patterning events such as cell division, cell fate maintenance and differentiation. Strict transcriptional and epigenetic control permits the selective decoding of batteries of genes that assemble within gene regulatory networks underlying these developmental decisions. In our lab, we focus on the patterning of the stem cell-like seam cells of the *C. elegans* epidermis as a model to dissect how gene regulatory networks, epigenomic regulation and the resulting expression profiles bring about robust developmental outcomes. Here, we tackle these questions by employing the multifaceted methodology of Targeted-DamID (TaDa) for the first time in *C. elegans*. We find that TaDa is capable of effectively identifying protein-DNA interactions of interest within a tissue of interest, requiring little starting material and preventing Dam-associated toxicity. Using TaDa we identify putative targets of the transcription factors LIN-22 and NHR-25 within the epidermis, thereby expanding our understanding of the underlying gene regulatory network and proposing new biological functions for these epidermal regulators. We further identify by assaying RNApol occupancy gene expression profiles for specific cell types of the epidermis, such as the seam cells and the major hypodermal syncytium hyp7, which reveals novel regulators of epidermal fate. Lastly, for those same cell types we probe chromatin accessibility to uncover aspects of the epigenomic regulation and identify tissue-specific regulatory elements. Our findings expand our current understanding of the genetic regulation of epidermal cell fate patterning and underline the potential value of TaDa to address diverse questions in *C. elegans*.

Keywords: cell fate and patterning, gene regulation, genomics, novel genetic technologies

85 Condensin DC spreads linearly and bidirectionally from recruitment sites to create loop-anchored TADs in C. elegans

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Condensins are molecular motors that compact DNA for chromosome segregation and gene regulation. In vitro experiments have begun to elucidate the mechanics of condensin function but how condensin loading and translocation along DNA controls eukaryotic chromosome structure in vivo remains poorly understood. To address this question, we took advantage of a specialized condensin, which organizes the 3D conformation of X chromosomes to mediate dosage compensation (DC) in C. elegans. Condensin DC is recruited and spreads from a small number of recruitment elements on the X chromosome (rex). We found that ectopic insertion of rex sites on an autosome leads to bidirectional spreading of the complex over hundreds of kilobases. On the X chromosome, strong rex sites contain multiple copies of a 12-bp sequence motif and act as TAD borders. Inserting a strong rex site and ectopically recruiting the complex on the X chromosome or an autosome creates a loop-anchored TAD. However, unlike the CTCF system, which controls TAD formation by cohesin, direction of the 12-bp motif does not control the specificity of loops. In an X;V fusion chromosome, condensin DC linearly spreads and increases 3D DNA contacts, but fails to form TADs in the absence of rex sites. Finally, we provide in vivo evidence for the loop extrusion hypothesis by targeting multiple dCas9-Suntag complexes to an X chromosome repeat region. Consistent with linear translocation along DNA, condensin DC accumulates at the block site. Together, our results support a model whereby strong rex sites act as insulation elements through recruitment and bidirectional spreading of condensin DC molecules and form loop-anchored TADs.

86 Mis-regulation of mtDNA 6mdA methylation causes enhanced oxidative stress and ageing in *C. elegans*

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Cellular energy production, ion balance, innate immunity, and metabolite synthesis are heavily reliant on mitochondrial function, which is dependent on the transcription, replication and maintenance of the mitochondrial genome (mtDNA). Owing to the compartmentalization of mitochondria and the proteobacterial ancestry of mtDNA, regulatory mechanisms applicable to nuclear DNA are not compatible within mitochondria. The most prominent epigenetic modification on bacterial DNA is adenosine methylation (m6dA), which is associated with DNA repair and transcriptional regulation. Using multiple highly sensitive and sequence-specific assays, we detected m6dA modifications on the *C. elegans* mtDNA that were distributed throughout the genome and enriched within certain regions. We identified potential m6dA methyltransferases and demethylases in the *C. elegans* genome that localize to mitochondria, and when overexpressed, reduce growth rates and cause premature ageing. Our results suggest that mis-regulation of mitochondrial m6dA can perturb correct mtDNA expression resulting in mito-nuclear protein imbalance, enhanced oxidative stress production, and activation of the mitochondrial unfolded protein response. Importantly, we have discovered the presence of mtDNA m6dA in a wide range of animal and plant species suggesting that its existence and biological role may be evolutionarily conserved.

87 *C. elegans*as a Nestor Guillermo Progeria Syndrome Model

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BAF-1 (Barrier to Autointegration Factor) is a highly conserved chromatin binding protein implicated in nuclear envelope (NE) breakdown, assembly and repair as well as chromatin compaction. It acts as a homodimer and its NE localisation is interdependent of lamins and LEM-domain proteins (LAP2, emerin, and MAN1). Nevertheless, BAF-1 is also present in the nucleoplasm. Strikingly, a single amino acid substitution in human BAF (A12T) causes Nestor-Guillermo Progeria Syndrome (NGPS). This premature ageing illness affects a variety of tissues, leading to growth retardation, severe skeletal defects and scoliosis.

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 ~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. Although the *baf-1(G12T)* mutation does not affect lifespan, age-dependent nuclear morphology deterioration is accelerated in mutant animals. Moreover, we found that *baf-1(G12T)* mutants are hypersensitive to NE perturbations, particularly to modifications affecting lamin/LMN-1.

Using Bimolecular Fluorescence Complementation (BiFC), we discovered that the interaction of the BAF-1(G12T) protein with itself is weaker when compared to wild type BAF-1 homo-dimerisation. Our BiFC experiments also revealed novel chromatin interaction partners.

To explore if the NGPS mutation affects BAF-1's association with chromatin, we determined the binding profiles for wild type and mutant BAF-1 through tissue-specific DamID. Globally, the profiles for the two proteins are very similar, but we also identified discrete genomic regions with altered association to BAF-1. We are currently correlating these observations with tissue-specific changes in gene expression.

89 The Pipeline CURE: lowering institutional barriers to research by reiteratively incorporating original *C. elegans* experiments throughout a biology curriculum

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Participation in research provides personal and professional benefits for undergraduates. However, some students face institutional barriers that prevent their entry into research, particularly those from underrepresented groups who may stand to gain the most from research experiences. Course-based undergraduate research experiences (CUREs) effectively scale research availability, but many only last for a single semester, which is rarely enough time for a novice to develop proficiency. To address these challenges, we established a Pipeline CURE. The Pipeline CURE integrates *C. elegans* epigenetics research being conducted by the Katz Lab at Emory University throughout the biology curriculum at Oglethorpe University, a nearby liberal arts college. Students are introduced to *C. elegans* with their first course in the major. After revisiting the research system in several subsequent courses, students can choose to participate in an upper-level research experience, led by an NIH **I**nstitutional **R**esearch and **A**cademic **C**areer **D**evelopment **A**ward (IRACDA) postdoctoral fellow from the Katz Lab. Preliminary

data taken during implementation of The Pipeline CURE suggests that the reiterative CURE strategy can recapitulate benefits of a traditional apprenticeship lab experience entirely within the curriculum in a classroom setting. These benefits associated with The Pipeline Cure include including replacing beginner confidence with mastery and resilience via repeated exposure to the same research system. As a result, the Pipeline CURE levels the playing field by giving underrepresented students the chance to experience original research, and potentially conduct honors thesis research, without having to seek out a traditional apprenticeship lab experience. The research experience gained by students can serve as an entry to STEM careers, or provide a platform for competing for admission to graduate programs. In addition, the data generated can open new areas of research and help facilitate publication of manuscripts. As an added benefit, The Pipeline CURE also exposes postdoctoral fellows to challenges that underrepresented groups face in the classroom. Thus, by uniting evidence-based teaching methods with ongoing scientific research, the Pipeline CURE provides a new model for overcoming barriers to participation in undergraduate research, that is flexible enough to be implemented at a range of institutions using a variety of research questions.

90 Strategies to improve equity in faculty hiring

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Through targeted recruitment and interventions to support their success during training, the fraction of trainees (graduate students and postdoctoral fellows) in academic science from historically underrepresented groups has steadily increased. However, this trend has not translated to a concomitant increase in the number of faculty from these underrepresented groups. Here, I focus on proven strategies that departments and research institutions can develop to increase equity in faculty hiring and promotion to address the lack of racial and gender diversity among their faculty.

91 Building intentional networks and partnerships within and across scientific societies to reach true diversity, equity, and inclusion in STEM

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Studies have shown that scientific discovery and creativity is enhanced when individuals from various backgrounds are involved. However, national data shows that specific demographics have been excluded in science. Within the U.S., funding agencies such as National Institutes of Health, National Science Foundation and the Howard Hughes Medical Institute recognized both the importance of diversity to science and that resources were needed if science was to benefit from the potential that diversity holds. For over five decades, funding agencies have provided financial incentives to researchers and academic institutes to broaden participation within the science, technology, engineering, math (STEM) fields and to identify institutional barriers that negatively impact diversity within STEM. While funders and academic institutions are critical entities in the scientific ecosystem, professional scientific organizations also play a role in attracting to and developing talent in STEM. While discipline specific scientific organizations were developed to serve the community of scientists writ large, some multi-disciplinary societies were established to create a dedicated space where marginalized scientists felt that they belonged. An example of such as society is the 46-year old inclusive non-profit organization Society for Advancement of Chicano/Native Americans in Science (SACNAS). Scientific organizations such as SACNAS focus attention on inclusion and belonging as a way to make the scientific enterprise diverse. As a long-term member of GSA and leaders within the SACNAS organization (SACNAS Presidential role), we put forward ideas and actionable items of engagement for scientific societies and their members. These items include 1) assessments of practices that impact equity, inclusion and diversity, 2) the importance of intentional partnerships and communications to collectively form stronger and sustainable networks within and across scientific societies, and 3) individual actions that can be taken to promote inclusion within STEM at their home institutes. Aligning these actions as funders, academic institutions and scientific organizations will collectively lead to greater engagement and awareness of the general public with science, broadening participation within STEM, and advancement in discovery. In this presentation data, ideas, concepts, and examples of operational processes will be presented.

93 Plasticity of Argonautes and their associated small RNA pathways in nematodes

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Nematoda is a diverse phylum that includes many free-living as well as parasitic species. The model nematode *C. elegans* has a diverse set of small RNAs and an expanded group of Argonautes, particularly the worm-specific clade Argonautes (WAGOs) and their endogenous siRNAs that are largely amplified by RNA-dependent RNA Polymerases (RdRPs). Our understanding of small RNA types, Argonautes, and pathways in other nematodes is limited. We carried out comparative studies of the small RNA pathways in the Clade III parasitic nematode *Ascaris*. *Ascaris* has 10 Argonautes. However, a PIWI Argonaute and piRNAs are absent. Five of the Argonautes are from the WAGO clade. We generated antibodies against all five *Ascaris* WAGOs as well as AsALG-1 (miRNAs) and AsALG-4 (26G-RNAs) and used them to identify their associated small RNAs in the early embryo, ovary, and testis including discrete developmental stages during spermatogenesis. We found that in general, AsALG-4, AsCSR-1 and AsWAGO-3 small RNAs target mRNAs while AsWAGO-1, AsWAGO-2 and AsNRDE-3 small RNAs target repetitive sequences. Notably, AsNRDE-3 small RNAs change their targets from repetitive sequences to mRNAs during male meiosis at the pachytene stage. RNA-seq data identified a group of genes expressed at pachytene during spermatogenesis which are rapidly degraded at the end of pachytene. Degradation of these mRNAs is associated with AsALG-4 26G-RNAs specifically expressed only in late pachytene and diplotene. The timing and expression of AsCSR-1 and AsALG-4 Argonautes and their small RNAs during spermatogenesis suggests they likely function independently, with AsCSR-1 and its 22-24G-RNAs fine-tuning expression of a broad set of transcripts and AsALG-4 and its 26G-RNAs down-regulating male meiosis-specific mRNAs. Genomic regions with transposons and their derivates are in general enriched for H3K9me3 and are targeted by an expansive set of 22G-RNAs associated with AsWAGO-1, AsWAGO-2 and AsNRDE-3 throughout development. Overall, there is clear conservation in the miRNA, 22G-RNA, and 26G-RNA pathways between the distantly related *Ascaris* and *C. elegans* nematodes. However, our data demonstrate the complexity and plasticity of small RNA pathways in a Clade III nematode without PIWI and a piRNA pathway and provide an in depth of analysis of the dynamics of small RNA pathways throughout spermatogenesis.

94 A Systematic Analysis of Argonaute Proteins in *C. elegans*

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RNA interference (RNAi) pathways, consisting of Argonaute (AGO) proteins and small RNAs (sRNAs) that provide sequence specificity, play key roles in gene regulation across all domains of life. Studies of AGOs in many species have revealed that they impact gene expression at nearly every stage in the life cycle of a transcript—from transcription to translation. Due to their central role in RNAi and profound impact on development and differentiation in numerous organisms, uncovering new and conserved molecular mechanisms of AGOs advances our fundamental knowledge of cellular function and has the potential to provide more precise means to manipulate gene expression, relevant for biotechnology and therapeutics.

The *C. elegans* genome encodes an expanded family of 27 *ago* genes, 19 of which produce functional proteins. We have undertaken a systematic study of every *C. elegans* AGO to develop a comprehensive portrait of the molecular mechanisms of these mostly uncharacterized proteins throughout development. Using CRISPR/Cas9 genome-editing, we epitope-tagged each AGO with GFP-3xFLAG. We used confocal microscopy to characterize the expression patterns of each AGO throughout development and found that 16 AGOs are expressed in the germline. Of these, eight AGOs localized to phase-separated germ granules. High-throughput sequencing of 1) sRNAs associated with each AGO, and 2) total sRNA pools from *ago* mutants versus wild type uncovered the stratification of subsets of AGOs into distinct gene regulatory modules. Phenotypic analyses of *ago* mutants under normal and stressful conditions revealed previously unappreciated phenotypes, including a transgenerational loss of fertility known as the Mortal Germline (Mrt) defect, and resistance to the bacterial pathogen *Pseudomonas aeruginosa*. Overall, our systematic and pioneering studies provide an unprecedented view of the sRNA regulatory landscape throughout the development of a complex animal.

95 Arginine methylation promotes siRNA-binding specificity for a spermatogenesis-specific isoform of the Argonaute protein CSR-1

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RNA silencing is a critically important mechanism through which cells regulate gene expression and protect the genome against aberrant RNAs, transposons, and viruses. RNA silencing is mediated by small non-coding RNAs, which are bound by Argonaute proteins and regulate complementary mRNAs at the level of transcription, translation, and RNA stability. CSR-1 is an essential Argonaute protein in *C. elegans* that binds to a subclass of small interfering RNAs (siRNAs) targeting most germline-expressed genes. Here we demonstrate that CSR-1 has two isoforms with distinct expression patterns; CSR-1B is ubiquitously expressed throughout the germline and during all stages of development while CSR-1A expression is restricted to germ cells

undergoing spermatogenesis. Furthermore, CSR-1A associates preferentially with siRNAs mapping to spermatogenesis-specific genes whereas CSR-1B-bound siRNAs map predominantly to oogenesis-specific genes. The specificity of the two CSR-1 isoforms is interesting, considering they share nearly complete sequence homology and co-localize at the P granule, perinuclear germ granules required for RNA regulation and silencing, in both L4 larval and male germlines. We found that the exon unique to CSR-1A is modified at arginine/glycine (RG) motifs by dimethylarginine, and loss of the dimethylarginine results in the loss of CSR-1A specificity for its preferred spermatogenic small RNA partners, resulting in CSR-1A indiscriminately binding to both spermatogenic and oogenic siRNAs. Thus, we have discovered a regulatory mechanism for *C. elegans* Argonaute proteins that allows for specificity of small RNA binding between similar Argonaute proteins with overlapping temporal and spatial localization.

96 Reprogramming the piRNA pathway for multiplexed and transgenerational gene silencing in *C. elegans*

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Short guide RNAs target Cas proteins to specific genomic loci enabling efficient gene editing in animals and plants. We have developed a conceptually similar method, called piRNA interference (piRNAi), where 21nt 'guide' piRNAs target the PRG-1 Argonaute to specific mRNAs for transcriptional and post-transcriptional gene silencing of up to four genes simultaneously. Six guide piRNAs are encoded in short synthetic gene fragments and expressed from simple extrachromosomal arrays without cloning. We test specific models for piRNA function, targeting requirements, evolutionary conservation, and inherited silencing using piRNAi. First, we demonstrate that endogenous genes (*him-5*, *him-8*, *spe-8*, and *spe-12*) are not protected from piRNA-mediated silencing. piRNAi can silence a ubiquitously expressed *gfp* in the female and male germline, with silencing persisting through embryogenesis until approximately the 100-cell stage. Silencing requires the piRNA pathway (*prg-1* and *prde-1*), but H3K9 methyltransferases (*set-25*, *set-32*, *met-2*), P-granule components (*pgl-1*, *mut-7*, *znfx-1*), and the nuclear RNAi pathway (*nrde-1*, *nrde-2*, *nrde-3*, *hrde-1*) are not required. Second, piRNAi silences genes robustly when targeting exons but not introns or untranslated regions. Furthermore, piRNA silencing tolerates 1-2 mismatches in the seed region and up to four mismatches overall. Third, we confirm that the piRNA pathway is evolutionarily conserved by silencing a *him-5* homolog in *C. briggsae* using *C. elegans* piRNA transcriptional units. Finally, we use piRNAi to develop a new assay for studying transgenerational silencing. Previous studies have demonstrated that piRNAs can semi-permanently silence *gfp* (RNAe or paramutation). We identify two novel genes, *him-5* and *him-8*, that show four and six generations of *hrde-1* dependent heritable silencing, respectively, but no paramutation. piRNAi should be a useful tool in *C. elegans* for transient gene silencing in the germline and may be particularly suitable for studying piRNA biology and heritable epigenetic silencing.

97 LOTR-1, the *C. elegans* TDRD5/7 homolog, helps maintain 22G siRNA distribution and fertility

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LOTUS and Tudor domain-containing proteins are intrinsic to germline development and are commonly found within germ granules. *Drosophila* versions of these proteins, Tejas and Tapas, localize Vasa to germ granules and play a role in piRNA-mediated retrotransposon silencing. Mammalian TDRD5 and TDRD7 proteins contain both LOTUS and Tudor domains and have essential roles during spermatogenesis. Here we describe D1081.7, the homolog of these proteins in *C. elegans*, named LOTR-1 for its LOTUS and Tudor domains. Within germ granules, LOTR-1 docks next to P granules to colocalize with the Z-granule component ZNFX-1. LOTR-1's Z-granule association requires its Tudor domain, but both LOTUS and Tudor deletions affect brood size when coupled with the knockdown of *glh-1*. The localization of PGL-1, DEPS-1 and GLH-1 to P granules is not impacted in *lotr-1* mutants; however, LOTR-1 retains both ZNFX-1 and PRG-1 at the nuclear periphery. Quantitative LOTR-1 IP-mass spectrometry confirmed the Tudor-dependent association of ZNFX-1, PRG-1, DEPS-1, and other WAGO-class Argonautes. Like *znfx-1*, *lotr-1* mutants redistribute the coverage of 22G-RNAs toward the 5' end of Mutator targets and impact transgenerational epigenetic inheritance. Unlike *znfx-1*, the 5' shift in 22G-RNA coverage does not extend to CSR-1 targets. We hypothesize that LOTR-1 facilitates interactions between PRG-1/WAGO-class Argonautes, ZNFX-1 and target 3'UTRs to balance 22G-RNA distribution. These key aspects of LOTR-1 in *C. elegans* provide insight into TDRD5 and TDRD7 function during spermatogenesis and potential impact as cancer-testis (CT) antigens.

98 Proteolysis dependent gene silencing in *C.elegans* germline

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Animal germ cells employ small RNAs and Argonaute proteins to surveil transcripts and silence transposons and foreign genetic elements. Here we report that, in *C.elegans* germline, expression of a transgene encoding codon optimized Cas9 gene fused to GFP is silenced via small RNA dependent and small RNA independent mechanisms. Interestingly, small RNA-dependent silencing was initiated and small RNAs targeting Cas9::GFP sequence were generated even in the absence of *prg-1* activity. Furthermore, disarming the small RNA pathway led to Cas9::GFP expression only in the most distal region of the gonad, suggesting that a second small RNA-independent silencing mechanism maintains the silencing throughout the pachytene region and in oocytes. A forward genetic screen revealed that inactivation of the ubiquitin-proteasome pathway causes full de-silencing of the CAS9::GFP throughout the germline, suggesting that the second silencing mechanism is mediated by protein degradation. Remarkably, insertion of virus derived T2A sequence that causes ribosome skipping, between Cas9 and GFP, lead to the expression of the downstream GFP moiety throughout the gonad. This observation suggests that the upstream Cas9 ORF is triggering protein degradation and that the mRNA is not degraded and can still encode downstream GFP. Thus ribosome subunits are likely not dissociated as in the RQC response to ribosome stalling. We propose that some modification within the upstream Cas9 mRNA signals for co-translational ubiquitylation and degradation of the peptide. Interestingly, a Cas9 transgene that contains multiple extended AT rich introns has been shown to escape this mode of silencing, pointing toward mRNA splicing as the triggering event and linking the phenomenon to the recently described intronless gene silencing. We are currently investigating the nature of the triggering signal and whether our findings extend to the regulation of endogenous targets.

99 Negative feedback between NHR-23 and *let-7* regulates developmental pace and number of molts in *C. elegans*

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The *let-7* microRNA was discovered as a regulator of the L4 larval stage to adult transition. *let-7* transcription oscillates across larval development, with a peak in every stage. But the transcriptional activator of *let-7* and functional significance of its oscillatory expression are not characterized. Here, we report that the transcription factor NHR-23 activates transcription of *let-7*, and in turn, *let-7* represses the expression of *nhr-23*, thus forming **a feedback loop**

. NHR-23 is the homolog of the Retinoid-related Orphan Receptor (ROR) and is predicted to bind consensus ROR Response Elements (RORE) in the promoter of *let-7.* Chromatin immunoprecipitation of NHR-23 followed by qPCR of the *let-7* promoter supports direct binding of NHR-23 to the *let-7* promoter. Scrambling 2 of the *let-7* RORE sites abrogates NHR-23 binding to the *let-7* promoter. In addition, the *let-7 RORE* mutants exhibit penetrant defects in vulval development and increased seam cell number, consistent with the partial loss of *let-7* activity. *let-7* targets the 3'UTR of the *nhr-23* transcript through *let-7* complementary sequences (LCSs). Expression of a reporter gene fused to the *nhr-23* 3'UTR is enhanced when one of the LCSs is mutated. In the endogenous context, *let-7* dampens oscillations of *nhr-23* as worms go through development. Mutating the *nhr-23* LCSs diminishes dampening of *nhr-23* transcript oscillations and leads to supernumerary molts. Furthermore, overexpressing *nhr-23* also leads to aberrant supernumerary molts in adults, similar to *let-7* hypomorphs. The NHR-23–*let-7* negative feedback loop also affects the pace of development. *let-7* hypomorphs, *let-7 RORE* mutants, and *nhr23* overexpressing strains all precociously exit quiescent phases between molts (lethargus). RNAi of *nhr-23* leads to delayed exit from lethargus that is rescued by *let-7* mutations. Taken together, these data suggest that the NHR-23—*let-7* negative feedback loop regulates both the number of molts and the pace of the molting cycle. The negative feedback loop between RORs and *let-7* may be conserved, as evidenced by the presence of homologous *cis*-regulatory elements in mammalian genes. Transcription of a reporter driven by the mouse *let-7* promoter in *C. elegans* is responsive to the levels of *nhr-23*. LCSs in the 3'UTRs of human RORβ and mouse RORα also regulate reporter gene expression in *C. elegans.* Therefore, the NHR-23–*let-7* negative feedback loop may represent a conserved genetic oscillator that regulates biological rhythms.

100 Screening by deep sequencing reveals mediators of miRNA tailing in *C. elegans*

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Most micro-RNAs (miRNA) result from the processing of longer primary transcripts transcribed from miRNA genes by the RNA polymerase Pol II. These transcripts harbor hairpin structures which are recognized and processed twice to yield mature miRNAs: (1) once in the nucleus by Drosha, with the support of Pasha (PASH -1 in *C. elegans*) and (2) again in the cytoplasm by Dicer. The resulting ~22nt mature miRNAs are effective regulators of gene expression. When loaded onto the RNA-induced silencing complex (RISC), mature miRNAs direct RISC activity to transcripts with complementary sequence elements to repress their expression. In turn, their levels must also be regulated. Despite this, the processes responsible for miRNA turnover remain poorly understood.

In instances where they are turned over, miRNAs are frequently modified by the addition of untemplated nucleotides to their 3' end, but the role of this tailing is often unclear. Here we characterized the prevalence and functional consequences of microRNA tailing *in vivo*, using the *C. elegans* model. Our data showed that miRNA tailing in *C. elegans* consists mostly of mono-uridylation of mature miRNA species, with rarer mono-adenylation – likely added to microRNA precursors. Through a targeted RNAi screen, we discovered that the TUT4/TUT7 gene family member CID-1 is required for uridylation, whereas the GLD2 gene family member F31C3.2 is required for adenylation. Thus, the TUT4/TUT7 and GLD2 gene families have broadly conserved roles in miRNA modification.

We also examined the role of tailing in turnover in the absence of ongoing miRNA production, using a temperature sensitive *pash-1* strain. At permissive temperatures, PASH-1 is functional, and biogenesis is uninterrupted. When upshifted to 25°C, PASH-1 activity is abrogated, and mature miRNA production ceases. We determined the half-lives of miRNAs after acute inactivation of microRNA biogenesis, revealing that half-lives are generally long (median=20.7h), as observed in other systems. Despite an increased proportion of tailed species in older microRNAs, we detected no changes to microRNA abundance or decay dynamics upon disrupting tailing. Thus, tailing is not a global regulator of miRNA decay in *C. elegans*. Nonetheless, by identifying the responsible enzymes, this work lays the groundwork to explore whether tailing plays more specialized context- or miRNA-specific regulatory roles.

101 Independent nuclear and cytoplasmic silencing mechanisms contribute to transgenerational RNAi

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RNA-mediated interference is a conserved mechanism used broadly to regulate gene expression. In *C. elegans*, RNAi is heritable and has been shown to target both nascent transcripts in the nucleus and mature transcripts in the cytoplasm (Billi et al., 2014). To explore how targeting of nuclear and cytoplasmic RNAs is coordinated during RNAi, we used fluorescence *in situ* hybridization (FISH) to examine the distribution of germline mRNAs targeted by feeding-induced RNAi. We observed a transient increase in nascent transcripts 4-8 hours after the onset of feeding followed by a subsequent decrease. Cytoplasmic transcripts accumulated in nuage (perinuclear condensates) and were rapidly turned over in the bulk cytoplasm. In progeny of fed mothers, we observed reduced transcript levels at the locus, low levels of RNA present in the nuage, and RNA strongly depleted in the bulk cytoplasm. These findings confirm that RNAi affects the accumulation and distribution of nascent and cytoplasmic transcripts in both animals exposed to the RNAi trigger and their progeny.

HRDE-1/WAGO-9 is a nuclear Argonaute required for transgenerational RNAi (Buckley et al., 2012). As expected, we found that HRDE-1 is required for nascent transcripts to respond to RNAi. Despite no change in nascent transcripts, *hrde-1* mutants accumulated targeted mRNAs in the nuage in both fed mothers and F1 progeny as in wild-type. ZNFX-1 is a nuage-localized SF1 helicase also required for transgenerational inheritance of RNAi (Ishidate et al., 2018; Wan et al., 2018). We found that *znfx-1* mutants have a phenotype opposite that of *hrde-1*: a wild-type nuclear response, but a failure in both recruiting RNAs to nuage and degrading the RNAs in the cytoplasm. Consistent with HRDE-1 and ZNFX-1 functioning in distinct branches of the RNAi pathway, *znfx-1; hrde-1* double mutants were defective in both nuclear and cytoplasmic responses and failed to show any silencing in F1 progeny. These observations suggest that RNAi triggers two distinct responses required for transgenerational RNAi: a nuclear response dependent on *hrde-1* that reduces production/accumulation of nascent RNAs at the targeted locus, and a cytoplasmic response dependent on *znfx-1* that retains targeted mRNAs in nuage and prevents their accumulation in the cytoplasm. The nuclear and cytoplasmic responses can be inherited independently of one another, and both are required for maximum silencing in progeny.

103 ELT-3 regulates cuticle collagen expression in response to environmental stimuli

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The *C. elegans* cuticle is a collagen-rich structure that acts as a physical barrier to protect the animal from pathogens, desiccation, and other stresses. Over 170 cuticular collagens are predicted in the *C. elegans* genome, but the role of each individual collagen is unclear, as is the need for so many collagens. Stage-specific and dauer-specific specialization of the cuticle, achieved by modulating collagen gene expression, has been reported, suggesting that the *C. elegans* cuticle may be tailored to the specific needs of the animal during development. We find that there is likely also specialization of the cuticle in response to environmental factors. Taking advantage of missense mutations in collagen genes that result in a rolling phenotype, we were able to identify environmental factors that result in changes in the cuticle, either enhancing or suppressing the effects of these mutations. We find that diet, early developmental arrest, and population density can differentially influence the penetrance of rolling in these mutants. These effects are in part due to changes in collagen gene expression that are mediated by the GATA family transcription factor ELT-3. We propose a model by which ELT-3 regulates collagen gene expression in response to environmental stimuli to promote the assembly of a cuticle specialized to a given environment.

104 A single cell multiomics approach to resolve genomic drivers of *C. elegans* development

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Animals develop from a single totipotent zygote that gives rise to all of the diverse cell types of the organism, which are genetically identical. During cell divisions in the embryo, most cells become specified to particular fates and initiate differentiation programs. Other cells maintain pluripotency and generate differentiated cells later in life. However, molecular events at decision making points when cells either maintain their cellular potency or commit to differentiation programs are poorly understood. To understand these processes, we need to determine the step-by-step changes in genomic remodelling that drive changes in gene expression from the birth of the pluripotent cell through its period of quiescence, activation, and production of different cell types.

We are using 10X genomics single cell technology to jointly measure chromatin accessibility and RNA output in individual cells of different *C. elegans* lineages in order to follow molecular events that drive cell fate commitment and expression at various steps of development. Our data reveal differences in DNA accessibility before and after zygotic genome activation (ZGA). Following the activation of zygotic transcription, regulatory elements controlling key lineage-defining marker genes become accessible in subpopulations of cells and RNA output shows high temporal correlation with chromatin accessibility. Following the E lineage as an example revealed dynamic processes that result in the precise activation of specific master transcription factors at various steps of the E lineage trajectory.

Using these data, we are developing new computational tools to recapitulate the real-time trajectory of individual cells throughout development by mapping cells onto the lineage tree and temporally ordering their age. With these methods we will be able to follow the precise molecular events resulting in the commitment of cells to specific developmental programs and finally understand and even predict the logic underlying cellular potency in *C. elegans*.

105 Oscillatory expression of molting cycle genes is coordinated with pharynx growth in larvae

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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 transcriptional reporters, 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 and marginal cells, respectively. Moreover, measuring size of the pharynx using time-lapse imaging of larval development revealed that 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 many 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, in time-lapse imaging of transcriptional reporters we observed that continued pharynx growth after starvation-induced arrest of body growth is accompanied by one cycle of oscillatory gene expression. 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.

106 Analysis of OEF-1 as a potential epigenetic reader of H3K36me3 in the *C.elegans* germ line

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The trimethylation of Lys 36 residue on H3 (H3K36me3) is a signature histone mark for gene expression in the *C.elegans* germline. Its genome distribution is mediated specifically by MES-4 and MET-1 histone methyltransferases. This modification regulates several events related to transcription, including promoting splicing, suppressing cryptic transcription initiation, and suppressing DNA damage. H3K36me3 is likely linked to these downstream pathways by epigenetic "readers", proteins that bind a specific epigenetic mark and regulate downstream pathways in response. However, H3K36me3 reader proteins in the *C.elegans* germline are poorly characterized. We explored the possibility that Oocyte-Excluded Factor 1 (OEF-1), a germline-specific factor discovered in our lab (McManus and Reinke, 2017), might function as a reader of H3K36me3. OEF-1 has a role in germline progression, binds preferentially to autosomal genes, and co-localizes with H3K36me3. To study the functional relationship between OEF-1 and H3K36me3, we developed a computational pipeline comprehensive of ChIP-seq and RNA-seq experiments, which we then analyzed. ChIP-seq data for H3K27me3 and H3K36me3 in *oef-1* mutants and wild-type adults were obtained from purified germ nuclei, and binding profiles were then further analyzed. RNA-seq was performed from dissected gonads from *oef-1* mutant and wild-type young adults and analyzed for gene expression and splicing differences. From the ChIP-seq data, we found a strong correlation between OEF-1 and H3K36me3 binding profiles. From the RNA-seq data, we found that, even though the loss of OEF-1 has minimal effect on transcript abundance, it greatly increases the frequency of retained introns predominantly at the level of autosomal germline-specific genes. Thus, our data to date implicates OEF-1 in a role in promoting splicing fidelity in the *C.elegans* germ line, potentially by its association with H3K36me3 at germline-expressed genes.

107 LOTUS-domain containing proteins recruit *C. elegans* Vasa to germline granules and control the formation and size of the condensates

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Limkain, **O**skar and **TU**DOR 5 and 7 are LOTUS-domain containing proteins that have been described as important regulators of germline development and nucleators of ribonucleoprotein complexes in the germ cells of many metazoans. LOTUS-domain proteins have not been previously described in the *C. elegans* germ line.

MIP-1 and MIP-2 are two previously uncharacterized paralogs that contain two LOTUS domains and extensive intrinsically disordered regions. Using co-immunoprecipitation, we found these proteins to be strong interactors of MEG-3, another intrinsically disordered protein that is a core component of embryonic P granules, and we named them **M**EG-3 **I**nteracting **P**roteins 1 and 2. Fluorescently tagged and deletion alleles of the MIPs generated by CRIPSR-Cas9 show that the MIPs are constitutive components of germline granules in both embryos and the germ line.

The simultaneous depletion of MIPs produces temperature sensitive sterility, a strong mortal germ line phenotype, and defects that affect all major developmental switches in germline development, including germline stem cell maintenance, the progression of cells through meiosis, and gametogenesis. The MIPs are jointly required for the condensation of other core P granule components, including MEG-3, the *C. elegans* Vasa homolog GLH-1, and PGL proteins. Interestingly, loss of function of either MIP individually affects the distribution and the size of remaining granules in the germ line in complementary ways. In oocytes, *mip-1* deletion produces larger granules that are mostly cytoplasmic and *mip-2* deletion produces smaller granules mostly attached to the nuclear membrane. Further biochemical and yeast-two hybrid analyses have shown that the MIPs

physically interact with each other by forming homo- and heterodimers, and they directly bind GLH-1 through their N-terminal region, which contains the LOTUS domains. We propose that these proteins act as organizing centers in ribonucleoprotein networks and form a scaffold that helps recruit and balance essential RNA processing machinery within germline granules to regulate key developmental transitions in the germline.

108 Transgenerational regulation of sex determination

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Multicellular organisms transmit genetic information through an immortal germ line which must undergo self-renewal at each generation. Failure of germ cells to maintain telomeres, small RNA expression, or repair DNA leads to a progressive loss of fertility as germ cells become unable to proliferate or enter diapause from accumulation of stress, a phenomenon known as germline mortality. We have identified a novel class of mutants that display transgenerational sterility which is unrelated to accumulation of stress. Ablation of the ZFAND3/5/6 homologs *F22D6.2* and *F56F3.4*, which we propose to name *mstr-1* and *mstr-2* (multigenerational sterility, temperature regulated) leads to a progressive transformation of the nematode male germline to female over multiple generations thus causing sterility. Unlike canonical mortal germline mutants, sterile *mstr-1; mstr-2* mutants are near-fully fertile when outcrossed to wild-type males. In these mutants, the commitment of germ cells to spermatogenesis can be rescued by novel mutations in the proteasome or the terminal sex regulating transcription factor GLI/*tra-1*. We then show that MSTR-1 coimmunoprecipitates with the mRNA of QKI/GLD-1, a key sex determinant upstream of *tra-1*, and spatiotemporally restricts its expression from proximal germ cells fated for spermatogenesis. In *mstr-1; mstr-2* mutants, each generation of maintenance at 25°C shows accumulation of ectopic GLD-1 expression in spermatogenic cells, which is rescued by the proteasomal suppressors. Perplexlingly, *gld-1* mRNA expression remains constant over multiple generations while its protein product increases. Therefore, we propose a model where MSTR-1 binds to mRNA and targets nascent proteins for proteasomal degradation to ensure proper spatial and temporal-generational expression of cell fate determinants. We are currently investigating the mechanistic basis of this novel post-transcriptional transgenerational regulation of gene expression. Overall, our work shows that in sexually reproducing organisms, germline immortality not only requires the clearance of stress but also renewal of germ cell commitment to spermatogenesis every generation.

109 Defining the function of EXC-4/CLIC in Ga-Rac signaling using TurboID to identify physical interactors

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Tubulogenesis, the process of biological tube formation and maintenance, is regulated by conserved signaling pathways. We use the *C. elegans* excretory canal (*ExCa*) cell, a unicellular tube, as a model to identify and study conserved tubulogenesis regulators. EXC-4, a *C. elegans* Chloride Intracellular Channel (CLIC) protein, was first defined for its role in *ExCa* tubulogenesis and subsequent work using human endothelial cells (hEC) and knockout mice showed that the *exc-4* orthologs *CLIC1* and *CLIC4* regulate angiogenesis. Our recent collaborative studies (Arena and Shaye, *in preparation*, Mao et al., *in press*) show that EXC-4/CLICs are conserved regulators of Gα and Rac signaling, but how CLICs function in this pathway remains unknown. Previous work showed that EXC-4 constitutively localizes to the *ExCa* apical plasma membrane, and that an N-terminal putative transmembrane domain (PTMD) is necessary and sufficient for this localization, but that the EXC-4 PTMD alone does not rescue *exc-4* null (*0*) mutants—indicating that the C-terminus provides functionality. Moreover, it was shown that the C-terminus of human CLIC1 can rescue *exc-4(0)*, indicating conserved function, but only when recruited to the apical membrane via the EXC-4 PTMD. CLIC1 and CLIC4 are cytoplasmic at steady state in hEC, but they are rapidly recruited, lasting <5', to the plasma membrane upon activation of Gα and Rho/Rac signaling. Therefore, in both *C. elegans* and human cells, EXC-4/CLIC membrane localization is critical for their role in Gα-Rho/Rac signaling. To understand how CLICs perform this conserved function we want to identify direct EXC-4/CLIC interactors. We hypothesize that functional EXC-4/CLIC-containing complexes will be readily identified in the *ExCa* because EXC-4 is constitutively localized to the membrane in this cell, where Gα-Rho/Rac signaling is initiated. To identify interactors, we are using TurboID, which rapidly and efficiently biotinylates proteins within ~10nm *in vivo*. Using an *ExCa*-specific promoter we are expressing various EXC-4::TurboID fusions as "baits" to identify interactors, and we have already found that full-length EXC-4::TurboID is functional and well-expressed. We expect to present initial results from this approach at the meeting.

110 The secreted modular calcium binding protein (SMOC-1) can function as both a long-range and a short-range modulator of BMP signaling in *C. elegans*

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The bone morphogenetic protein (BMP) pathway is a highly conserved signaling cascade that regulates key developmental and homeostatic processes in metazoans. The BMP pathway is conserved in *Caenorhabditis elegans*, and is known to regulate body size and mesoderm development. We have identified the *C. elegans* SMOC-1 (secreted modular calcium-binding protein-1) protein as a positive modulator of the BMP pathway. SMOC-1 is a predicted secreted matricellular protein with two predicted functional domains: the thyroglobulin-like type I repeat (TY) domain and the extracellular calcium binding (EC domain). Through a series of molecular genetic experiments, we have demonstrated that SMOC-1 functions in a positive feedback loop to promote BMP signaling: it acts through the BMP ligand DBL-1 to regulate BMP signaling, and its expression in the intestine is positively regulated by BMP signaling. *smoc-1* is expressed in multiple tissues. Using cell type specific promoters to drive *smoc-1* expression, we found that SMOC-1 functions cell non-autonomously to regulate body size. Surprisingly, a membrane-tethered SMOC-1 driven by the *smoc-1* promoter can also partially function. These findings suggest that SMOC-1 can act both locally and over a distance to regulate BMP signaling. Intriguingly, the SMOC-1 EC domain alone is sufficient to regulate the BMP pathway when freely secreted, but not when membrane tethered. Conversely, the TY domain alone is not sufficient to promote BMP signaling, yet this domain is required for SMOC-1 to function locally. Together, these findings suggest that the SMOC-1 TY and EC domains contribute to different facets of SMOC-1 functions. We are using immunoprecipation/mass spectrometry (IP/MS) to identify interacting partners of the various functional forms of SMOC-1. Comparative analysis of the IP/MS results will yield molecular insights on how SMOC-1 promotes the BMP pathway.

111 COP9 signalosome component CSN-5 stabilizes stem cell regulators FBF-1 and FBF-2

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RNA-binding proteins FBF-1 and FBF-2 are required for stem cell maintenance in *C. elegans*, although the mechanisms by which FBF protein levels are regulated remain unknown. Using a yeast two-hybrid screen, we identified an interaction between both FBFs and CSN-5, a component of the COP9 (constitutive photomorphogenesis 9) signalosome. This highly conserved COP9 complex can affect protein stability through a range of mechanisms including deneddylation, deubiquitination, and phosphorylation (Wolf et al., 2003). We discovered that CSN-5 promotes the accumulation of FBF-1 and FBF-2 proteins in *C. elegans* stem and progenitor cells. Phenotypic analysis results were consistent with *csn-5* contributing to FBF function since *csn-5* germlines are masculinized (produce only sperm similar to *fbf-1/2* loss of function) and show reduced numbers of stem cells. Similar phenotypes were observed in worms mutant for another COP9 holoenzyme component, *csn-6*. Curiously, phenotypes of the *csn*-2 mutant were clearly distinct, where oocytes were still forming and stem cell numbers were not as affected. Additionally, FBF protein levels were not as affected in the *csn-2* mutant as observed in the *csn-5* mutant. This suggests that *csn-5*'s effect on FBFs might be independent of the COP9 holoenzyme. Mapping protein-protein interactions between FBFs and CSN-5 suggested that the MPN (Mpr1/Pad1 N-terminal) metalloprotease domain of CSN-5 interacts with the RNA-binding domain of FBFs at physiologically relevant (micromolar) concentrations. Furthermore, these conserved domains of the human homologs PUM1 and CSN5 interact as well, thus identifying a protein complex conserved in evolution. Investigating CSN-5 contribution to FBF protein activity and stem cell maintenance will have implications for human stem cell biology and improve our understanding of diseases like cancer.

113 An Electron Microscopy Pseudo Time Series of the *C. elegans* Embryo

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Advances in Electron Microscopy (EM) bring about the possibility of temporal analysis using staged samples over time as well as offering opportunities to gain insights by synthesizing EM with other imaging modalities. We present a pseudo time series of C. elegans embryonic development, four volumes covering around 2 hours particularly rich in development between 320 and 475 minutes post first cleavage. This period encompasses neurulation, neural organogenesis and neuropil formation key events that build most of the major structures of the nervous system as well as critical events for many other organ systems. We correlate this EM data with florescence data spanning the first eight hours of embryogenesis. Every cell in the florescence data is identifiable via lineaging. To correlate these data sets we develop a novel computational method for alignment of identities between data sets in the challenging presence of spatial and temporal variation. This approach involves co-optimization of spatial alignment and the structure of labeled data based on a model of dynamic anatomy in the form of an adjacency graph with expected variation. This model captures both variable elements and consistent spatial proximity relationships. We identify every cell in three of the four time points. Identity results are accurate, ranging from 72 to 78 percent correct when assessed

against a large set of manual annotations based on position and morphology. This is better than any previously reported results for identifying all cells in an organism based on position alone. The resulting single cell level annotation allows efficient navigation of this large EM data set. We use the sequence to probe the interactions over time between different components within the nerve ring elucidating the relationship between the temporal and spatial location of initial outgrowths into the ring and ultimate structure. We also examine the interaction between cells during the formation of the amphid dendrite structure providing insight into the timing and succession of events at the inter and intra cellular level. Our observations only scratch the surface of the details available in the data set. We will provide the EM data with single-cell level annotation as a resource for the community.

114 A retrograde zippering mechanism regulates neurite placement in the *C. elegans* nerve ring

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A fundamental design principle of nervous systems is the grouping of neuronal contacts into layers within nerve bundles. The layered arrangement of neurites requires nanoscale precision in their placement within bundles, and this precision, which cannot be exclusively explained by simple tip-directed outgrowth dynamics, underpins synaptic specificity and circuit architecture. Here we implement novel imaging methods to document the specific placement of single neurites during the assembly of the *C. elegans* nerve ring. We uncover a retrograde zippering mechanism that controls precise placement of neurites along specific layers. Nanoscale precision in neurite placement is orchestrated via temporally-regulated expression of Ig adhesion molecule SYG-1. SYG-1 acts as an instructive signal, defining layer boundaries and guiding neurite zippering onto target neurons. SYG-1 acts through its canonical ligand SYG-2 and SYG-1 extracellular domains are sufficient for instructing neurite placement. Our study suggests that adhesion-mediated retrograde zippering might be an important developmental mechanism that coordinates neurite placement and synaptic connectivity within brain neuropil bundles *in vivo*.

115 Temporal Maturation of the C. elegans Post-Embryonic Nervous System

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In most animals, the majority of the nervous system is generated and assembled into neuronal circuits during embryonic development. However, during juvenile stages, nervous systems still undergo extensive anatomical and functional changes to eventually form a fully mature nervous system by the adult stage. The molecular changes in post-mitotic neurons across post-embryonic development and the genetic programs that control these temporal transitions are not well understood. Using the model organism *C. elegans*, we comprehensively characterized the distinct functional states (locomotor behavior) and corresponding distinct molecular states (transcriptome) of the post-mitotic nervous system across temporal transitions from early post-embryonic periods to adulthood. We observed pervasive changes in gene expression, many of which are controlled by the developmental upregulation of the conserved heterochronic miRNA *lin-4/mir-125* and the subsequent promotion of a mature neuronal transcriptional program through the repression of its target, the transcription factor *lin-14*. The functional relevance of these molecular transitions are exemplified by a temporally regulated target gene of the *lin-14* transcription factor, *nlp-45,* a neuropeptide-encoding gene. We found that *nlp-45* is required for temporal transitions in exploratory activity across larval stages, across sexual maturation, and into a diapause arrest stage. Combined, these studies provide new insights into regulatory strategies that control neuron-type specific gene batteries to modulate distinct behaviors states across temporal, sex and environmental dimensions of post-embryonic development, and also provide a rich atlas of post-embryonic molecular changes to uncover additional regulatory mechanisms.

116 cAMP controls a trafficking mechanism that directs the neuron specificity and subcellular placement of electrical synapses

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Despite the functional importance of electrical synapses, the molecular mechanisms that direct the formation of neuron-specific gap junctions remain largely unknown. To address this question, we identified targets of the UNC-4 transcription factor that controls connectivity in the C. elegans motor circuit. UNC-4 functions in VA motor neurons to direct the formation of gap junctions on the VA axon with the interneuron AVA (VA-AVA). Locomotion is disrupted in *unc-4* mutants because VAs are miswired with electrical input from the interneuron AVB (VA-AVB) which aberrantly form on the VA soma. Thus, UNC-4 controls both the specificity and subcellular placement of electrical synapses. We determined that UNC-4 blocks expression of two antagonists of cAMP, the phosphodiesterase, PDE-1, and the Go/Gi-coupled GPCR, FRPR-17, to prevent assembly of ectopic VA-AVB gap junctions. This finding suggests that cAMP signaling promotes the formation of functional wild-type VA-AVA gap junctions. We validated this hypothesis by showing that optogenetic elevation of cAMP rescues the Unc-4 movement defect and thus restores VA-AVA circuit function. In addition, forced depletion of cAMP in VAs phenocopies *unc-4* mutants. Because gap junction placement is shifted from the VA axon to cell soma in unc-4 mutants, we reasoned that trafficking of gap junction components could be perturbed. Live-cell imaging of the gap junction protein, GFP-UNC-9, confirmed that trafficking into the VA axon is strikingly impaired in *unc-4* mutants. Genetic activation of cAMP signaling is sufficient to restore GFP-UNC-9 trafficking in VAs. Thus, we propose that cAMP directs both the specificity and placement of electrical synapses by activating mechanisms that transport gap junction components into the VA axonal compartment. Although studies in cultured cells have implicated cAMP in gap junction assembly, our in vivo experiments now firmly establish that cAMP regulates the biogenesis of electrical synapses in an intact nervous system.

117 How do neurexins promote presynaptic development?

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Synapse development is critical for the formation of neuronal networks while defects in synapse formation lead to neurodevelopmental disorders. Cell adhesion molecule signaling plays a prominent role in synapse formation, and strong evidence has emerged linking dysfunction of the cell adhesion molecule neurexin to autism spectrum disorders and intellectual disability. However, the precise role of this molecule in regulating synapse formation remains elusive. In humans, three neurexin genes are expressed as thousands of splice isoforms, all of which share a conserved intracellular domain. Understanding the intracellular signaling pathway mediated by neurexin may lead to the development of therapeutics for a multitude of neurexin-associated disorders. *C. elegans* has long been established as a powerful tool for gene discovery. To elucidate which proteins interact with neurexin's intracellular domain (ICD) we are employing a recently developed enzyme-catalyzed proximity labeling method called TurboID. In TurboID, a protein of interest is tagged with a promiscuous labeling enzyme – the *E. coli* biotin ligase BirA – which biotinylates neighboring proteins located within a few nanometer radius. Biotinylated proteins are pulled down using streptavidin. To determine the appropriate position for insertion of BirA within neurexin's ICD while not interfering with neurexin's function, we engineered transgenes with various BirA locations. Transgenes carrying BirA inserted just before the C-terminal PDZ binding motif fully rescued the synaptic assembly defects observed in neurexin null mutants, so we chose this location for endogenous insertion via CRISPR/Cas9. The endogenously-tagged BirA strain was then validated using our previously developed presynaptic assembly markers and showed no synaptic defects when compared to wild-type animals. Streptavidin pull-downs followed by mass spectrometry were performed using our endogenously-tagged neurexin-BirA strain and 70 potential neurexin ICD interactors were identified when compared to controls (cytosolic BirA and wild type strains). Of those, we are following up on 21 hits that, by gene ontology analysis, include cytoskeletal-related, endo/exocytitc, metabolic and adhesion processes. This approach will reveal the signaling pathway downstream of neurexin with the potential of identifying therapeutic targets widely applicable to various neurexin-associated disorders.

118 Sensory cilia architecture shapes olfactory response dynamics

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Structurally and functionally diverse sensory cilia house signal transduction molecules and play essential roles in olfaction, hearing, and photoreception. Complex cilia morphologies dictate the concentration and organization of signaling molecules within them, and are thus considered critical for precisely shaping sensory responses. However, the contribution of unique cilia morphologies to sensory signaling is poorly understood. Chemosensory neurons in *C. elegans* are specialized to respond to unique subsets of chemical and other stimuli and exhibit a range of morphologically distinct cilia. Cilia are built by the process of intraflagellar transport (IFT) which traffics structural and signaling components into and out of cilia. Mutations in IFT genes result in severe cilia structural defects. We and others have found that while mutations in IFT genes abolish chemical responses in a subset of chemosensory neurons, surprisingly, primary odorant responses in neurons containing complex 'wing' cilia are

unaffected in these mutants. Instead, these neurons exhibit defects in sensory adaptation and desensitization. To decouple the contributions of cilia morphology and IFT to odorant responses, we generated a temperature-sensitive mutation in a *C. elegans* kinesin motor protein. Upon shift to the restrictive temperature, IFT is acutely blocked without immediate disruption of cilia morphology. We find that a minimal cilium length is required for odorant detection in rod-like simple cilia. However, in wing cilia, their morphological complexity shapes desensitization but not adaptation of the sensory response. Instead, regulated trafficking of G protein-coupled receptors (GPCRs) may drive adaptation to odorants. Our results suggest that signaling-dependent removal of GPCRs is mediated by phosphorylation via the G-protein coupled receptor kinase GRK-2, followed by BBSome-mediated ciliary exit of GPCRs. We also identify a role for arrestin-mediated endocytosis of GPCRs at the cilia base in shaping sensory response dynamics. Together, our work demonstrates how specialized cilia morphologies contribute to the unique responses of individual chemosensory neurons in *C. elegans,* and highlights the importance of ciliary structural diversity in shaping sensory behaviors.

119 Mechanisms of selective neuron-glia attachment

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Glial cells form specialized attachments to selective subtypes of neurons, but how such selective neuron-glia pairing occurs remains unknown. To address this question, we have focused on two sensory neurons, called URX and BAG, that extend dendrites to the nose tip and form exclusive stereotyped attachments to a single glial partner, the lateral ILso glial cell. Using forward and candidate genetic screens, we identified several factors that are required during embryogenesis for this neuron-glia interaction, including the cytoplasmic scaffolding proteins GRDN-1 and MAGI-1 and the adhesion molecules SAX-7 and HMR-1. We find that, in wild-type embryos, URX and BAG dendrites attach near the developing nose and stretch to their full lengths during embryo elongation, while in mutants the URX and BAG dendrites detach from the nose as early as the two-fold stage of embryogenesis, resulting in severely shortened dendrites in mature animals. Interestingly, in two-fold stage embryos, the BAG dendrite ending is already adjacent to lateral ILso glia, while the URX dendrite ending is near a different group of glia, leading to the hypothesis that the mature attachment of URX to ILso glia develops in a stepwise manner through transient interactions with other glia. Consistent with this hypothesis, we find that expression of GRDN-1 in all glia, but not in ILso alone, rescues URX dendrite development. We have developed novel imaging tools that enable us to visualize these intermediary contacts of URX with other glia. Preliminary data suggest that *grdn-1* mutants exhibit expansion of the apical region of glia, reminiscent of apical constriction defects that have been reported upon knockdown of the vertebrate homolog of GRDN-1 in neuroepithelia. Further, we find that glia-specific depletion of the canonical apical polarity determinant PAR-3 causes URX dendrite extension defects that recapitulate the defects seen in *grdn-1* mutants. Together, these results point to an unexpected role for apical polarity pathways in glia being required to guide the URX dendrite to form its mature neuron-glia attachment.

120 The optogenetic voltage clamp (OVC) – A closed-loop all-optical approach for true optogenetic control of muscles and neurons in live animals

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Unlike voltage-clamp electrophysiology, optogenetics allows perturbation, but not true control of neural activity. We thus established an all-optical optogenetic voltage clamp (OVC), in which fast, live readout of the cell membrane potential is used to generate light-based feedback, which is sent to optogenetic actuators for de- or hyperpolarization to clamp the cell at a distinct potential. To this end, we combined two opposing rhodopsins with a genetically encoded voltage indicator, embedded in a software-based live closed-loop feedback system. This way, the OVC synergistically combines the non-invasive character of imaging methods with the control capabilities of electrophysiological methods in live animals.

To probe the performance of the OVC, we used different excitable cell types. First, we established the approach in easily accessible muscle cells, where different configurations allowed reliable clamping of their membrane potential. Second, we turned to the spontaneously spiking pharyngeal muscle, that produces action potentials at around 4 Hz, and which the OVC could follow and clamp in a dynamic fashion. Third, we tested the OVC on cholinergic and GABAergic motor neurons, where

it equally reliably allowed to clamp their membrane potential based on the voltage sensor fluorescence. Finally, we tested applicability of the OVC in the GABAergic motor neuron DVB, that activates expulsion muscle contraction during the defecation motor program, by producing action potential like activity every 45-50 s. Last, we also calibrated fluorescence signals to electrically measured membrane voltages.

Live computation of acquired fluorescence data streams allowed achieving a refresh rate of up to 90 Hz, where the OVC can reliably clamp relative changes in fluorescence between -5 to 5 % $\Delta F/F_0$ in all targeted tissues. An additional version of our software enables the live selection of distinct holding values by the experimenter, facilitating an instant response to observed activities of the clamped cell. Our software works independent of the combination of optogenetic tools and hardware components, thus the OVC approach should be easily transferrable to other organisms, but also to cultured cells. Outperforming standard patch-clamp electrophysiology in terms of non-invasiveness, throughput and ease of application, the OVC paves the way for true all-optical control of individual neurons in freely behaving animals.

121 Real-time volumetric whole-animal imaging at cellular resolution with SCAPE microscopy in NeuroPAL worms

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Recording neural activity at single cell resolution during unrestrained behavior holds tremendous potential for investigating the C. elegans neural code on a global scale. As fluorescent calcium indicators and 3D microscopy speeds have improved, recording from 100's of cells in moving worms has become feasible. However, 2 problems remain 1) being able to extract robust information from individual cells in a moving worm, and 2) knowing the defined identity of each individual tracked cell. Recently, a novel C. elegans strain termed NeuroPAL was developed that labels individual neuronal identities via a stereotypical multi-color fluorescence map. To harness the power of this worm we developed a high-speed multispectral volumetric microscopy platform with sub-cellular resolution, optimized for the NeuroPAL worms. Our SCAPE microscopy-based approach uses a scanning oblique light sheet which provides low phototoxicity and optical sectioning capabilities in a convenient single-objective geometry compatible with common C. elegans sample mounting procedures. The system's multi-laser launch, spectral image splitter and high-speed intensified camera make it possible to rapidly acquire a fully 3D NeuroPAL image in under 0.3 s. These scans can be interspersed with dual channel imaging of GCaMP and RFP with 0.33 x 0.67 x 0.25 µm sampling density over a 310 x 210 µm FEP-covered agarose arena at 13 volumes per second. The resulting data suggests much simpler tracking of uniquely identifiable cells throughout the worm, and analysis of the cellular calcium dynamics during free behavior.

123 HPK-1 prevents the decline of proteostasis through neuroendocrine control of the proteostatic network

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The progressive decline of cellular proteostasis is a hallmark of normal organismal aging, and is the basis for the onset and progression of a growing number of neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's disease. These diseases share a common characteristic: the accumulation of proteotoxic aggregates that result in cellular dysfunction and death. Proteotoxic disease is opposed by a cellular proteostatic network (PN) of approximately 1500-2000 proteins, which maintain the proteome by balancing rates of protein synthesis, degradation, folding, and sequestration. We have identified the transcriptional cofactor HPK-1 (homeodomain-interacting protein kinase) as an important PN component that preserves proteostasis and extends longevity. We find HPK-1 is primarily expressed in the nervous system during adulthood. Loss of neuronal *hpk-1* shortens lifespan, while neuronal overexpression of *hpk-1* is sufficient to increase lifespan. Neuronal HPK-1 is responsive to both acute heat shock and chronic nutritional stress, suggesting HPK-1 may act within the nervous system to integrate diverse signals and coordinate adaptive responses within the PN. We investigated whether HPK-1 acts cell autonomously in neurons to mediate stress response and proteostasis, or alternatively functions cell non-autonomously from neurons to regulate these processes in peripheral tissues. Neuronal loss of *hpk-1* hastens the collapse of both neuronal and muscle proteostasis. Conversely, neuronal overexpression of *hpk-1* delays the progressive decline of both neuronal and muscle proteostasis. Neuronal HPK-1 overexpression produces a paracrine signal to hyper-induce molecular chaperone expression

locally and a neuroendocrine signal to induce autophagy in peripheral tissues. To further investigate the non-cell autonomous regulation of neuronal HPK-1, we expressed HPK-1 in different types of neurons and found that overexpression of HPK-1 in serotonergic and GABAergic neurons is sufficient to preserve proteostasis in the muscle. Interestingly, overexpression of HPK-1 in serotonergic neurons, but not in GABAergic neurons, is sufficient to increase heat stress resistance, suggesting HPK-1 exerts a different PN function in these types of neurons to preserve proteostasis. Overexpression of HPK-1 in single types of neurons is not sufficient to increase lifespan, suggesting that its combinatorial roles in neuronal cell-types is required to extend longevity. Collectively, our results position HPK-1 at a central regulatory node acting from the nervous system, upstream of the greater PN, by exerting distinct but complementary roles in different types of neurons.

124 What *C. elegans* can tell us about the misfolded tau toxicity?

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Abnormal tau phosphorylation and aggregation into bundles of filaments in the central nervous system is a common feature of a heterogeneous group of pathologies called tauopathies. Similarly to other misfolded proteins, tau oligomers more than fibrillar assemblies, have been suggested to be the main responsible of toxicity. Hyperphosphorylated tau is able to spread in the brain exerting its toxic function through a *non-cell-autonomous* mechanism.

With hypothesis that abnormal tau conformers play a causal role in driving toxicity, we conceived an original, integrated approach involving the use of recombinant human wild-type (WT) tau or tau carrying P301L mutation, cells overexpressing tau P301L, brain homogenates from WT or transgenic mice overexpressing tau P301L and *C. elegans*. Cerebral homogenates from chronic traumatic brain injured (TBI) mice, showing widespread tau pathology, were also employed.

We found that recombinant tau oligomers, but not monomers, induced functional deficits in *C. elegans* consisting on neuromuscular impairment and altered synaptic transmission. Results were similar when worms were exposed to brain homogenates from P301L or TBI mice. These defects were not mediated by the *C. elegans'* endogenous tau homolog protein with tau-like repeats (PTL-1), which has high level of sequence homology with the repeat region of mammalian microtubule associated protein (MAP)2, MAP4 and tau, as indicated by experiment performed in *ptl-1* knock-out worms.

Harsh protease digestion to eliminate the protein component of the brain homogenates from TBI or P301L mice, pre-incubation with anti-tau antibodies or tau depletion by immunoprecipitation, abolished the toxicity indicating a pivotal role of abnormal tau conformers.

These findings indicate that *C. elegans* represents a tractable model to investigate *in vivo* the toxicity of misfolded/aggregated tau, assessing its impact on neuromuscular function. They support a vital role of abnormal tau species in chronic neurodegeneration and set the groundwork for the development of a *C. elegans*-based platform for screening anti-tau compounds to interfere with the consequences of tauopathies.

125 The Mitochondrial Permeability Transition Pore Activates a Maladaptive Mitochondrial Unfolded Protein Response

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Mitochondrial activity determines aging rate and the onset of chronic diseases. The mitochondrial permeability transition pore (mPTP) is a pathological channel that forms in the inner mitochondrial membrane in response to excessive cytosolic Ca^{2+} or high ROS conditions. Sustained opening of the mPTP leads to outer mitochondrial membrane rupture, release of Ca^{2+} into the cytosol, and cell death. As mitochondrial function declines with age, the frequency of the mPTP increases. The mPTP is central to early-stage pathologies associated with several age-related diseases, including Alzheimer's and Parkinson's disease (AD, PD) and late-stage pathologies of ischemia-reperfusion injuries, including heart attack and stroke. The mPTP is thought to be composed of the F-ATP synthase (complex V) and its membrane-bound H^+ rotor is thought to facilitate pore opening. Oligomycin sensitivity-conferring protein (OSCP), another subunit of F-ATP synthase, helps protect against the mPTP, while its destabilization helps promote the mPTP. We have found that loss of OSCP in the nematode *Caenorhabditis elegans* initiates the mPTP and shortens lifespan specifically during adulthood, in part via initiation of the mitochondrial unfolded protein response (UPR^{mt}). Pharmacological or genetic inhibition of the mPTP, including loss the H^+ rotor, inhibits the adult UPR^{mt} and restores

lifespan. Loss of the H^+ rotor during adulthood also extends lifespan, suggesting that the mPTP normally promotes aging. Our findings reveal how the mPTP/UPR^{mt} nexus may contribute to aging and age-related diseases and how inhibition of the UPR^{mt} may be protective under certain conditions.

126 TCER-1-regulated alternative splicing promotes stress resilience

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Alternative RNA splicing augments proteomic biodiversity in eukaryotes, and has an emerging role in regulating longevity. This process has been shown to have various roles based on physiological demands including those in response to external stimuli. Here, we show that in the nematode *Caenorhabiditis elegans*, exposure to the Gram-negative pathogen, *Pseudomonas aeruginosa,* induces a shift in alternative splicing. In vivo analysis of transgenic reporters of splicing fidelity showed significant splicing changes in multiple tissues. Based on our recent discovery that TCER-1, worm homolog of the human transcription elongation and splicing factor, TCERG1, represses innate immunity in worms, we examined the role of TCER-1 in the modulation of splicing upon pathogen exposure. *tcer-1* mutants exhibited aberrant splicing regulation in multiple tissues, and transcriptomic analysis identified 54 exon-skipping and 468 intron retention events upon *tcer-1* inactivation. In vivo analyses of these events revealed a TCER-1 dependent splicing pattern that correlated with our stress resilience phenotypes. Further, CRISPR-mediated recreation of alternative splicing observed in *tcer-1* mutants enhanced immunoresistance and motor function. Together our findings bring to the forefront the important role of alternative splicing in stress resilience and healthspan, and reveal TCER-1- to be a major regulator of this process.

127 Embryo Integrity Regulates Maternal Proteostasis and Stress Resilience

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The proteostasis network (PN) comprises the cellular machineries that regulate protein synthesis, folding, and degradation, to promote proteome integrity. Reduced functionality of the PN during aging results in the accumulation of misfolded and aggregated species that are detrimental for cellular health, and is a hallmark of many age-associated diseases. In multicellular organisms, the PN is regulated by transcellular communication to coordinate proteostasis across tissues and organs in response to physiological and environmental stimuli. The reproductive system in particular is a critical tissue for proteostasis regulation, and signals from the germline initiate the decline of somatic proteostasis and cellular stress responses at reproductive maturity in *C. elegans*. Here we show that stress resilience and proteostasis are also regulated by embryo-to-mother communication in reproductive adults. To identify genes that act directly in the reproductive system to influence somatic proteostasis, we performed a tissue-targeted RNAi screen for germline modifiers of muscle polyglutamine aggregation. We found that inhibiting the formation of the extracellular vitelline layer of the fertilized embryo inside the uterus suppresses aggregation in multiple somatic tissues and improves maternal stress resilience in an HSF-1-dependent manner. Damage to the vitelline layer of the embryo also prevents the collapse of the heat shock response that normally occurs in early adulthood. This embryo-to-mother pathway relies on DAF-16/FOXO activation in vulva tissues to maintain organismal stress resilience, suggesting that the vulva senses the integrity of the fertilized embryo to detect damage and initiate the organismal response. Gene expression analysis of vitelline layer defective animals using RNA sequencing also revealed that genes involved in lipid metabolism are activated, which is accompanied by elevated fat stores, suggesting a link between fat metabolism and proteostasis in these animals. Our findings reveal a previously undescribed transcellular pathway that links the integrity of the developing progeny to somatic proteostasis regulation and lipid metabolism in the parent. This pathway may serve to reassess commitment to reproduction and promote somatic endurance when progeny production is compromised.

128 A neuronal thermostat controls membrane fluidity in *C. elegans*

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Cells adapt to temperature shifts by adjusting lipid desaturation levels and the fluidity of membranes in a process dependent on fatty acid desaturase enzymes, and which is thought to be controlled cell autonomously. We have discovered that subtle, step-wise increments in ambient temperature can lead to the conserved heat shock response being activated in head neurons of *C.elegans*. This response is exactly opposite to the expression of the lipid desaturase FAT-7 in the worm's gut with respect to temperature.

We use neuronal overexpression of *hsf-1,* the master regulator of the heat shock response*,* as a tool to study the consequences of the heat shock response in neurons. We find that over-expression of *hsf-1*, in neurons, causes extensive fat remodeling to occur across tissues. These changes include a decrease in *fat-7* desaturase expression and an increase in acid lipase expression in the intestine, as well as a shift in the levels of unsaturated fatty acids in the plasma membrane. These shifts are in line with membrane fluidity requirements to survive in warmer temperatures. This is further supported by our lifespan data showing that neuronal over-expression of *hsf-1* is more beneficial at warmer temperatures. Knocking down *hsf-1* specifically in neurons revealed that endogenous HSF-1 in neurons is not only sufficient, but also partially necessary to control the fat remodellling response in distal tissues.

We find that the cGMP receptor, TAX-2/TAX-4, expressed in a subset of at least six sensory neurons, as well as TGF-β/BMP signaling, are key players in the transmission of neuronal stress to peripheral tissues. This suggests that a thermostat-based mechanism can centrally coordinate membrane fluidity in response to warm temperatures across tissues in multicellular animals.

129 Neuronal HLH-30/TFEB Regulates Longevity and Heat Stress Resistance Via Distinct Non-Cell Autonomous Mechanisms

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Transcription factor EB (TFEB) is the central transcriptional activator of the autophagy-lysosomal pathway and is important for organismal lifespan regulation and stress resistance. However, the tissue-specific autonomous and non-autonomous mechanisms orchestrated by TFEB are not well understood. As neurons constitute an important tissue for coordinating organism-wide processes, we investigated the neuronal role of TFEB in stress resistance and longevity. To this end, the *C. elegans* TFEB orthologue, *hlh-30*, was pan-neuronally expressed against a ubiquitous *hlh-30* loss-of-function background. Findings indicated that while neuronal HLH-30/TFEB is important for the enhanced longevity of *daf-2* insulin/insulin-like growth factor receptor signaling (IIS) mutants, it was insufficient to extend the shortened lifespan of *hlh-30* loss-of-function mutants. Surprisingly, neuronal HLH-30/TFEB reconstitution mediated robust improvements in thermoresistance in *hlh-30* but not *daf-2* mutants, suggesting divergent mechanisms of longevity and thermoprotection that can be decoupled with the IIS pathway. Since DAF-16/FOXO is the primary transcription factor responsible for the extended lifespan and improved thermotolerance of *daf-2* IIS mutants, we sought to investigate its non-cell autonomous requirement for neuronal HLH-30/TFEB-mediated longevity and thermoresistance. Preliminary findings suggest a synergism of neuronal HLH-30/TFEB with non-neuronal DAF-16/FOXO in lifespan extension but not thermoresistance. Instead, the uncharacterized gene *W06A11.1* was identified as a bona fide mediator of thermoresistance which is non-cell autonomously required by neuronal HLH-30/TFEB for effecting thermoprotection. Taken together, these findings indicate a decoupling of the longevity and thermoprotective mechanisms of neuronal HLH-30/TFEB. As TFEB is an emergent target for the treatment of neurodegenerative and age-related diseases, findings herein reveal novel and potentially targetable TFEB-associated mechanisms.

130 *Caenorhabditis elegans* processes sensory information to choose between freeloading and self-defense strategies

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Hydrogen peroxide is the preeminent chemical weapon that organisms use for combat. Individual cells rely on conserved defenses to prevent and repair peroxide-induced damage, but whether similar defenses might be coordinated across cells and tissues in animals remains poorly understood. Here, we screen a collection of sensory neuron genetic ablations in the

nematode *C. elegans* to determine their effects on resistance to peroxide. We identify a neuronal circuit that processes information perceived by two of those sensory neurons to control the induction of hydrogen peroxide defenses in the organism. In the presence of *E. coli*, *C. elegans'* food source, the animal's neurons signal via TGFb-insulin/IGF1 relay to target tissues to repress expression of catalases and other hydrogen peroxide defenses. We found that catalases produced by *E. coli* can compensate for the animal's downregulation of catalases by depleting hydrogen peroxide from the local environment and thereby protecting *C. elegans*. This adaptive strategy is the first example of a multicellular organism modulating its defenses when it expects to freeload from the protection provided by molecularly orthologous defenses from another species.

131 Inheritance of associative memories in *C. elegans*

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Organisms often face changing environments; hence, the ability to predict future conditions is essential for survival. Associative memories play a central role in this regard, as memory reactivation generates fast physiological responses that aid in coping with impending developments. But could these valuable associative memories be transferred to subsequent generations? We show that parental associative memories of traumatic experiences are indeed inheritable. We trained worms to associate a naturally favorable odor with starvation. Subsequent odor-evoked memory reactivation induced stress. Surprisingly, the stressful associative memory was also transmitted to the F1 and F2 generations, even though these animals were never exposed to the odorant before. Moreover, the stress responses provided both the parents and the offspring with a fitness advantage. The sperm, but not the oocytes, transmitted the associative memory, and a candidate-gene screen revealed that H3K9 methylation and the RNAi machinery underlie these heritable responses. Furthermore, activation of a single chemosensory neuron (AWC^{OFF}) sufficed to induce a systemic stress response in both the parents and their progeny, suggesting that this neuron is part of the memory engram. Our findings provide an important evidence, to the yet debatable idea, that associative memories can be inherited

133 Repeated Sampling of *Caenorhabditis elegans* Across the Hawaiian Islands Reveals Spatiotemporal Patterns of Genetic Diversity

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Caenorhabditis elegans isolated from the Hawaiian Islands are known to harbor a high degree of genetic diversity relative to non-Hawaiian isolates. It was recently suggested that Hawaiian *C. elegans* can be partitioned into at least four genetically distinct groups. An analysis of geospatial environmental data further suggested that the genetic groups might associate with environmental parameters such as elevation and temperature, although the sample size for that study was small (n = 43 isolates). To better characterize the niche and genetic diversity of Hawaiian *C. elegans* and further define the associations of genetic groups with environmental parameters, we sampled different substrates and niches across the Hawaiian Islands six times over a three-year period. In total, we isolated 7,107 nematodes from 2,400 of 4,506 substrate samples (53% success rate). Among the nematodes we isolated, we identified five *Caenorhabditis* species, including 499 *C. elegans*, 377 *C. briggsae*, and 55 *C. tropicalis* isolates. We measured several environmental parameters at each sampling site and combined them with environmental parameters from geospatial databases to reveal that *C. elegans* is typically found in cooler and relatively drier climates at higher elevation than the other two selfing *Caenorhabditis* species. We isolated *C. elegans* most frequently from montane-alpine mesic forest habitat dominated by plant species native to the Hawaiian Islands. When possible, we cryopreserved *C. elegans* isolates and sequenced their genomes. To date, including Hawaiian isolates from collaborators, we have sequenced the genomes of 505 Hawaiian *C. elegans* isolates. With these data, we grouped the isolates into 163 isotypes (strains belonging to a single isotype have >0.9997 genome-wide concordance). We found that some of the isotypes were collected from the same locations over the three-year sampling period, and most of the collections of the same isotype were found within 500 meters of each other. Principal component analysis (PCA) of genetic variation revealed that the 163 isotypes fall into seven genetically distinct groups, three more than previously found on the islands with a smaller sample. Taken together, our findings begin to outline the spatiotemporal patterns of *C. elegans* genetic diversity on the Hawaiian Islands and raise new questions about evolutionary forces driving the genetic structure we have uncovered. For example, are these groups isolated by ecological or geographic distances, or perhaps both, and to what extent do reproductive incompatibilities contribute to the structure we have observed?

134 Natural genetic variation in *irld* genes modifies insulin signaling to influence starvation resistance

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The genetic basis of natural variation in starvation resistance is not well understood though it is a fundamental, biomedically important trait. We developed a population selection and sequencing approach (MIP-seq) to measure starvation resistance for a large number of wild *C. elegans* strains in a single culture. We identified three quantitative trait loci (QTL) affecting starvation resistance. These QTL overlap with hyper-divergent regions and contain multiple members of several large gene families involved in environmental interactions. In particular, we identified 16 members of the insulin/EGF receptor-like domain (*irld*) family with variants within starvation resistance QTL. We generated and assayed loss-of-function mutations for four *irld* family members, all of which increased starvation resistance. We show that the transcription factor *daf-16/FoxO*, a critical effector of insulin/insulin-like growth factor signaling (IIS) known to promote starvation resistance, is required for increased resistance of *irld-39; irld-52* mutants, that these mutants affect DAF-16 target gene expression, and that the IIS receptor *daf-2/InsR* is epistatic to these *irld* genes. We propose that IRLD proteins bind insulin-like peptides (ILPs) to modify signaling in the sensory nervous system thereby affecting organismal physiology. This work demonstrates the efficacy of using population sequencing to investigate natural variation of a complex trait, and it identifies *irld* genes that regulate IIS and starvation resistance. Furthermore, it shows that variation in a rapidly evolving large gene family modifies activity of a deeply conserved signaling pathway to affect a fitness-proximal trait.

135 Complex interactions among quantitative trait loci explain natural variation in *C. elegans* germ stem cell niche activity

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A fundamental goal of evolutionary biology is to understand the divergence of genetic and developmental mechanisms underlying the great diversity of organismal life histories. Comparison among distant taxa cannot easily address this issue due to high complexity; however, it is possible to investigate the quantitative genetic architecture underlying life history and developmental phenotypes within species. Here we show that *C. elegans* wild isolates from around the globe display extensive size variation of the germline mitotic zone (MZ), indicative of variation in germline proliferative activity. To learn more about the molecular genetic differences underlying such natural variation in the *C. elegans* germ stem cell niche, we focused on two wild isolates, JU1200 and JU751, with strong differences in the size of total germline and mitotic compartment. Quantification of MZ size in ~70 F2 recombinant inbred lines (RILs) derived from the parental cross between these two isolates and subsequent quantitative trait locus (QTL) mapping identified a large-effect QTL on chromosome II (~7.25 Mb) that acts additively with a QTL on chromosome V (~2.6Mb). Together, these two loci explain 32% of the observed phenotypic variation, suggesting that there are other small effect loci in the genetic background important in determining MZ size. While efforts to identify causal variants in the chromosome II QTL region were unsuccessful, we were able to investigate a promising candidate variant within the chromosome V QTL. Through CRISPR-Cas9 gene editing, we demonstrate that this variation, a 150bp deletion upstream of *lag-2* containing a BHLH-2 binding site, strongly affects MZ size in the JU1200 genetic background but not in the JU751 background. We find a similar but weaker interaction between this locus and the chromosome II QTL. Finally, we demonstrate surprisingly complex three-way interactions between the genetic background, the chromosome II QTL, and the 150 bp deletion upstream of *lag-2*. Together, our results identify a specific molecular variant affecting a cellular process that ultimately regulates reproductive potential, and they shed light on the complex, quantitative genetic architecture underlying natural variation in a germ stem cell niche.

136 Genomic analysis of natural *Stenotrophomonas* bacteria and their effects on wild and domesticated *C. elegans*

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Natural isolates of *Caenorhabditis elegans* are found in rotting vegetation in association with a multitude of bacteria (Dirksen et al., 2016). Interestingly, the microbiomes of nematodes isolated from different substrates appear to harbor a common set of bacterial genera (Zhang et al., 2017). Among these are members of the *Stenotrophomonas* genus. While not specifically identified yet as a component of the *C. elegans* microbiome, certain strains of *Stenotrophomonas maltophilia* have been

shown to reduce *C. elegans* survivorship and evade host defense responses (White et al., 2016, Radeke and Herman, 2020). We have found that *S. maltophilia* strains are differentially virulent to *C. elegans* and show both common and strain-specific transcriptomic responses (White et al., 2018, Radeke and Herman, 2020). In addition, comparison of *S. maltophilia* genomes revealed that most *S. maltophilia*genes are strain-specific, suggesting that many potential virulence factors are unique.

In order to determine whether these observations reflect natural interactions, we explored the interactions between three natural *C. elegans* strains and 12 *Stenotrophomonas sp*. previously isolated from the *C. elegans* microbiomes of each strain or surrounding environment (obtained from the Félix, Samuel and Schulenberg laboratories). Survivorship analysis and brood counts, used as a measure of fitness, revealed that these natural *Stenotrophomonas* isolates showed variations in virulence and fecundity to the *C. elegans* natural strains and N2. Moreover, we found variation in survival and fecundity between each natural nematode strain and N2 in response to certain *Stenotrophomonas* isolates. This suggests differential adaptation of the *C. elegans* strains to natural *Stenotrophomonas* isolates. We determined the genome sequences of all 12 *Stenotrophomonas* strains to discover whether differences in observed pathogenicity between strains can be explained by differences in genetic features. Our phylogenetic analysis includes these, and 142 genomes that represent all identified lineages of *Stenotrophomonas.* We are testing for the presence of 48 genes previously characterized as virulence genes in other systems and testing whether our *C. elegans* life history data can aid in finding others. Further analyses of the bacterial genomic differences coupled with genome comparison of the nematode strains could help to understand effects of local adaptation to organismal-microbiome interactions.

137 Dissecting the Sequential Evolution of a Selfish Mitochondrial Genome in *Caenorhabditis elegans*

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Despite 1.4 billion years of endosymbiosis, selfishly acting mitochondrial genomes occasionally arise, outcompeting other mitotypes and increasing their own intracellular frequency despite neutral or deleterious effects on their host cell. Unfortunately, mitochondrial genomes are challenging to engineer, preventing experiments aimed at uncovering the factors that contribute to the advantage of one mitotype over another. We discovered a selfishly acting mitochondrial genome, comprising a large deletion, two indels, and a missense mutation, that arose spontaneously in a line of *Caenorhabditis elegans* following 346 generations of experimental evolution. The regular cryopreservation of the experimental line provided an opportunity to dissect the sequential origin and fitness effects of the mutations within this selfish mitochondrial genome. We investigated whether subsequent mitochondrial mutations compensate for the deleterious effects of preceding ones, as has been seen with mutations associated with dosage compensation and mitonuclear compensation, and whether a particular class of mutations confers selfish behavior in mitochondria. We assayed four life-history traits in backcrossed lines with wild-type nuclear genomes and heteroplasmic mutation-bearing mitochondrial genomes. The addition of each subsequent mitochondrial mutation reduced overall fitness in backcrossed lines. We then propagated ancestral lines containing each combination of mutations by bottlenecking to reduce interindividual competition. Tracking the intraindividual frequency of heteroplasmy over evolutionary time, we attributed a mean increase in heteroplasmic frequency across replicates to an intraindividual selfish drive in three of the four mutations tested. Interestingly the only line that did not show clear evidence of selfish drive was a frameshift insertion in *nd5,* but a subsequent insertion at the same location did behave selfishly. We found that selfish drive can be seen in a variety of classes of mitochondrial mutations, but we found no evidence for compensatory effects of any subsequent mutations. We were unable to disentangle the contribution of the original deletion from the selfish behavior of each subsequent mutation, and it is possible that subsequent mutations lack a selfish drive of their own but serve to enhance that of the original deletion.

138 T-box radiation: A window into evolution in real time

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Little is known about the genetic changes that catalyse speciation events. While diverging populations are known to accumulate genomic variation over time, examples of causative loci that lead to speciation have been seldom characterised. This is due, in large part, to how speciation can only be studied retrospectively in most systems. However, naturally occurring populations of *C. elegans*, or wild isolates, diverging for only thousands of years, present a unique opportunity to capture processes which may precede new species divergence, thereby uncovering the genetic hallmarks associated with its onset.

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, suggestive of their rapid evolution. Among the suite of ten *C. elegans* specific T-box genes not found in the rest of the genus are three paralogue pairs all expressed during embryogenesis. In contrast with N2 which retains both functional paralogues, we show that these three pairs display remarkable patterns of mutation accumulation in wild isolates, with one or other genes in the pair accumulating loss-of-function mutations, but never both. The phenotypic consequences of this reciprocal pattern of mutation accumulation could have far-reaching implications for our understanding of speciation if, for example, such patterns led to hybrid inviability. To investigate this, we have characterised the roles of the *tbx-35/tbx-36* gene pair.

The reciprocal nature of deleterious mutation accumulation in wild isolates in the *tbx-35*/*tbx-36* gene pair suggests that they act redundantly. However, *tbx-35* has been shown to be involved in muscle specification during mid-embryogenesis, distinct from the role and expression of *tbx-36* in early embryos that we describe here. Strikingly however, we find that overlapping functionality is indeed revealed when the environmental conditions are changed, suggesting that *tbx-36* has in fact retained a role in muscle specification that is not necessarily seen under standard laboratory conditions. If the early embryonic role of *tbx-36* is N2-specific, this could be a lab adaptation, raising the possibility that N2 is on the road to becoming a new species due to the lab environment. Thus, the rapidly evolving T-box gene family provides a paradigm for investigating evolution and speciation in action in *C. elegans* populations today.

139 Genetic determinants of host-microbiome interactions in *Caenorhabditis elegans*

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Microbes shape many aspects of host physiology, development and predisposition to disease. Yet the complexity of host-associated communities (microbiomes) makes studying host-microbe interactions challenging. As such, we employed the *Caenorhabditis elegans* system to investigate molecular mediators of host-microbial interactions with a simplified 12-member microbiome (CeMbio). Previous studies showed the microbiome exerts significant influence on the physiology and development of *C. elegans*, but lack of molecular tools has limited the ability to identify the microbial factors responsible. To address this challenge, we developed resources to enable genetic manipulation of CeMbio strains. Thus far, we identified several genetic determinants of microbe-microbe interactions, host-specific association and impact on physiology in the dominant microbiome member *Ochrobactrum*.

For genetic manipulation of the CeMbio strains, we utilized broad host range vectors to make an effective panel of fluorescent reporter strains. We used these strains to test pairwise interactions with other microbiome members both *in vitro* and within the *C. elegans* gut. *Ochrobactrum* growth was inhibited by three strains while synergistic with one strain in rich media. In the *C. elegans* gut, however, microbial interactions were often dramatically different. Notably, *Myroides* suppresses *Ochrobactrum* growth *in vitro*, while *Ochrobactrum* benefits from the presence of *Myroides* to colonize the gut. This suggests that host factors may be driving the enrichment for and interactions between members of its microbiome.

To examine the molecular determinants of host association and competition, we developed tools for random transposon mutagenesis in *Ochrobactrum* and several CeMbio strains. We screened a 96-clone mutant library of *Ochrobactrum* for fitness changes relative to the wild-type in ability to colonize *C. elegans* hosts with and without *Myroides*. None of the mutants altered *Myroides* inhibition of *Ochrobactrum in vitro*, but several (12) mutants exhibited host association and inter-microbial competition defects. These mutants highlight specific metabolic pathways that *Ochrobactrum* relies on to colonize and compete for nutrients in association with *C. elegans*. Our work has demonstrated the ability to use broad host range molecular tools to manipulate CeMbio strains allowing us to visualize and identify molecular mechanisms underlying microbiome assembly and impact upon the *C. elegans* host.

140 Commensal versus pathogenic bacterial adherence to the intestinal epithelium of *C. elegans*

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Functional and morphological parallels to the intestines of humans, as well as the variety of microbes that make up its natural microbiome, have led to the emergence of *C. elegans* as a model system to study host-microbe interactions in vivo. Through ecological sampling, we have identified three bacterial species that bind longitudinally to the intestinal epithelial cells of *Caenorhabditis* isolates. DIC microscopy imaging reveals these adhering bacteria bind in a hair-like pattern along the intestinal

wall. We were interested in whether these bacteria had any effects on host fitness when monocolonized in the *C. elegans* lumen. Interestingly, we found adherent bacteria 1 (LUAb1), negatively affects the life span and brood size of *C. elegans*, whereas the two others, adherent bacteria 2 and 3 (LUAb2 and LUAb3), have a neutral effect on *C. elegans* fitness and are best classified as commensal bacteria.

We conducted 16s rRNA sequencing by extracting worm intestines, conducting PCR with universal bacterial primers, and Sanger sequencing of the amplicon. This approach revealed all three bacterial isolates to be novel. LUAb1 and LUAb3 belong to the Enterobacteriaceae family, whereas LUAb2 belongs to the Alphaproteobacterial class. Fluorescence in situ hybridization (FISH) probes were designed to these three bacterial strains to allow for visualization in the intestines. We found LUAb1 colonized nearly 90% of the anterior-posterior intestinal length in more than 90% of the population. Although LUAb2 and LUAb3 colonized the intestines of the worm less than 90% of the intestinal length, the frequency of colonization is the same. Moreover, LUAb3 is the only strain currently culturable in vitro. Whole genome sequence of LUAb3 revealed it is not a new species, but rather a Gram-negative bacterium *Lelliottia jeotgali*. Absent from the complete *L. jeotgali* genome sequence, however, was a plasmid encoding a Type IV secretion system, pilins, and adhesins found in LUAb3. We plan to investigate if this plasmid facilitates adherence in the *C. elegans* lumen.

To summarize, we have found three bacterial isolates that exhibit a directional binding phenotype in the intestinal epithelium of *C. elegans*. We plan to elucidate the host and bacterial receptors that facilitate this adherence mechanism through a forward genetic screen in *C. elegans*. This research could expand our current understanding of the *C. elegans* microbiome and inform host-microbe interaction studies in other animals.

141 A closer look at cuticle-resident microbes and their impact on host physiology

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All animals are in contact with communities of microbes, termed a microbiome. Microbiomes play a large role in the determination of host physiology, health, and behavior. While the gut microbiome takes the spotlight of current research, the skin microbiome is an unrecognized area of study despite harboring a diverse community of microorganisms. This is the case for the model nematode, *Caenorhabditis elegans*; despite subtle yet convincing implications of surface-adherent bacteria, microbial interactions with its cuticle (skin) remain understudied and a “skin microbiome” unacknowledged in literature. *C. elegans* is constantly surrounded by microorganisms in their natural habitat, proven by their common isolation from rotting plants filled with microbes. These microbes inevitably come into contact with the worm cuticle and so the adherence of these microbes is a likely reality. Existence of these surface-adherent microbes is further supported by the adoption of extensive washing protocols which aim to rid the cuticle surface of all residual microbes. We seek to identify, characterize, and define a role for these cuticle-resident microbes in *C. elegans* using a natural model microbiota, CeMbio. Significantly, we characterize a discrepancy between the sheer number of bacteria between surface-bleached and unbleached animals via Colony Forming Unit (CFU) counts. We demonstrate that a large number of cutaneous bacteria reside on the *C. elegans* skin. Furthermore, our preliminary results suggest that bacterial isolates within CeMbio can be primarily gut- or skin-dominating based on the relative bacterial abundances from 16S rRNA sequencing. To understand how skin-dominating bacteria affects host physiology, we use Hoechst 34580 uptake to assess how CeMbio variably affects cuticle integrity in mutant animals. From our results, we hypothesize that CeMbio bacteria interact with the cuticle structures of *C. elegans* to impact worm integrity both positively and negatively. These studies can provide a deeper understanding of how environmental microbes elicit changes in host physiology and explain the role of natural microbes in an animal’s primary defense—the skin.

143 Cell fate plays critical roles in promoting collective cell movements in *C. elegans* gastrulation and ventral cleft closure during embryogenesis

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Wnt signaling can promote cell fate decisions and collective cell movements through distinct mechanisms. In *C. elegans* embryogenesis, Wnt acts primarily through the Wnt/β-catenin asymmetry pathway, which is known to regulate cell fate decisions, but it is unclear how this pathway impacts collective cell movements. We examined a cGAL Wnt mis-expression mutant by time-lapse fluorescent microscopy and found that left-right axis rotation, gastrulation, and ventral cleft closure

were disrupted. To determine whether these defects were due to a direct regulation of collective cell movements by Wnt or an indirect disruption of cell fates, we examined embryonic cell movements in fate mutants for five major cell types using a time-lapse imaging approach with automated lineage reconstruction. We were able to recapitulate previously described disruptions to gastrulation in *end-1/end-3* intestinal fate mutants, and also observed defects in ventral cleft closure. In *hlh-1/unc-120* muscle fate mutants, we observed that the putative muscle cells underwent gastrulation normally, but the embryos had an extrinsic defect in ventral cleft closure. In *nhr-25* or *elt-1* hypodermal fate mutants, we observed that the putative hypodermal cells adopted the correct positions on the dorsal side of the embryo, but on the ventral side, some mesodermal cells failed to complete gastrulation and ventral cleft closure failed. In *cnd-1/ngn-1/lin-32* neuronal fate mutant embryos, we observed failure in ventral cleft closure by the presumptive neuroblasts. In *pha-4* pharynx fate mutants, we observed that some of the putative pharyngeal cells failed to undergo gastrulation by the end of comma stage. Taken together, these finding indicate that cell fate plays key roles in regulating gastrulation and ventral cleft closure, but not left-right axis rotation, indicating that this collective cell movement could be directly regulated by Wnt signaling. We found that ventral cleft closure is a complex process that depends on intestine, muscle, hypodermal, and neuronal fates, possibly through the regulation of juxtacrine signaling, cell adhesion, or extracellular matrix remodeling by the muscle and intestinal cells and long-range signaling from the hypodermal cells. Our results also indicate that mesodermal gastrulation is also dependent on long range signals from the hypodermal cells. Thus, our results identify two novel roles for the hypodermis in organizing the early embryo prior to ventral enclosure and highlight novel intrinsic and extrinsic roles for cell fate regulators in collective cell movements.

144 A novel biosensor reveals the timing and dynamics of LIN-12/Notch activation underlying resolution of the AC/VU decision during gonadogenesis

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LIN-12/Notch is a transmembrane receptor that is cleaved upon DSL ligand binding, freeing the intracellular domain to translocate to the nucleus and activate transcription of target genes. Studying LIN-12 activation in cell fate decisions in live *C. elegans* is difficult because rapid turnover of the intracellular domain in the nucleus reduces detectable output of fluorescent translational fusions. Also, target gene transcriptional reporters have intrinsic delays from transcription, translation, and fluorescent protein maturation. To circumvent these difficulties, we developed a genetically-encoded, rapid response biosensor that uses changes in the subcellular localization of GFP as a readout for Notch activation. This system, "**S**ensor **A**ble to detect **L**ateral **S**ignaling **A**ctivity" (**SALSA**), accurately reports LIN-12 activity in multiple tissues in *C. elegans*.

We used SALSA to address questions about the timing and dynamics of Notch activation during a paradigmatic lateral specification event in gonadogenesis. During the AC/VU decision in *C. elegans,* Z1.ppp and Z4.aaa, which we call the «α cells,» each have the potential to be the **a**nchor **c**ell (AC) or a **v**entral **u**terine precursor cell (VU), and interact with each other via LIN-12/Notch to resolve their fates. While both cells initially express both LIN-12 and its ligand, LAG-2/DSL, feedback mechanisms amplify stochastic differences in LIN-12 activity to restrict LIN-12 expression to the presumptive VU and LAG-2 expression to the presumptive AC. Lineage analysis (Attner et al., 2019) revealed that when the interval between the birth of the α cells is "long" (greater than ~30 minutes), the first-born α cell is strongly biased towards the VU fate; when the interval is "short," the AC/VU decision appears random with respect to birth order. We used SALSA to investigate how differences in birth order and birth time interval affected the rate and extent of LIN-12 activation to create the VU cell fate bias. We found that when there is a long birth time interval, the first born α cell has already experienced significant LIN-12 activity prior to the birth of the second α cell, thus accounting for the cell fate bias. Furthermore, the α cells do not differ in their relative LIN-12 activity at their births or the rate at which LIN-12 activation increases after their birth, indicating that the difference reflects activation of LIN-12 in the first-born α cell itself and not in its parent. Thus, SALSA allowed us to demonstrate that the stochastic element of birth time is resolved into a deterministic element through the activation of LIN-12 signal transduction upon the birth of the α cell.

145 Translation-dependent mRNA localization to *Caenorhabditis elegans* adherens junctions

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mRNA localization is an evolutionarily widespread phenomenon that facilitates sub-cellular protein targeting and the assembly of protein complexes. mRNA targeting within cells relies either on "zip codes" within mRNA untranslated regions (UTRs) or on translation-dependent cues. While extensive work has focused on UTR-dependent processes, much less is known about translation-dependent mRNA localization. We examined mRNA localization in *Caenorhabditis elegans* embryonic epithelia. From an smFISH-based survey, we identified different mRNAs associated with the cell membrane/cortex or apical junctions in a

stage- and cell type-specific manner. Mutational analyses for one of these transcripts, *dlg-1*/*discs large*, revealed that it relied on a translation-dependent process and did not require its 5' or 3' UTR. DLG-1 carboxy-terminal sequences were necessary and sufficient for mRNA association with the cortex, while amino-terminal sequences were important for its enrichment near the junction. The absence of an amino-terminal signal peptide and the persistence of localized mRNA in amino-terminal deletion mutants indicated that the translation-dependent localization of *dlg-1* mRNA differs from an ER-like signal-sequence mechanism. Rather, we suggest a two-step model in which *dlg-1* transcripts are first targeted at or near the cell membrane through SH3 and, possibly, Hook and GuK domains, and then enriched at the apical junction by L27. These studies identify an unconventional mechanism for mRNA localization within developing epithelia and the necessary cis-acting sequences within the studied mRNA. Altogether out data provide an alternative prospective through which gene expression can be spatially controlled.

146 A folder mechanism ensures size uniformity among *C. elegans* individuals by coupling growth and development

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Animals increase by orders of magnitude in their volume during development. Hence, even small differences in the growth rates between individuals could generate large differences in their adult body size. Yet, such volume divergence among individuals is usually not observed in nature.

We combined theory and experiment to understand the mechanisms of body size uniformity. Using live imaging, we measured the volume growth of hundreds of individuals of *C. elegans* over the entire span of their postembryonic development. We find that *C. elegans* grows exponentially in volume with a coefficient of variation of the growth rate of ~7%, but that individuals diverge much less in volume than expected from this heterogeneity. The mechanism counteracting size divergence does not involve size thresholds for developmental milestones. Instead, an inverse coupling of the growth rate and the duration of development produces a constant volume fold change per larval stage.

The duration of larval stages of *C. elegans* is determined by the period of a developmental oscillator. Using mathematical modelling, we show that an anti-correlation between the growth rate and the oscillatory period emerges as an intrinsic property of a genetic oscillator. We propose that the robustness of body volume fold change is a hard-wired characteristic of the oscillatory circuit and does not require elaborate mechanisms of size control by cellular signalling. Indeed, the coupling of growth and development was unaltered by mutation of canonical pathways of growth control. This novel concept of size homeostasis may broadly apply to other multicellular systems controlled by genetic oscillators.

147 The mitotic spindle and the cytokinetic furrow cooperatively align the dorsoventral axis with embryo geometry

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Establishment of the major body axes is essential for embryonic development. The *C. elegans* dorsoventral axis is established during the two-cell stage, and its orientation is defined by the division of the anterior AB blastomere. Earlier work in various holoblastic animals has shown that embryo geometry strongly affects early embryonic division patterns. However, whether and how embryo geometry affects the orientation of the dividing AB cell, and thereby the dorsoventral axis is unknown. To study this, we performed quantitative 3D live imaging of the cytoskeletal machinery in dividing AB blastomeres of embryos that are compressed perpendicular to the anteroposterior axis. In agreement with previous studies, we find that the AB cell division, and therefore the future dorsoventral axis, aligns parallel to the long cellular axis (which is perpendicular to the compression direction). Moreover, we show that, when viewed down the anteroposterior axis, the mitotic spindle of the AB blastomere is initially oriented randomly with respect to the long axis. During spindle elongation, it undergoes a rapid, large scale rotation to finally align parallel with the long axis. This mitotic spindle rotation is accompanied by a rotation of the cytokinetic furrow, ultimately resulting in the cell division axis to be aligned with the long cellular axis. Although the molecular mechanisms are still topic of debate, the mitotic spindle in many contexts is known to find the long axis of the cell. However, by performing conditional genetic perturbations, we find that force generation in the actomyosin layer is required and governs the kinetics of alignment of the mitotic spindle along the future DV axis. We speculate that the cooperative action of spindle and cortex promotes timely alignment of the cytokinetic machinery in fast-dividing cells.

148 BBLN-1 is essential for intermediate filament organization and apical membrane morphology

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Epithelial tubes are essential components of metazoan organ systems that control the flow of fluids and the exchange of materials between body compartments and the outside environment. The size and shape of the central lumen confer important characteristics to tubular organs and need to be carefully controlled. Here, we identify the small coiled-coil protein BBLN-1 as a regulator of lumen morphology in the *C. elegans* intestine. Loss of BBLN-1 causes the formation of bubble-shaped invaginations of the apical membrane into the cytoplasm of intestinal cells, and abnormal aggregation of the subapical intermediate filament (IF) network. BBLN-1 interacts with IF proteins and localizes to the IF network in an IF-dependent manner. The appearance of invaginations is a result of the abnormal IF aggregation, indicating a direct role for the IF network in maintaining lumen homeostasis. Finally, we identify bublin (BBLN) as the mammalian ortholog of BBLN-1. When expressed in the *C. elegans* intestine, bublin recapitulates the localization pattern of BBLN-1 and can compensate for the loss of BBLN-1. In mouse intestinal organoids, bublin localizes subapically, together with the IF protein keratin 8. Our results therefore may have implications for understanding the role of IFs in regulating epithelial tube morphology in mammals.

149 Developmentally programmed H3 expression changes embryonic plasticity and reinforces cell fate specification

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Early embryonic cells are pluripotent, possessing the transient capacity to generate all the cells of an organism. A fundamental question in developmental biology concerns identifying the epigenetic factors that underlie this temporary developmental plasticity, as well as understanding how commitment to a specific cell lineage is achieved and maintained. Using *C. elegans* as a model to dissect the role of distinct histone incorporation during gametogenesis and embryogenesis, we have uncovered an unappreciated developmental regulation of the incorporation of key molecular carriers of epigenetic information, the replication-coupled histone H3, and histone variant, H3.3, during gametogenesis that influence the epigenetic organization in the early embryo, with a lasting effect on pluripotency and lineage commitment.

To characterize the dynamics of endogenous histones throughout the *C. elegans* lineage, 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. In doing so, I 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. Unlike somatic cells where all 15 copies of H3 are expressed, germ cells uniquely restrict canonical H3 expression to 4 loci. Furthermore, upon cellular differentiation, I have identified a 400-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.

150 A molecular clock to control skin regeneration

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The skin of *C. elegans*, composed of a cuticle and the underlying epidermis, undergoes regular cycles of regeneration, the molt. These cycles involve extensive rhythmic accumulation of mRNAs, affecting thousands of genes or ~25% of the transcriptome. Yet, the 'molting clock' that generates rhythmicity and directs timely molts has remained elusive. Here, we provide insight into its molecular components and function. We also argue that the molting clock is not a nematode-specific invention but that similar mechanisms account for rhythmic skin regeneration across the animal kingdom. Specifically, in a targeted screen, we identified BLMP-1 through its defect in molting timing. We show that BLMP-1 accumulates and functions rhythmically and that its loss causes slow and asynchronous progression through the molting cycle and impairs cuticular barrier function.

These phenotypes result from perturbed oscillatory gene expression. Thus, by combining ChIP-seq and temporally resolved gene expression profiling, we identify a set of 250 BLMP-1 target genes of which nearly 90% are oscillating genes. Reflecting BLMP-1's rhythmic activity, these genes exhibit a clear peak phase preference (i.e., their expression peaks at a specific time relative to the larval development cycle). A larger set of 1,400 genes is also dysregulated upon loss of BLMP-1 and contains >70% oscillating genes, but these are not bound by BLMP-1 and, accordingly, lack such a peak phase signature. We conclude that BLMP-1 functions both in generating oscillations, as a putative core clock gene, and in relaying them to a specific set of oscillator output genes, among which molting/cuticle genes are vastly overrepresented. These activities ensure timely molting and an intact skin barrier.

Strikingly, the *blmp-1* orthologue *Prdm1/Blimp1* is dynamically expressed in different mouse skin compartments, including hair follicles. Postnatal mammalian hair follicles undergo regular regenerative cycles of growth and regression under the control of a clock of unknown mechanism, and this rhythmicity, and skin barrier function, are both perturbed in *Prdm1* mutant mice. Similar observations apply to additional hits from our screen. Hence, we propose that we have identified components of an evolutionarily conserved skin regeneration clock. These findings further suggest that despite its simplicity, the *C. elegans* skin is a powerful experimental model of animal skin regeneration.

151 Conserved extracellular proteins determine mechanoelectrical transduction channel localization and function in *C. elegans* touch receptor neurons

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The sense of touch is made possible by neurons that use ion channels to convert mechanical stimuli into electrical signals. Many other proteins affect mechanosensation, either through direct force transfer by acting as physical tethers, or indirectly by modulating cell stiffness and morphology, protein trafficking, and channel positioning. Subcellular localization of mechanosensory ion channels is especially important for localized force sensation, since channels further away from the point of stimulation are recruited as stimulus intensity and speed increase. Here we examine the micro-environment of ion channels to investigate the molecular basis of channel positioning, leveraging the well-characterized MEC-4 mechanoelectrical transduction channel in *C. elegans* touch receptor neurons (TRNs).

We show that MEC-4 channels localize to punctae distributed along TRN neurites *in vivo* but not in cultured TRNs *in vitro*, suggesting that *in vivo* channel localization depends on factors that are absent *in vitro*. Both time-lapse imaging and fluorescence recovery after photobleaching shows that most *in vivo* MEC-4 puncta are immobile over several minutes, suggesting that they are anchored to stable structures. To identify these structures, we screened mutants affecting plasma membrane proteins, cytoskeletal proteins, and extracellular matrix (ECM) proteins to investigate what factors regulate the positioning and stability of the MEC-4 puncta. In doing so, we discovered that the ECM protein nidogen regulates MEC-4 puncta distribution and both behavioral and electrical responses to touch. Nidogen associates with laminin networks in basement membranes and we show that both nidogen and laminin co-localize with MEC-4 puncta, in a manner that depends upon another ECM protein, MEC-1. To learn more about the physiological significance of channel puncta, we modeled mechanical strain distribution along a neurite *in silico* and found that physical attachment of the neurite to the ECM at puncta generates localized regions of increased strain. We speculate that this amplifies and focuses the mechanical stimulus for ion channel opening. Future experiments will determine whether these ECM proteins physically anchor the MEC-4 channels, accounting for the immobility of the MEC-4 puncta, and whether such a physical tether is responsible for ion channel opening in response to a mechanical stimulus.

153 Intracellular calcium management is key in diapause-induced neuroprotection

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Calcium acts as a double-edged sword, playing critical roles in both neuronal degeneration (Goodman *et al*., 2002; Bianchi *et al*., 2004; O'Hagan *et al*., 2005; Xu *et al*., 2001; Calixto *et al*., 2012) and regeneration (Hammarlund *et al*., 2009; Tang and Chisholm, 2016; Caneo *et al*., 2019). We previously showed in *C. elegans* that diapause prevents degeneration and even promotes regeneration of Touch Receptor Neurons (TRNs) expressing mutated *mec-4d* channels (Caneo *et al*., 2019). In this

work, we aim to understand how intracellular calcium is managed during neuroprotection. To monitor calcium, we performed 2-photon imaging of TRNs expressing GCaMP6m and tagRFP in wild type and *mec-4d* worms at L2 stage as well as dauers. Consistent with a contribution of calcium in *mec-4d* -induced neurodegeneration, we found an increase in intracellular calcium in L2 *mec-4d* worms compared to WT controls. To our surprise, basal calcium was also increased in diapausing *mec-4d* compared to L2 *mec-4d* animals. This suggests that non-physiological calcium concentrations are maintained in the soma while TRNs regenerate. Enhanced calcium buffering by mitochondria may underlie the regenerative capacity in high calcium. We next investigated mitochondrial alterations in *mec-4d* worms using a GFP fused with an MLS in the TRN (Fatouros *et al*., 2012). In *mec-4d* L2 animals, there is a 23% decrease in mitochondrial length along with a 22% reduction in number compared to WT L2; in contrast, WT dauers exhibit 15% longer but 16% fewer mitochondria compared to WT L2 animals. Notably, *mec-4d* dauers show an increase of 31% and 36% in mitochondrial length and number, respectively, compared to *mec-4d* L2 animals. Taken together, these findings suggest that *mec-4d* mutation disrupts mitochondrial function, and entering diapause compensates for mitochondrial dysfunction. We next wondered whether calcium might be acting in axonal protection. Using RNAi, we silenced the calcium transporters *sca-1*, from the endoplasmic reticulum, and *mcu-1*, from the mitochondria, in the TRNs and assessed axon morphology at 72 hours after hatching. Reducing the expression of *sca-1* and *mcu-1* resulted in a reduction of WT-like axons (p-value=0.0007), suggesting that calcium transport from the cytoplasm into these organelles is key for neuronal protection. Thus, our results provide new insights into the calcium-dependent mechanisms that tilt the balance between neuronal death and repair.

154 B-Raf contribution to motoneuron degeneration

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The B-Raf orthologue *lin-45* belongs to MAPK/ERK pathway, is involved in the regulation of cellular proliferation and differentiation and shows a Ras GTPase binding activity. Beside its well-known role in vulva formation, *lin-45* is also expressed in neurons, including motoneurons, and is critical for chemo- and thermo-sensory behaviors and locomotion. Using a phospho-antibody array combined with a network-biology approach on Spinal Muscular Atrophy (SMA) mice, we identified B-Raf as a signaling hub among different dysregulated pathways involved in motor neuron degeneration. In SMA, motor neurons degenerate because of mutations in the Survival Motor Neuron 1 (*SMN1*) gene. We investigated in *C. elegans* the role of B-Raf/*lin-45* in degenerating motoneurons (MNs) and observed that *lin-45* expression is reduced in pre-symptomatic *smn-1(KO)* animals. We rescued the neurodegeneration and locomotion defects caused by *smn-1* silencing in D-type MNs by re-expressing *lin-45* specifically in MNs. We thus demonstrated that *lin-45* can play a cell-autonomous neuroprotective role when *smn-1* is downregulated. Consistently, a hyperactive isoform of *lin-45* (S312A), which is devoid of an inhibitory phospho-site, maximized the rescue effects. The neurodegeneration caused by *smn-1* silencing increases with animal ageing and we were able to rescue it by expressing *lin-45* from L2 stage, through inducible transgenics. This is important since at L2 stage the degeneration has already started and all 19 D-type motoneurons should have been generated, thus suggesting that *lin-45* protects from motoneuron loss rather than interfering with neurogenesis and can block the degeneration when it is already started. Using MAPK pathway drug inhibitors and *mek-2* mutants we abrogated the rescue obtained after *lin-45* overexpression. Thus, genetic and pharmacological approaches showed that *lin-45* rescue is mediated by the MAPK/ERK pathway. The central role of B-Raf was confirmed in a SMA model of SMA and in patient cells and strongly support a role of B-RAF in neurodegeneration and in particular in the *Smn1* pathway.

155 Dendrite regeneration in PVD neuron is controlled by the RAC GTPase CED-10 and the RhoGEF TIAM-1

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Neurons are vulnerable to physical insults which compromise the integrity of both dendrites and axons. Unlike well characterized axon regeneration, our knowledge in dendrite regeneration is limited. To understand the mechanisms of dendrite regeneration, we used PVD neurons with stereotyped branched dendrites. Using femtosecond laser, we severed the primary dendrites and axon of this neuron. After the primary dendrite was severed near the cell body, we observed a sprouting of new branches from the cut site within 3 hours. By 24 hours, the primary dendrite regrew to cover the original territory in

complex pattern unlike the uninjured dendrites. We quantified the regeneration in broadly two aspects- the territory covered and fusion phenomena (Oren-Suissa et al.,2017, Kravtsov et al.,2017).

Axon injury causes a retraction of the severed end followed by a Dual leucine zipper kinase-1(DLK-1) dependent regrowth from the severed end or conversion of neighboring dendrite to axon. However, Dendrite regeneration was independent of DLK-1 and other conventional axon regeneration pathways including cAMP elevation, *let-7* miRNA, Akt-1 and Phosphatidyl serine exposure/PS.

Among various candidates tested, *ced-10* mutants showed a defect in dendrite regeneration. It is a RAC GTPase involved in regulation of neuronal cytoskeleton and cell engulfment. Cell-specific rescue experiments suggest that cell-autonomous and epidermal expression of CED-10 is required for dendrite regrowth and fusion, respectively. Moreover, the PVD-specific expression of an activated version of CED-10 led to both increased branching and fusion. We ventured further to find the upstream players which can control the function of RAC GTPase in dendrite regeneration. We found out that the TIAM-1 RhoGEF is required for dendrite regeneration and the activated CED-10 can bypass the requirement of TIAM-1 in dendrite regrowth. Our work provides a framework for understanding the cellular mechanism of dendrite regeneration using PVD model.

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156 The metalloprotease ADAM17/ADM-4 promotes regenerative axonal fusion by stabilising the fusogen EFF-1

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Axonal damage, such as in nerve injuries, interrupts the communication between a neuron and its target tissue. Functional recovery is achieved when the regenerating axon re-innervates its original target tissue. However, intervention to repair such damage is still not achievable. Axonal fusion is an efficient means of repair following axonal transection, whereby the proximal axon, still attached to the cell body, regrows and re-establishes membrane and cytoplasmic continuity with its own separated axonal fragment, restoring neuronal function. The molecular mechanisms of this process are not fully elucidated. In *C. elegans*, a key function has been established for the fusogen EFF-1, which mediates the merging of the plasma membranes of the two separated fragments. Using a candidate gene approach, and the *C. elegans* PLM mechanosensory neurons as a model system, we identified ADM-4 as a key regulator of EFF-1-mediated axonal fusion. ADM-4 is a member of the ADAM (A Disintegrin and Metalloprotease) family, and ortholog of the human ADAM17/TACE (Tumor necrosis factor Alpha-Converting Enzyme). *adm-4* loss-of-function leads to a severe reduction of axonal fusion in PLM neurons without affecting axonal regrowth. We demonstrate that ADM-4 regulates this process in a cell-autonomous fashion and observe dynamic changes in the subcellular localisation of ADM-4 after axotomy. Furthermore, overexpression of ADM-4 selectively in the PLM neurons is sufficient to enhance axonal fusion in wild-type animals. We have previously shown that phosphatidylserine (PS) exposure on the damaged axon functions as a “save-me” signal to promote fusion. Our recent data show that putative PS-binding sites in ADM-4, as well as its metalloprotease activity, are essential for its function. We propose that PS exposure triggered by injury binds ADM-4 and activate its proteolytic function. Finally, biochemical analysis reveals that ADM-4 binds and stabilises EFF-1 to promote axonal fusion. Our results uncover an essential function for ADM-4 in promoting axonal fusion, and set the foundation for the design of novel therapeutics for nerve injuries.

157 The extracellular matrix protein MIG-6/papilin mediates the maintenance of neuronal architecture

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After the initial assembly of the nervous system during embryogenesis, neuronal circuits need to persist lifelong in the face of maturation, growth, body movements, and aging. How neuronal organization is protected throughout life is not well understood. Our research has demonstrated that molecular mechanisms actively maintain the architecture of the nervous system, acting with great cellular specificity (Bénard and Hobert, 2009). *sax-7* mutants lack the cell-adhesion molecule SAX-7/L1CAM, and specific neuronal structures that initially develop normally subsequently become disorganized. Through a genetic screen, we uncovered that loss of *mig-6*/papilin suppresses neuronal disorganization in *sax-7* mutants, suggesting antagonistic roles for these genes: whereas SAX-7 mediates adhesion among neurons, MIG-6 may confer increased flexibility between neurons and their surrounding environment. MIG-6/papilin harbors a papilin cassette, composed of thrombospondin type I and lagrin domains, which is shared with ADAMTS metalloproteinases that remodel the extracellular matrix. In neuronal maintenance, *mig-6* functions post-developmentally, and the short isoform of *mig-6* is secreted from muscles into the extracellular matrix to non-autonomously impact neuronal maintenance in a *mig-17*/ADAMTS-dependent manner. Loss of *mig-6* leads to the accumulation of extracellular collagen type IV/EMB-9 fibrotic-like structures, which do not occur in the wild type, nor in *sax-7* mutants. Post-developmental depletion of collagen IV reduces these fibrotic-like structures and reinstates neuronal maintenance defects in *sax-7; mig-6* mutants. Moreover, loss of the collagen-IV-crosslinking-extracellular enzyme peroxidasin/PXN-2 also re-establishes *sax-7* neuronal maintenance defects in the double mutants *sax-7; mig-6*, and interestingly, PXN-2 is upregulated in *mig-6* mutants. Thus, MIG-6 may ensure a state of flexibility of the extracellular matrix ensheathing neuronal structures that balances neuron-to-neuron adhesion, enabling neuronal architecture to endure lifelong stress. Consistent with this notion, loss of *mig-6* bestows enhanced protection of neuronal organization in conditions of increased body movements compared to wild type. Understanding general principles of the maintenance of neuronal architecture and connectivity may help identify key factors influencing the onset and progression of neurodegenerative conditions.

158 The nuclear ubiquitin ligase adaptor SPOP is a conserved regulator of C9orf72 dipeptide toxicity

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A hexanucleotide repeat expansion in the C9orf72 gene is the most common cause of inherited amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Unconventional translation of the C9orf72 repeat produces dipeptide repeat proteins (DPRs). Previously, we showed that the DPRs (PR)50 and (GR)50 are highly toxic when expressed in *C. elegans* and this toxicity depends on nuclear localization of the DPR. In an unbiased genome-wide RNAi screen for suppressors of (PR)50 toxicity, we identified 12 genes that consistently suppressed either the developmental arrest and/or paralysis phenotype evoked by (PR)50 expression. All of these genes have vertebrate homologs and 7/12 contain predicted nuclear localization signals. One of these genes was *spop-1*, the *C. elegans* homolog of SPOP, a nuclear localized E3 ubiquitin ligase adaptor only found in metazoans. *spop-1* is also required for (GR)50 toxicity and functions in a genetic pathway that includes *cul-3*, which is the canonical E3 ligase partner for SPOP. Genetic or pharmacological inhibition of SPOP in mammalian primary spinal cord motor neurons suppressed DPR toxicity without affecting DPR expression levels. SPOP is commonly mutated in prostate, endometrial, and renal cancer. We find that CRISPR/Cas9 knock-in of two of the most common cancer-causing SPOP missense mutations also protect against (PR)50 toxicity, suggesting that similar mechanisms mediate the roles of SPOP in cancer and C9 toxicity. Finally, we find that genetic inhibition of *bet-1*, the *C. elegans* homolog of the known SPOP ubiquitination targets BRD2/3/4, suppresses the protective effect of spop-1 mutations. Together, these data suggest a model in which SPOP promotes the DPR-dependent ubiquitination and degradation of BRD proteins. We speculate the pharmacological manipulation of this pathway, which is currently underway for multiple cancer subtypes, could also represent a novel entry point for therapeutic intervention to treat C9 FTD/ALS.

159 Neurohormonal signalling modulates polyQ aggregation by controlling fat metabolism

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The maintenance of protein homeostasis is essential for organism survival. Misfolded proteins may alter this balance because they are susceptible to form toxic aggregates. This pathological context is common in many neurodegenerative disorders such as Huntington disease, Alzheimer, Parkinson and ALS. Aggregation dynamics may be monitored *in vivo* using *C. elegans* that expresses prone-to-aggregation molecules, tagged with fluorescent proteins. We did a random mutagenesis screening to isolate modifier genes that modulates protein aggregation of polyglutamines (polyQs). We isolated a mutant allele (*vlt10*), of the electrical synapse modulator *unc-1*/Stomatin Like protein 3. This allele enhances polyQ aggregation in muscle cells in a neuron-specific manner. We show that *vlt10* induces an excess of neurohormonal signalling from the cytosolic sulfotransferase SSU-1, which functions in ASJ neurons. This hormonal signal targets into the nuclear hormone receptor 1 (NHR-1), which appear to regulate genes of the lipid metabolism, which in turns modulate polyQ aggregation. Additionally, we have identified an opposite modulator role from another hormonal pathway which leads to DAF-12. Our findings suggest that steroid hormones induce changes in fat metabolism that impact over protein homeostasis. Some components of this pathways are enzymes or receptors susceptible to be modulated by compounds, and therefore they are potential druggable targets to treat neurodegenerative diseases.

160 Stress-induced increases in neuronal exopher extrusion require lipid biosynthesis and FGF/RAS/MAPK signaling

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In human neurodegenerative diseases, neurons can transfer toxic protein aggregates to surrounding cells, promoting pathology via poorly understood mechanisms. In Caenorhabditis elegans, proteo-stressed neurons can expel neurotoxic proteins in large membrane-bound vesicles called exophers. We investigated how specific stresses impact neuronal trash expulsion to show that neuronal exopher production can be markedly elevated by oxidative and osmotic stress. Unexpectedly, we also found that fasting dramatically increases exophergenesis. Mechanistic dissection focused on identifying nonautonomous factors that sense and activate the fasting-induced exopher response revealed that DAF- 16/FOXO dependent and independent processes are engaged. Fasting-induced exopher elevation requires the intestinal peptide transporter PEPT-1, lipid synthesis transcription factors Mediator complex MDT-15 and SBP-1/SREPB1, and fatty acid synthase FASN-1, implicating remotely initiated lipid signaling in provoking neuronal trash elimination. A conserved FGF/RAS/MAPK signaling pathway that acts downstream of, or in parallel to, lipid signaling also promotes fasting-induced neuronal exopher elevation. Our data define a non-autonomous network that links food availability changes to remote, and extreme, neuronal homeostasis responses relevant to aggregate transfer biology.

161 Investigating The Phase Transition of EFA-6 and Its Role In Microtubule Regulation

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The concept of phase separation provides a new framework to understand the role of proteins with intrinsically disordered regions (IDRs). We have previously identified the signaling protein Exchange Factor for ARF-6 (EFA-6) as a potent inhibitor of axon regrowth. We have also shown that axon injury triggers a rapid redistribution of EFA-6 protein from a generally even plasma membrane localization to more discrete puncta within the cytosol. Both the redistribution of EFA-6 and inhibition of axon regrowth are mediated by its intrinsically disordered N-terminal domain and requires a conserved 18aa motif at the N terminus. We further demonstrated that EFA-6 inhibited axonal microtubule growth and interacted with the MT-associated proteins TAC-1 and ZYG-8, both of which are required for axon regeneration. EFA-6 is known to limit microtubule growth at the cell cortex. The *D. melanogaster* ortholog of EFA-6 has also been reported to inhibit microtubule polymerization at the cortex. However, how EFA-6 plays its role in regulating microtubules remains unclear. We recently purified recombinant EFA-6 proteins and observed that EFA-6 formed liquid-like droplets and phase separated with TAC-1. Interestingly, we found that EFA-6 could phase transition from liquid-like droplets to gel-like aggregates, and the transition was dependent on the conserved 18aa motif. We are currently investigating whether and how EFA-6 phase transition regulates its function.

163 A Large Family of Enzymes Responsible for the Modular Architecture of Nematode Pheromones

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The nematode *Caenorhabditis elegans* produces a broad family of pheromones, known as the ascarosides, that are modified with a variety of groups derived from primary metabolism. These modifications are essential for the diverse activities of the ascarosides in development and various behaviors, including attraction, aggregation, avoidance, and foraging. The mechanism by which these different groups are added to the ascarosides is poorly understood. Here, we identify a family of over 30 carboxylesterase domain-containing (CEST) enzymes, which are homologous to mammalian carboxylesterase and acetylcholinesterase enzymes, and show that a number of these enzymes are responsible for the selective addition of specific modifications to the ascarosides. Analysis of ascaroside production in *cest-3*, *cest-9.2*, and *cest-8* mutant strains shows that they specifically do not produce indole-3-carbonyl (IC)-modified (i.e., icas), (*E*)-2-methyl-2-butenoyl (MB)-modified (i.e., mbas), octopamine succinyl (OS)-modified (i.e., osas) ascarosides, respectively. These defects can be rescued through *cest* overexpression. Furthermore, a *cest-3[S208A]* strain in which the Ser residue in the catalytic triad of CEST-3 was mutated to an Ala residue does not produce the IC-ascarosides. This strain is also less likely to localize to a lawn of bacterial food than wild type. This result is consistent with the role of the IC-ascarosides in promoting attraction, promoting aggregation on food, and suppressing foraging. Through stable isotope-labeled ascaroside feeding experiments, we demonstrate the *in vivo* activity of the CEST enzymes and provide direct evidence that the acyl-CoA synthetase ACS-7, which was previously implicated in the attachment of certain modifications to the ascarosides in *C. elegans*, instead activates the side chains of certain ascarosides for shortening through β-oxidation. Our data provide a key to the combinatorial logic that gives rise to different modified ascarosides, which should greatly facilitate the exploration of the specific biological functions of these pheromones in the worm.

164 Identification of modular glucoside in *C. elegans* a new class of putative signaling molecules

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Diverse small-molecule signals have been shown to regulate almost every aspect of *C. elegans* biology, including ascarosides, the polyketide nemamide, or the male-produced developmental accelerator nacq#1[1-3]. Here we present evidence for another class of *C. elegans* signaling molecules, the modular glucosides.

We initially discovered the modular glucosides when comparing the metabolomes of wildtype *C. elegans* and *glo-1* mutants, which lack lysosome-related organelles. When analyzing *glo-1* worms, we found that they are deficient in the production of almost all modular ascaroside pheromones (e.g. the dispersal signal osas#9 and the aggregation signal icas#3)[4,5]. Unexpectedly, we detected another, much larger family of compounds whose biosynthesis was also abolished in *glo-1* worms. Follow-up analyses via MS and NMR spectroscopy revealed that these *glo-1*-dependent compounds represent a large class of novel modular metabolites that are derived from a glucose scaffold decorated with building blocks from amino acid, nucleoside, and fatty-acid metabolism.

Analogous to the biosynthesis of modular ascarosides, enzymes of the carboxylesterase (CEST) family play a central role in the assembly of the modular glucosides[6]. Furthermore, biosynthesis of modular glucosides via CEST enzymes is evolutionarily conserved, similar to that of ascaroside pheromones[5,7].

Given their highly specific enzymatic assembly, we hypothesize that modular glucosides serve important biological functions. Notably, modular glucosides are primarily retained in the worm body, in contrast to ascarosides, which are primarily excreted, suggesting that the modular glucosides serve as intra-organismal signaling molecules.

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165 Nutrient-induced rewiring of microbial metabolic pathways modulate 5-fluorouracil efficacy in *C. elegans*

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Microbiota derived metabolites have been shown to influence cancer susceptibility, tumour progression and therapy outcome. Diet is a major environmental factor influencing gut microbiota composition, dictating the abundance of potential oncometabolites. The complex interactions between diet and the microbiota need to be clarified so that dietary intake may be shaped to influence disease outcome. Having previously demonstrated the impact of bacterial metabolism in 5-fluorouracil (5-FU) cancer drug efficacy, we set out to investigate the role of nutrition in host-microbe responses to this drug, an antimetabolite widely used for colorectal cancer (CRC) chemotherapy. Host-microbe-drug-nutrient interaction and its influence on drug efficacy were assessed using a high-throughput 4-way screening approach. To understand gene-nutrient interactions at the bacterial level that regulate the effect of a drug on host physiology, genetically modified *E. coli* from the Keio deletion library were used to probe the role of bacterial metabolic pathways in 5-FU effects in a nutrient-dependent manner. We found that glycolytic nutrients, including sugars, importantly antagonise the toxic effects of 5-FU action on the host. Sugars induce a shift in metabolic pathways that favour the production of nucleotides, specifically UMP and UTP derived from the activation of pyrimidine de novo biosynthesis pathway in bacteria, counteracting pro-drug activation through the salvage pathway. We also found that bacterial mutations in the TCA cycle and pyruvate metabolism decrease drug efficacy in amino acid-based media. A combination of bacterial genetic work and metabolomics has revealed the presence of a novel bacterial metabolite with the capacity to increase 5-FU drug efficacy in both *C. elegans* and human cancer cells. Importantly, flux balance analysis of the cancer associated microbiota predicts an increased production of this metabolite in several organs. In particular, investigations of the gut microbiome from four independent human CRC cohorts shows an increased production of this metabolite, compared to healthy patients, further implicating this metabolite in drug cancer therapy. These findings highlight the potential of manipulating gut microbiota through diet to improve cancer therapy outcome.

166 Interkingdom transfer of molybdenum cofactor from bacteria to *C. elegans*

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The molybdenum cofactor (Moco) is a 520 dalton prosthetic group that is synthesized in a multi-step enzymatic pathway present in Archaea, Bacteria, and Eukarya. In animals, four oxidases (among them sulfite oxidase) use Moco as a prosthetic group. Moco is essential in animals; humans with mutations in genes that encode Moco-biosynthetic enzymes display lethal neurological and developmental defects. Moco supplementation seems a logical therapy, however free Moco is too fragile to be purified and administered therapeutically. Surprisingly, Moco biosynthesis is not essential in the nematode *Caenorhabditis elegans* if they are fed bacteria that synthesize Moco. Our genetic and biochemical studies demonstrate that protein-bound Moco is the stable, bioavailable species of Moco taken up by *C. elegans* from bacteria and is an effective dietary supplement in a *C. elegans* model of Moco deficiency. These findings establish an animal pathway for Moco transport and suggest a novel therapeutic strategy for Moco deficiency.

167 Interneuron Control of Diapause Entry

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To maximize fitness, animals integrate various external stimuli to sculpt their physiological and behavioral responses throughout development. Under adverse environmental conditions, C. elegans larvae can choose to enter an alternate stress-resistant diapause state. C. elegans constitutively secretes dauer larvae-inducing pheromone, which serves as a proxy for high conspecific density. This information about local competition is integrated with other inputs to assess the environment's suitability for future reproductive growth. Although the roles of sensory neurons have been studied, little is known about the function of other neuron classes in this developmental paradigm.

Here, we show that the AIA interneurons integrate pheromone cues from multiple sensory neuron classes and propagate this information via neuropeptidergic pathways to regulate the diapause entry decision. Pre- vious studies have shown that FMRFamide-like neuropeptide flp-2 null mutants are significantly more likely to enter diapause compared to wild-type animals. A flp-2 GFP reporter expressed in AIA, and AIA-specific flp-2 cDNA expression is sufficient to rescue the flp-2 mutant diapause entry phenotype. We also show that chemogenetic inhibition of AIA recapitulates the flp-2 mutant phenotype. AIA is the major postsynaptic partner of the glutamatergic ASK and ADL pheromone-sensing neurons. To investigate the link between the AIA ac- tivity and input signals from these pheromone sensing neurons, we used a microfluidic device to deliver crude pheromone extract in a controlled environment. While ASK and ADL are activated by acute pheromone pre- sentation, we find that AIA is inhibited likely through glutamate-gated chloride channels. To further confirm the dependency of the flp-2 expression on the AIA activity, and that the inhibition of AIA during the L1 larval stage initiates the diapause entry, we've performed periodic imaging of activity in AIA as worms proceed through development in microchambers subject to either dauer-inducing or reproductive chemical environments. Us- ing this platform, we show that AIA is quiescent under dauer-inducing conditions but active under reproductive growth conditions.

Identification of a key stimulus integrator in this developmental decision provides an opportunity to further probe its underlying computational processes.

168 The CHARGE syndrome gene *chd-7* plays a role in dauer formation and longevity

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CHARGE syndrome is a complex developmental disorder caused by mutations in the chromodomain helicase DNA-binding protein-7 (Chd7) and characterized by retarded growth and malformations in the heart and nervous system tissues. However, despite the public health relevance of this disorder, relevant targets of CHD7 that relate to disease pathology are still poorly understood. Here we report that *chd-7*, the nematode ortholog of Chd7, is required for dauer morphogenesis, lifespan determination, and stress response. Genetic epistasis places *chd-7* in the DAF-7/TGF-b pathway. Consistent with our discoveries, we found *chd-7* to be allelic to *scd-3*, a previously identified dauer suppressor from the DAF-7/TGF-b pathway. Interestingly, DAF-12 transcriptionally regulates *chd*-7, which is necessary to repress *daf-9* for execution of the dauer program. Transcriptomics analysis comparing *chd-7*–defective and normal dauers show multiple collagen genes, consistent with a conserved role for the TGF-b pathway in expression of the extracellular matrix. To validate a conserved function for *chd-7* in vertebrates, we used *Xenopus laevis* embryos, an established model to study CHARGE syndrome's features.

169 The kynurenine pathway and biosynthesis of NAD^+ and Rhodoquinone in worms

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The kynurenine pathway, also known as the main degradation route of tryptophan (Trp), has stood out since 1853 for its role in the novo synthesis of NAD^+ the universal redox cofactor. Moreover, recent studies have revealed its importance for the biosynthesis of Rhodoquinone (RQ), a prenylated benzoquinone structurally related to Ubiquinone, present in anaerobic facultative animals such as helminths and essential to harvest energy under hypoxic conditions. Thus, in some lineages, the kynurenine pathway is at the core of energy metabolism.

In order to gain information about the kynurenine pathway genes and the biosynthesis of RQ and NAD^+ in helminths, we analyzed genomes of seven platyhelminths and nine nematodes, including parasites and free-living organisms.

We found that of all the enzymes analyzed only the kynureninase (KYNU) is encoded in all genomes. This finding supports the discovery that this enzyme is essential for RQ biosynthesis in *C. elegans*. Additionally, the analysis revealed that the first step of the pathway in helminths is catalyzed by either indolamine dioxygenase, IDO or tryptophan dioxygenase, TDO. This mutually exclusive pattern contrasts the presence of both enzymes in mammals. Interestingly, the formamidase (AFMD) and kynurenine monooxygenase (KMO) genes, encoding enzymes upstream of KYNU in the pathway, were not present in all helminth lineages. These results, together with the presence in these lineages of a specific polyprenyltransferase isoform that uses arylamines from the kynurenine pathway, suggest that a complete kynurenine pathway is not needed for RQ biosynthesis in helminths. Indeed, we determined that the parasitic platyhelminth *Mesocestoides corti* that lacks AFMD and KMO can synthesize large amounts of RQ. Nitrogen-labeled Trp in this helminth will allow us to confirm the amine group of RQ derives from this amino acid.

Recent studies performed in *C. elegans* demonstrated that the enzyme Uridine monophosphate synthetase (UMPS), and not the missing quinolinate phosphoribosyl transferase (QPRT), was responsible for de novo biosynthesis of NAD^+, catalyzing the conversion of quinolinic acid to nicotinamide mononucleotide in the NAD^+ branch of the kynurenine pathway. Although we determined that UMPS is encoded in all the genomes analyzed, most helminth lineages lack key enzymes that are necessary for the NAD^+ biosynthetic pathway.

To assess the de novo NAD^+ biosynthesis from Trp in organisms that do not encode a complete kynurenine pathway, we will use deuterated Trp in *M. corti* and examined the presence of deuterated NAD^+. Finally, examination of the NAD^+ salvage pathway in helminths suggests that recycling of nicotinamide is used for NAD^+ biosynthesis in helminths

170 Glycerol-3-phosphate phosphatase / PGPH: a novel calorie restriction mimetic enzyme in *C. elegans*.

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Metabolic stress due to nutrient excess and lipid accumulation is at the root of many age-associated disorders and the identification of therapeutic targets that mimic the beneficial effects of calorie restriction has clinical importance. Here, using *C. elegans* as a model organism, we studied the roles of a recently discovered enzyme at the heart of metabolism in mammalian cells, glycerol 3-phosphate phosphatase (G3PP) (gene name *Pgp*) that hydrolyzes glucose-derived glycerol-3-phosphate (Gro3P) to glycerol. Gro3P is a key metabolite that regulates flux of various metabolic pathways and particularly, the glycerolipid/fatty acid (GL/FA) cycle associated with obesity, type-2-diabetes, and cardiometabolic disorders. We identify three *Pgp* homologues in *C. elegans* (*pgph1, pgph-2, and pgph-3*) and demonstrate *in vivo* that their protein products have G3PP activity, essential for glycerol synthesis and contributes to stress responses and healthy aging. Hyperosmotic and high glucose stresses induce *pgph* transcripts, glycerol production, and salt stress adaptation in a *PGPH*-dependent manner. Using targeted metabolomics, we find that Gro3P accumulates in *pgph* mutant animals in basal conditions and more prominently following salt and glucose stresses, while most intermediary metabolites are rarely altered by PGPH loss. Using an unbiased transcription factors RNAi screen, we further identify transcriptional regulators of *pgph-2* and *pgph-3* salt-mediated expression. Loss of PGPH increases fat deposition, exacerbates glucotoxicity, decreases resistance to various stresses, shortens median lifespan and decreases healthspan parameters. Importantly, *pgph-2* overexpression reduces fat deposition with age at basal and glucose excess conditions without restricting animal feeding or decreasing reproduction. Overexpression of *pgph-2* improves healthspan and protects from glucotoxicity retarding age-related locomotor decline in normal and high glucose conditions. Strikingly, our data suggest that the overexpression of *pgph-2* partly mimics the beneficial effects of dietary restriction. Overall, the results demonstrate that G3PP/PGP is a novel evolutionary conserved regulator of glucose and fat metabolism that protects against nutrient and environmental stresses and is involved in glucose detoxification and healthy aging.

171 The SR protein RSP-2 regulates the expression and physiological responses of the truncated DAF-2 isoform

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We have previously characterized a truncated isoform of the worm insulin receptor, DAF-2B which alters insulin signaling phenotypes via sequestration of insulin-like peptides. The DAF-2B isoform is generated by alternative splicing, whereby inclusion of exon 11.5 is followed by a failure to splice to exon 12, leading to the retention of a short in-frame intronic sequence. However, the factors which control these splicing events are not known. In *C. elegans, rsp* genes encode members of the SR protein family which control both constitutive and alternative splicing. To determine if SR-proteins play a role in regulation of the DAF-2B isoform, we examined the effect of RNAi against *rsp* genes in a fluorescent *daf-2b* splicing reporter strain. We found that knockdown of the SRp40 homologue, *rsp-2* significantly increased fluorescence expression in all larval stages, most prominently in early larval stages. Increased reporter expression, as well as increased expression of the endogenous *daf-2b* transcript, was confirmed using a deletion mutation, *rsp-2(Δ).* These observations suggest that the normal function of RSP-2 is to inhibit expression of the *daf-2b* transcript.

Since *rsp-2(Δ)* is associated with higher *daf-2b* expression, we hypothesized that *rsp-2(Δ)* would mimic phenotypes associated with high expression of DAF-2B, such as increased dauer entry and reduced dauer exit. Consistent with this, we found that *rsp-2(Δ); pdk-1* double mutant animals had higher rates of dauer formation and reduced dauer recovery. Epistasis experiments between *daf-2b* and *rsp-2* in the *pdk-1* background show that *daf-2b* is downstream of *rsp-2* but only partially required, suggesting that RSP-2 may more broadly be a target of the insulin signaling pathway. To investigate this link, we used CRISPR/Cas9 to generate mutations that target a predicted Akt phosphorylation site in *rsp-2.* Animals bearing a phosphorylation deficient mutation showed enhanced dauer entry and higher *daf-2b* expression whereas a phosphorylation mimetic mutation showed reduced dauer entry and no change in *daf-2b*. Therefore, RSP-2 regulation of DAF-2B appears to be linked to the phosphorylation cascade of the insulin-signaling pathway.

172A Deciphering how the Ubiquitin Proteasome System executes Linker Cell-type Death

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Programmed cell death is essential for animal development and homeostasis, and its disruption is associated with many disorders including neurodegeneration and cancer. Apoptosis is a prominent cell death form, however mutations in key apoptotic regulators only cause minor developmental defects in vertebrates. Non-apoptotic programs also exist, but their molecular basis remains poorly understood. Linker Cell-type Death (LCD) is a non-apoptotic and caspase-independent cell death program operating in *C. elegans* development. Remarkably, the morphological hallmarks of LCD are observed throughout vertebrate development, suggesting LCD is conserved. The Ubiquitin Proteasome System (UPS) is a key effector of LCD. The heat shock factor HSF-1 stimulates transcription of the E2 ligase *let-70*/Ube2D2. LET-70/UBE2D2 functions with Cullin Ring E3 ubiquitin ligases comprising the substrate recognition subunit BTBD-2 and other proteins to stimulate proteasome activity and effect LCD. The proteolytic targets of the UPS during LCD, however, and how their degradation trigger linker cell demise are unknown. To identify candidate targets of the UPS, we performed a yeast 2-hybrid screen against the substrate recognition subunit BTBD-2. Our preliminary evidence suggests that BTBD-2 interacts with an enzyme required for cellular methylation. Importantly, loss of this enzyme restores LCD to animals lacking the E2 ligase *let*-70, suggesting that this gene is a *bona fide* UPS target during LCD. To determine how loss of this enzyme precipitates LCD, we will confirm that the gene is degraded by the UPS, determine the role of its enzymatic activity in LCD, and assess similar genes and known interacting partners for a role in LCD. In parallel to these studies, we have found that EBAX-1, a substrate recognition subunit of Cul2-based E3 ligases, is also required for LCD. Two independent *ebax-1* loss of function mutations display inappropriate linker cell survival, and these defects are rescued by expression of wild-type *ebax-1* genomic clones. Furthermore, an endogenous translational reporter for *ebax-1* is expressed throughout the lifetime of the linker cell. To determine how *ebax-1* promotes LCD, we are performing epistasis studies with other LCD genes and are identifying its target substrates. Together these studies will reveal mechanisms that execute non-apoptotic cell death that can elucidate the etiology of associated disorders and eventually identify novel therapeutic targets.

173B Dietary Composition Modulates Neurodegeneration in a *C. elegans* Parkinson's Disease Model

Anthony Gaeta[1], Luke McKay[1], Candice Keogh[1], Kim Caldwell[1], Guy Caldwell[1] 1) University of Alabama.

The diet of any organism is crucial in providing the energy necessary to not only exist and persist in the environment, but to overcome stress when challenged. One such type of stress comes in the form of Parkinson's disease (PD), which is

characterized by the progressive loss of dopaminergic (DA) neuron function and integrity. Although it has been reported and observed that diet type has wide ranging effects on phenotypes in both humans and animal models, the effect that diet has on the progression of DA neuron loss in PD is largely unknown. However, evidence supporting a connection between the brain and the gut microbiome has become increasingly relevant to neurodegenerative diseases. Furthermore, the effect of parental diet on attributes of PD in subsequent generations has not been adequately investigated. The findings of this study indicate that a non-standard diet for *Caenorhabditis elegans* (*C. elegans*) has an impact on PD-associated pathology in this animal, in a transgenerational manner. Specifically, feeding worms an alternative bacterial diet of HB101 *E. coli*, as opposed to the laboratory standard OP50 *E. coli*, promotes significant, transgenerational protection of DA neurons in response to the stressor and pathological hallmark of PD, a-synuclein (a-syn) overexpression and accumulation. This protection is shown to be dependent on the normal function of Systemic RNA Interference Defective (SID) proteins, including SID-1, SID-2, and SID-3. These proteins are involved in the ability of double-stranded RNAs (dsRNAs) to traverse cellular boundaries, systemically, and silence target genes in *C. elegans* to varying degrees. Comparative transcriptomic analysis between worms reared on OP50 vs. HB101 *E. coli* in an a-syn model background has revealed genes and pathways associated with a wide range of processes. These included genes encoding proteins involved in metabolism, cell signaling, development, transcription, stress responses, and transmembrane transport, all of which were observed to be differentially expressed in worms reared on HB101 *E. coli*. Subsequent functional analysis of select targets by RNA interference revealed the contribution of individual genes to neuroprotection against a-syn-mediated neurodegeneration. This research lays a foundation for future investigation of the subtle distinctions in diet that can impact conserved pathways modulating neuron survival in response to stress.

174C Organismal death triggered by oyster mushrooms via mitochondrial dysfunction

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The oyster mushroom *Pleurotus ostreatus* is a nematophagous basidiomycete that produces toxins to paralyze nematodes. We previously reported that *P. ostreatus* triggers a massive calcium influx and rapid cell necrosis in the neuromuscular system of *C. elegans* via its sensory cilia. However, how do the mushroom hyphae trigger rapid cell necrosis remains unclear. Here, we show that *P. ostreatus* induced calcium influx in the mitochondrial matrix, resulting in massive mitochondria enlargement within five minutes of hyphal contact. In addition, the ATP level dropped promptly in the pharyngeal muscle cells of the paralyzed nematodes. Moreover, we observed a calcium wave propagated across the mitochondria of hypodermis. Mutants exhibited muscle contraction defects restricted the calcium wave, suggesting that body wall muscle contraction contributed to the propagation of calcium wave in hypodermis. Furthermore, *C. elegans* mutants with disrupted ER-mitochondria contacts decreased the calcium influx in the mitochondria of pharyngeal muscle cells. Our findings illustrate that *Pleurotus* toxins trigger drastically ion imbalance and disrupt mitochondrial function, leading to energy failure and cell necrosis throughout the entire animal.

175A Depletion of *cdc-25.2* in the intestine induces mitochondrial oxidative stress and germ cell apoptosis through a *cep-1*-dependent pathway

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A CDC25 phosphatase is essential for animal development as a cell cycle regulator by removing inhibitory phosphates from the cyclin-dependent kinase. However, we found that expression of CDC-25.2 remained in the somatic tissues at the post-developmental stage, suggesting a non-canonical role of CDC-25.2. At the adult stage, *cdc-25.2(g52)* mutant increased production of intracellular reactive oxygen species (ROS) and germ cell apoptosis (GA) which were suppressed by antioxidant, N-acetyl-L-cysteine treatment. These results suggest that GA was induced by intracellular ROS produced in the *cdc-25.2(g52)* mutants. Furthermore, we found that intestine-specific *cdc-25.2* RNAi induced both the high levels of mitochondrial ROS production and GA, and intestine-specific overexpression of *cdc-25.2* in *cdc-25.2(g52)* mutants suppressed mitochondrial ROS production and GA. Therefore, these findings indicate that CDC-25.2 in the intestine is required for the control of GA by modulating ROS production. Interestingly, this process was somatic CEP-1 dependent because depletion of *cep-1* in the soma by soma-biased RNAi did not increase both levels of ROS production and GA in the *cdc-25.2(g52)* mutants. Based on these findings, we suggest a non-canonical role of *cdc-25.2* in the intestine, that is, *cdc-25.2* controls mitochondrial oxidative stress in the soma to protect cell death in the germ line in which somatic CEP-1 activity is required. This study was supported by NRF2018R1A2B6007915 and NRF2021R1A2C1011658.

176B Investigation of the In vivo and In vitro effects of Essiac® Liquid Herbal Extract on Health and Cancer

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Essiac® is an herbal compound that has been widely used as a dietary supplement for health and immune system support, as well as a homeopathic cancer treatment. Despite multiple studies aiming to demonstrate its touted benefits, the results have been inconclusive. Some studies have shown Essiac® to impart gastroenterological protection, combat reactive oxygen species (ROS), increase immune cell subsets, and reduce *in vitro* cancer cell numbers, other studies have not been able to show reduced cancer load *in vivo*. Therefore, in this study using the fully-prepared proprietary blend, Essiac® Liquid Herbal Extract (LHE), we thoroughly explored its health benefits using the nematode animal model, *Caenorhabditis elegans* (*C. elegans*), as well as assess its antiproliferative abilities against three non-adherent (myeloma, lymphoma, and leukemia) and two adherent tumor forming (breast and prostate) cancer cell lines. Our findings show that when *C. elegans* were exposed to the recommended dosage of Essiac® LHE, there was an increase in their overall lifespan, and an increase in their ability to withstand oxidative stress induced mortality when challenged. Additionally, our work demonstrated that a 24% exposure of Essiac® LHE induced a significant decrease in cell viability and proliferation within all five cancer cell lines (RPMI 8226, Jurkat, CML, LNCaP, and MCF7). Furthermore, our results indicate that the anti-proliferative effects of Essiac® LHE are not being mediated through the induction of intrinsic apoptosis, but through an alternative cellular mechanism. Taken together, these *in vitro* and *in vivo* findings lend support to the overall health benefits and antiproliferative abilities of Essiac® LHE.

177C The loss of *psf-2* GINS leads to the inappropriate survival of cells programmed to die during *C. elegans* development

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The genetic pathway required for programmed cell death during *C. elegans* development is highly conserved. The key activator of this pathway is the pro-apoptotic BH3-only gene *egl-1.* Earlier studies showed that *egl-1* activity is controlled at the level of gene expression. Specifically, *egl-1* expression is controlled both at the transcriptional level through lineage-specific transcription factors and at the post-transcriptional level through microRNAs. In this study, we present evidence that *egl-1* expression is controlled at an additional level. The gene *psf-2* encodes a subunit of the GINS complex, which is essential for DNA replication. We identified a temperature-sensitive loss-of-function mutation of *psf-2, t3443*ts, and found that it does not only cause a cell cycle defect but a general block in cell death i.e. a Ced phenotype. Furthermore, we provide evidence that the Ced phenotype exhibited by *psf-2*(*t3443*ts) mutants is independent of these animals' cell cycle defects. Based on these observations we propose that *psf-2* GINS is required for programmed cell death during *C. elegans* development. To determine whether *psf-2(t3443ts)* affects the expression of *egl-1*, we quantified the number of *egl-1* transcripts in cell death lineages i.e. lineages in which a cell death occurs. We found that *psf-2(t3443ts)* abolishes a spike in the number of *egl-1* transcripts normally observed in cells programmed to die. This suggests that the loss of *psf-2* GINS blocks programmed cell death by abolishing the transcriptional up-regulation of *egl-1*in cells programmed to die. Based on our findings, we propose that the GINS complex is necessary for changes in chromatin state at the *egl-1* locus that permit the transcriptional up-regulation of the *egl-1* gene in cells programmed to die.

178A Autophagy and the degradation of apoptotic cells

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The autophagy pathway generates autophagosomes, double-membrane structures responsible for degrading protein aggregates, intracellular organelles, and other cellular components. This intracellular trafficking process is essential for the cell stress response, recycling the degraded material for use in different cellular processes. During C. elegans embryonic development, 113 somatic cells undergo apoptosis; these cells are then swiftly internalized by neighboring engulfing cells through phagocytosis, generating phagosomes. The newly formed phagosome then enters a maturation process during which acidic vesicles are fused to the phagosome promoting its degradation. Components of the autophagy pathway have previously been linked to phagosome maturation in mammalian cells in a process called LC3-associated phagocytosis (LAP). The autophagy machinery generates a single membrane-vesicle to conjugate LC3 directly onto the phagosome membrane promoting phagosome-lysosome fusion. Using live imaging on developing C. elegans embryos, we discovered that LC3-labeled

puncta generated in engulfing cells are recruited to the surface of phagosomes and subsequently fuse to them, depositing their content into the phagosomal lumen. The observed fusion pattern suggests that the puncta are of a double-membrane nature, indicative of double-membranous autophagosomes and not single-membrane LAP vesicles. We also observed that these autophagosomes form two distinct populations, those that are labeled with LGG-1 and those marked with LGG-2, both homologs of yeast Atg8 and mammalian LC3. Lacking either group of autophagosomes significantly delay the degradation of apoptotic cells, indicating that both LGG-1 and LGG-2 function in the clearance of apoptotic cells. Finally, we identified that a signaling pathway with a previously known role in phagosome-lysosome fusion also facilitates the integration of autophagosomes to phagosomes. Our findings have collectively added autophagosomes to the list of intracellular organelles involved in phagosome maturation. Furthermore, we have demonstrated that in *C. elegans*, it is autophagosomes, not LAP vesicles, that facilitate phagosome maturation, and we have revealed a novel function of the autophagy pathway in the clearance of apoptotic cells.

179B The cytoskeletal regulator UNC-53/Nav2 controls cell death processes in *Caenorhabditis elegans*

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C. elegans is used to unravel the complex signalling processes that control apoptosis and cell corpse engulfment. Apoptosis is partly directed by a pathway using CED-3/Caspase and CED-4/Apaf1 while the distinct process of corpse engulfment is mediated by pathways that include: (i) CED-1/SREC, CED-6/GULP, CED-7/ABC1, DYN-1/Dynamin and (ii) CED-2/CrkII, CED-5/DOCK180, CED-10/RAC, CED-12/ELMO and (iii) ABI-1/ABI and ABL-1/ABL. UNC-53/Nav2 is a cytoskeletal binding protein that controls cell migration and interacts with ABI-1 and UNC-73/TRIO. UNC-53 is homologous to the Neuron Navigator (Nav) genes, a gene family with diverse functions, including the growth and development of tumorigenic cells. To study a potential role for UNC-53 in cell death processes we first compared the number of residual cell corpses in the pharynxes of newly hatched L1 single mutants of *ced-1*, *ced-2*, *ced-5*, and *ced-10* to corresponding double mutants with *unc-53*. *unc-53* loss suppressed cell corpse engulfment of *ced-1*, *ced-2*, and *ced-10* but not *ced-5* mutants, placing *unc-53* outside of a ced-1 pathway but not independent of *ced-2* and *ced-5*. *unc-53* suppressed the distal tip cell guidance defects frequently observed in these mutants and had a striking effect on *ced-10* and *mig-2*/RHO animals, reducing the frequency of anteroposterior polarity reversals. *unc-53* loss also increased the amount of CED-10 as measured by increased GFP in *unc-53 (n152)*; CED-10::GFP strains compared to animals carrying the CED-10::GFP integrated array alone. We next used time-lapse microscopy to see if *unc-53* affected the timing, number or morphology of cell corpses. Morphology and timing were normal in *unc-53*, but we found that *unc-53 (n152)* null mutants had significantly fewer apoptotic corpses from the comma stage through to the 3-fold stage of embryogenesis. This latter observation suggests that *unc-53* may influence cell apoptosis directly. We next tested for a role for *unc-53 (n152)* in the apoptosis of neuronal cells. *unc-53 (n152)* enhanced the weak allele *ced-3 (n2427)* and blocked neuronal cell death in cells normally fated to die (P2.aap, P9-12.aap). *unc-53 (n152)* also significantly blocks CED-3 and CED-4 mediated killing when these proteins are ectopically expressed in the mechanosensory neurons. Our experiments suggest that *unc-53* impacts cell death directly through a pathway involving CED-3/CED-4 but may have an additional role in cell corpse engulfment.

180C Genetic Control of Caspase-mediated and Caspase-independent Cell Elimination in *C. elegans*

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The programmed elimination of unwanted or unneeded cells during animal development is a conserved process essential for proper development and tissue homeostasis. Insufficient or excessive cell elimination can result in diseases such as cancer and neurodegenerative disorders, respectively. While caspase-mediated apoptosis is the best characterized form of programmed cell elimination, other mechanisms, such as cell extrusion, also exist. Cell extrusion occurs in a wide range of metazoans, including mammals, fish, insects and nematodes. In *C. elegans*, at least eight embryonic cells, including the cell ABplpappap, normally fated for caspase-mediated apoptosis undergo extrusion in the absence of *ced-3* caspase activity.

To investigate the mechanisms underlying cell extrusion, we performed an F1 EMS mutagenesis screen of 15,000 haploid genomes using a *ced-3(lf)* caspase-deficient genetic background searching for mutants defective in the cell extrusion of ABplpappap. From this screen we isolated a mutant showing 64% penetrance of ABplpappap survival. Interestingly, this mutant also allows 30% penetrance of ABplpappap survival of caspase-mediated apoptosis in the presence of *ced-3(+)* caspase activity. These findings suggest that this gene controls two apparently distinct forms of programmed cell elimination of ABplpappap: cell extrusion and caspase-mediated apoptosis. To identify more genes involved in both of these processes we performed

an RNAi screen that identified 7 additional genes which, when knocked down, were capable of blocking both the caspase-mediated apoptosis of ABplpappap and the extrusion of ABplpappap in a caspase-deficient background.

By characterizing the role of these genes in the elimination of ABplpappap, we hope to elucidate new processes that contribute to programmed cell elimination. We hope that these studies will provide insight into diseases resulting from the perturbation of caspase-mediated apoptosis and cell extrusion, such as cancer.

181A *cep-1*/p53 mediated DNA damage response - understanding apoptosis in *Caenorhabditis elegans* germ cells

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The DNA damage response (DDR) is an elaborate system that functions to preserve genome integrity. The DDR is initiated by DNA damage checkpoint pathways which halt cell cycle progression to allow DNA repair to precede or trigger programmed cell death (apoptosis) depending on the cell type and the extent of the damage. The tumor suppressor p53 is a central regulator of the DDR and regulates apoptosis by transcriptionally inducing pro-apoptotic genes. p53 is the most commonly mutated gene in human cancers. Most cancer-associated p53 mutations are missense mutations that compromise the tumour suppressive transcriptional activity. Consequently, damaged cells fail to undergo apoptosis, leading to uncontrolled proliferation and cancer formation.

In this study, we employ *Caenorhabditis elegans*, to gain a deeper understanding of the mechanisms through which the DNA damage checkpoint signaling pathways impact the apoptotic DNA damage response. The core apoptotic pathway is distinctly conserved in *C. elegans*, where *C. elegans* p53-like, CEP-1*,* transcriptionally induces the BH3-only domain pro-apoptosis factors to trigger the apoptosome upon DNA damage. This model provides distinct advantages for the genetic investigation of apoptosis –the germline of the worm shows a specific and easy to visualize apoptotic response to DNA damage, enabling quantifiable evaluation of genetic and chemical interventions.

We recapitulated the most prevalent p53 hotspot mutation in the worm, and generated a genetic tool for high throughput screening of novel regulators of *cep-1*/p53 dependent apoptosis in whole live organism. Ionizing radiation-induced apoptosis is abrogated in worms with the hotspot mutation, and in the forward genetic screen performed, we were able to isolate mutant worms which showed restored apoptosis despite a mutated p53. Our data indicate that the restored apoptotic response occurs in a delayed manner and involve a dysregulated ERK/MAPK signaling pathway. Overall, our preliminary findings point towards a secondary delayed wave of DNA damage response that is set in motion if the primary response regulated by *cep-1*/p53 fails when it is mutated.

182B Identifying the key players of phosphatidylserine externalization in non-apoptotic dying cells

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Throughout development, cells are programmed to die for homeostatic control. Corpse clearance is key for animals to avoid inflammation and auto-immune disease. To better understand the “eat me” signal of phosphatidylserine (PS) exposure for cell clearance, we are attempting to disrupt the externalization of PS on *C. elegans* polar bodies. The second polar body undergoes a non-apoptotic form of cell death, externalizes PS, and is cleared by embryonic cells during early development. PS exposure is known to be caused by lipid scramblases, enzymes present in the plasma membrane that translocate phospholipids between the leaflets of the lipid bilayer. However, the scramblases that regulate PS exposure in non-apoptotic dying cells are unknown.

A combination of RNAi and the *ced-8(n1891)* loss-of-function allele suggested that the scramblase CED-8 plays a redundant role in externalizing PS on polar bodies with the mitochondrial factor WAH-1 as well as the scramblases SCRM-1, SCRM-2, and SCRM-3. As CED-8, WAH-1, and SCRM-1 are involved in PS externalization during apoptosis, we tested whether PS exposure depends upon the apoptotic caspase CED-3. However, PS was still externalized on polar bodies in *ced-3(n717)* mutants, confirming that scramblase activation is independent of the apoptotic pathway.

To confirm the role of the redundant scramblases, we generated a *scrm-4 scrm-1 scrm-2; scrm-3*; *ced-8* quintuple scramblase mutant strain. These mutants still externalized PS on polar bodies, indicating that there are other ways to externalize PS on these cells. We are currently testing other scramblase proteins, including SCRM-6, SCRM-7, SCRM-8, and CED-7. Thus, we will define the molecular players in the regulation of PS exposure for non-apoptotic cell death, which will allow us to define the role of PS in phagocytic uptake as well as later steps of phagosome maturation.

183C Calcium Ions Trigger the Exposure of Phosphatidylserine on the Surfaces of Necrotic Cells

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Intracellular Ca^{2+} level is under strict regulation through calcium channels and storage pools including the endoplasmic reticulum (ER). Mutations in certain ion channel subunits, which cause mis-regulated Ca^{2+} influx, induce the excitotoxic necrosis of neurons. In the nematode *Caenorhabditis elegans*, dominant mutations in the DEG/ENaC sodium channel subunit MEC-4 induce six mechanosensory (touch) neurons to undergo excitotoxic necrosis. Dominant mutations in other channel components induce the necrosis of various neurons. These necrotic neurons are subsequently engulfed and digested by neighboring hypodermal cells. We previously reported that necrotic touch neurons actively expose phosphatidylserine (PS), an "eat-me" signal, to attract engulfing cells. However, the upstream signal that triggers PS externalization remained elusive.

We constructed the GCaMP5 Ca^{2+} reporter that is specifically expressed in touch neurons and and the MFG-E8::mCherry reporter for PS on cell surfaces. After monitoring the cytoplasmic Ca^{2+} signal simultaneously with the PS signal on the surfaces of touch neurons in real time during embryonic development, we report that a robust and transient increase of cytoplasmic Ca^{2+} level occurs prior to the exposure of PS on necrotic touch neurons. Inhibiting the release of Ca^{2+} from the ER, either pharmacologically or genetically, specifically impairs PS exposure on necrotic but not apoptotic cells. On the contrary, inhibiting the reuptake of cytoplasmic Ca^{2+} into the ER induces ectopic necrosis and PS exposure. Remarkably, PS exposure occurs independently of other necrosis events. Furthermore, unlike in mutants of DEG/ENaC channels, in dominant mutants of two Ca^{2+} channels, PS exposure on necrotic neurons does not rely on the ER Ca^{2+} pool. Our findings indicate that high levels of cytoplasmic Ca^{2+} are necessary and sufficient for PS exposure. They further reveal two Ca^{2+}-dependent, necrosis-specific pathways that promote PS exposure, a "two-step" pathway initiated by a modest influx of Ca^{2+} and further boosted by the release of Ca^{2+} from the ER, and another, ER-independent, pathway. Moreover, our observations suggest that both the ER-mediated and ER-independent Ca^{2+} pathways promote PS externalization through activating ANOH-1, the worm homolog of mammalian phospholipid scramblase TMEM16F. Our work has revealed a novel Ca^{2+}-triggered PS exposure mechanism active in necrotic neurons, a mechanism different from the caspase-activated PS exposure mechanism employed by apoptotic cells. We propose that this mechanism might be conserved in other organisms including mammals.

184A The role of the Insulin Signaling Pathway in *C. elegans* Germline Stem Cell Mitosis

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The spindle assembly checkpoint (SAC) monitors kinetochore-microtubule attachments and halts mitotic progression in the presence of unattached or incorrectly attached chromosomes. The SAC ensures the fidelity of chromosome segregation during mitosis by guarding against chromosome mis-segregation and aneuploidy. While the events and mechanisms that control the SAC are relatively well understood, how they are influenced by developmental and environmental signaling networks remains unclear. The insulin signaling (IIS) pathway is a universally important and highly conserved pathway that integrates cell proliferation and development in many organisms. We are using *C. elegans* germline stem cells (GSCs) to ask how the IIS pathway affects mitotic duration and fidelity *in vivo* via the *in situ* live imaging of these mitotically dividing cells. This pathway has a known role in cell cycle progression, with *daf-2* mutants having fewer germ cells and a lower mitotic index, compared to stage-matched N2 animals, due primarily to a delay in the G2 phase of the cell cycle. Removal of *daf-18* or *daf-16* restores normal germ cell numbers suggesting canonical signaling from DAF-2 to DAF-16 affecting the rate of cell cycle progression. While clear links have been established between IIS and GSC proliferation, IIS involvement in events during mitosis, including the SAC, remains unclear. Evidence from cell culture models suggests that IIS may play a role during mitosis; however, it is not clear whether this occurs under *in vivo* physiological conditions.

We have found that *daf-2* mutants have an increased duration of mitosis and decreased number of cells entering mitosis. Both effects can be rescued by subsequent deletion of *daf-18* or *daf-16*. In addition, *daf-2* mutants have an increased incidence of chromosome segregation errors when the SAC is compromised, suggesting impairment of spindle assembly. Ongoing work will assess whether these effects are cell autonomous and will determine the underlying cause of the observed increase in chromosome segregation errors.

185B Investigating the regulation of CDC-20 recruitment to kinetochores

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The WD-40 repeat protein CDC-20 is the co-activator of the Anaphase Promoting Complex/Cyclosome (APC/C), the E3 ubiquitin ligase responsible for mitotic exit, as well as a core subunit of the mitotic checkpoint complex that restrains APC/C activity when chromosomes are not yet attached to the mitotic spindle. CDC-20 rapidly fluxes through the kinetochore region of chromosomes via association with a conserved binding motif, known as the ABBA motif, in BUB-1. CDC-20 flux through kinetochores is essential for mitotic checkpoint activation, which delays anaphase onset until all chromosomes attach to microtubules, and for promoting anaphase onset following kinetochore-microtubule attachment (Kim and Lara-Gonzalez et al, 2017, *Genes and Development 31*:1089–1094). Here, we investigate the regulation of BUB-1-dependent recruitment of CDC-20 to kinetochores. We found that mutating a conserved Polo-like Kinase 1 (PLK-1) docking site in BUB-1 eliminated CDC-20 kinetochore recruitment to the same extent as mutating the ABBA motif; in addition, the peak kinetochore recruitment of the mutant BUB-1 was reduced to ~50% of wildtype levels, likely due to a role for BUB-1-bound PLK-1 in promoting kinetochore recruitment of BUB-1. To address if the defect in CDC-20 kinetochore recruitment was a consequence of reduced BUB-1 kinetochore localization or was due to BUB-1-docked PLK-1 regulating the ABBA motif-CDC-20 interaction, we selectively mutated the BUB-1-associated protein BUB-3 to impair its recognition of the phosphorylated kinetochore scaffold KNL-1. The BUB-3 phospho-recognition mutant reduced peak BUB-1 kinetochore recruitment to ~30% of wildtype levels; however the consequences on anaphase onset and CDC-20 kinetochore localization were significantly less severe than observed for PLK-1-docking mutant BUB-1. These observations support a model in which BUB-1-associated PLK-1 is essential for CDC-20 kinetochore recruitment, either by direct phospho-regulation of the ABBA motif or by controlling access of the ABBA motif to CDC-20. Our current experiments are focused on distinguishing between these two potential mechanisms.

186C Interactions between the PAM-1 aminopeptidase and the cell-cycle machinery during oocyte maturation and early development

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The cell cycle is a tightly regulated process controlled by the transient activation of cyclin-dependent kinases (CDKs). Activation and deactivation of CDKs is coordinated by its associated cyclin as well as other kinases, phosphatases, and the degradation machinery. Coordination of these complexes drives the cell through each stage of the cell cycle and is necessary for development, tissue maintenance, and fertility. Puromycin-sensitive aminopeptidases (PSAs) are highly conserved metalloproteases implicated in cell-cycle regulation in numerous organisms. Despite widespread roles in this process, the mechanism by which PSAs interact with the cyclin/CDKs and other cell-cycle machinery is poorly understood. A key goal of our project is to uncover these interactions in the *C. elegans* model. The *C. elegans* PSA homolog, PAM-1, plays a role in meiotic exit regulation, sister chromatid separation and anterior-posterior axis establishment and when mutated, results in embryonic lethality. In a screen for suppressors of *pam-1,* we identified a mutation in *wee-1.3* that rescues the lethality of *pam-1* mutants. WEE-1.3 is an inhibitory kinase that negatively regulates CDK-1, part of the maturation promoting complex. *pam-1* and *wee-1.3* genetically interact in polarity establishment as the presence of the suppressor rescues many polarity landmarks in *pam-1* mutants. Since WEE-1.3 has been implicated in oocyte maturation we went on to test if the two proteins interact in this process. We found that *pam-1* mutants are protected from the precocious oocyte maturation defect associated with loss of WEE-1.3, suggesting PAM-1 and WEE-1.3 interact in more than one process. To further look at the role of PAM-1 in the cell cycle, we are closely examining meiotic and mitotic defects in *pam-1* mutants as compared to *pam-1* in combination with the loss of cell-cycle components such as WEE-1.3 and the cyclin dependent kinases. We are also examining the localization of the proteins in *pam-1* mutants. We expect to gain a new understanding of how PAM-1 regulates that cell cycle and how it interacts with known regulators that is likely to be applicable to other systems.

187A Role of Cohesin in Chromosome-Dependent Meiotic Spindle Assembly

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Accurate chromosome segregation during mitosis or meiosis requires a bipolar array of microtubules with microtubule plus ends oriented toward chromosomes and microtubule minus ends oriented toward the poles. Mutants that assemble spindles which contain more than or fewer than two poles typically produce aneuploid progeny. During mitosis and male meiosis, bipolar spindle formation is initiated by the duplication of the centrosomes. Because female meiotic spindles in *C. elegans* and vertebrates do not have centrosomes, we asked whether the discrete bipolar structure of bivalents is instead required for the initiation of bipolar spindle assembly. To address this question, we conducted time-lapse imaging of spindle assembly using GFP::tubulin or the spindle pole markers GFP::ASPM-1 or GFP::LIN-5 in cohesin mutants that have single chromatids during segregation stages of meiosis. Severson and Meyer (eLife.03467) previously reported that *rec-8, spo-11* double mutants and *rec-8, spo-11, coh-3, coh-4* quadruple mutants both have single chromatids at metaphase I and metaphase II. Our results

revealed that bipolar spindles assemble around *rec-8, spo-11* metaphase I single chromatids but not around *rec-8, spo-11, coh-3, coh-4* metaphase I single chromatids nor any of the metaphase II single chromatids. These results suggest that chromosome bipolarity is not required for bipolar spindle assembly and further suggest that non-cohesive cohesin can promote bipolar spindle assembly. To address how non-cohesive cohesin might promote bipolar spindle assembly, we monitored chromosome-associated AIR-2. AIR-2 normally concentrates in a ring between homologous chromosomes at metaphase I and between sister chromatids at metaphase II. We found intense labeling of AIR-2 on the assembly-competent metaphase I single chromatids of *rec-8, spo-11* mutants but minimal GFP::AIR-2 was associated with any of the assembly-incompetent single chromatids. We are currently asking whether AIR-2 is the only spindle assembly factor recruited by cohesin and trying to identify the downstream effectors of AIR-2.

188B Dissecting cell cycle entry: Insights from a *cdk-4* allele with a sex myoblast-specific proliferation defect

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Exit from G0 and entry into the cell cycle is tightly regulated during metazoan development. During G1, cells respond to various microenvironmental cues that control their decision to either proliferate or enter G0. After the G1 restriction point is passed, cells are committed to another round of division. The sole *C. elegans* cyclin/CDK orthologs, CYD-1/CDK-4 and CYE-1/CDK-2, are required for the initiation and progression of cells through G1 into S. The highly invariant *C. elegans* sex myoblast (SM) lineage provides a genetically tractable and visually accessible in vivo model to study cell cycle control and its effect on cell fate. During larval development, two bilaterally symmetrical SM cells are born and undergo a 65-micron migration to flank the center of the gonad. These cells proceed to undergo three rounds of division differentiating into the vulval and uterine muscle cells of the egg-laying apparatus. Our laboratory has recently discovered that a 769-base pair deletion in the *cdk-4* promoter results in a G0-arrested SM cell that is capable of migrating, but is no longer able to divide regardless of developmental stage. Using a novel translocation-based kinase sensor, we show that the cell-cycle arrested SM cell appears devoid of CDK-4 activity. We also demonstrate that a temperature-sensitive allele of *cyd-1(q626)* phenocopies *cdk-4(n1382)*. Further, in *cdk-4(n1382)* mutants lacking CKI-1, the primary *C. elegans* ortholog of p21/p27, we partially suppress the SM cell division defect. Our findings support a model in which CDK-4 deactivates CKI-1, preventing the inhibition of CDK-2. Thus, in the absence of CDK-4 and CKI-1, CDK-2 is sufficient to drive cell cycle progression. Taken together, these data provide new insights into how cells transition from quiescence-to-proliferation-to-terminal differentiation during animal development.

189C The Chromatin Remodeling Protein CHD-1 and the EFL-1/DPL-1 Transcription Factor Cooperatively Down Regulate CDK-2 to Control SAS-6 Levels and Centriole Number

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Centrioles are submicron-scale, barrel-shaped organelles typically found in pairs, and play important roles in ciliogenesis and bipolar spindle assembly. In general, successful execution of centriole-dependent processes is highly reliant on the ability of the cell to stringently control centriole number. This in turn is mainly achieved through the precise duplication of centrioles during each S phase. Aberrations in centriole duplication disrupt spindle assembly and cilia based signaling and have been linked to cancer, primary microcephaly and a variety of growth disorders. Studies aimed at understanding how centriole duplication is controlled have mainly focused on the post-translation regulation of two key components of this pathway: the master regulatory kinase ZYG-1/Plk4 and the scaffold component SAS-6. In contrast, how transcriptional control mechanisms might contribute to this process have not been well explored. Here we show that the chromatin remodeling protein CHD-1 contributes to the regulation of centriole duplication in the *C. elegans* embryo. Specifically, we find that loss of CHD-1 or inactivation of its ATPase activity can restore embryonic viability and centriole duplication to a strain expressing insufficient ZYG-1 activity. Interestingly, loss of CHD-1 is associated with increases in the levels of two ZYG-1-binding partners: SPD-2, the centriole receptor for ZYG-1 and SAS-6. Finally, we explore transcriptional regulatory networks governing centriole duplication and find that CHD-1 and a second transcription factor, EFL-1/DPL-1 cooperate to down regulate expression of CDK-2, which in term promotes SAS-6 protein levels. Disruption of this transcriptional regulatory network results in the production of extra centrioles.

190A The conserved histone deacetylase, HDA-1, functions in cell cycle-dependent and independent roles to promote invasive differentiation

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Cell invasion occurs naturally during development; however, it also contributes to tumor progression. Although understudied, data from different cancer subtypes suggests that a dichotomy exists between invasive and proliferative behavior. Our lab uses the *C. elegans* anchor cell (AC) to study this proliferative-invasive switch during uterine-vulval development. Post-embryonically, the AC invades into the vulval epithelium to form the mature vulva. We have shown that the AC exists in G0/G1-cell cycle arrest during invasion, which requires the nuclear hormone transcription factor, *nhr-67 (tailless/Tlx*). Loss of *nhr-67* results in mitotic, non-invasive ACs. *nhr-67* maintains AC arrest by regulating the expression of the cyclin-dependent kinase inhibitor, *cki-1 (p21/p27)*. Induced expression of CKI-1 is sufficient to restore invasion in an *nhr-67*-depleted background, suggesting that the G1/G0 state is required for invasive activity. Differentiation of invasive behavior is also regulated by chromatin modifiers that act downstream and/or parallel to G0/G1 arrest. We have found that the histone deacetylase, *hda-1*, promotes AC invasion by positively regulating pro-invasive gene expression; however, whether *hda-1* regulates invasion by controlling cell cycle arrest is unknown. We and others have recently shown that key pro-invasive transcription factors, *nhr-67/Tlx, hlh-2/E, fos-1a/Fos,* and *egl-43/Evi1,* maintain the AC in a post-mitotic, pro-invasive state by acting as part of gene regulatory network composed of two subcircuits. *nhr-67*, *egl-43*, and *hlh-2* function in a type 1 coherent loop with positive feedback to maintain the AC in a post-mitotic state and facilitate invasion, while *fos-1* acts in a cell cycle-independent subcircuit to promote invasion. Using genetic approaches, CRISPR-Cas9 genome engineering and high-resolution imaging, we explored the regulatory relationships between HDA-1 and these key transcription factors. We find that *hda-1* functions in the AC to maintain G1/G0 arrest, and that loss and depletion of *hda-1* results in mitotic, non-invasive ACs. We show that in the AC, *hda-1* regulates the activity of NHR-67, FOS-1a, and HLH-2. Interestingly, induced expression of CKI-1 fails to restore invasion in *hda-1*-deficient ACs, but is able to rescue the mitotic defect of *hda-1*-depleted animals. These results suggest that *hda-1* functions in both cell cycle-dependent and independent subcircuits to maintain the post-mitotic, pro-invasive state of the AC.

191B Multiple Phosphorylation Events Regulate Centriole Assembly.

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Centrioles are microtubule-based cylindrical structures that recruit a proteinaceous matrix called pericentriolar matrix (PCM). Together these two entities constitute a centrosome that serves as a microtubule-organizing center. In mitotic cells, centrosome form the poles of the spindle and in some terminally differentiated cells, centrioles serve as basal bodies to template sensory or motile cilia. Maintaining proper centriole number is critically important for spindle assembly and cilia function and is achieved through a highly regulated duplication process that occurs once per cell cycle. Too many or too few centrioles manifest in various human diseases such as cancer, primordial dwarfism and primary microcephaly. Studies in *C. elegans* have identified the conserved proteins – SAS-7, SPD-2, ZYG-1, SAS-6, SAS-5 and SAS-4 as indispensable for centriole biogenesis. The kinase ZYG-1, a homolog of human Plk4, is considered the master regulator of centriole assembly but the identity of its critical substrates and the cascade that triggers centriole assembly *in vivo* are not completely understood. A major hurdle in addressing this and other questions is the difficulty in obtaining functional recombinant centriole proteins. Using a novel method, we were successful in obtaining sufficient amounts of recombinant full-length proteins from *E. coli*. Using these proteins, we could show that ZYG-1 phosphorylates SAS-5 extensively *in vitro* and that most of phosphorylated residues in SAS-5 were conserved in nematodes. Interestingly, a few of these residues formed a part of a larger highly conserved 25 amino acid motif. Surprisingly, we found that phosphorylation of the residues in this motif modulate phosphorylation elsewhere in SAS-5. We also show that this motif is required for both ZYG-1 and SAS-4 to bind SAS-5. Using phosphomimetic mutations within the motif, we find that a specific array of mutations dictates the choice of binding partner. Our results indicate that phosphorylation of this region aids in establishing a mutually exclusive interaction pattern of SAS-5 with either ZYG-1 or SAS-4. We have also used CRISPR/Cas9 to systematically analyze the effect of knocking out phosphorylation sites in endogenous SAS-5 and have found that while many of these mutation impact centriole assembly, none of them complete block this process. Our work suggests that ZYG-1 does not regulate SAS-5 through a single phosphorylation event but rather controls multiple functions of SAS-5 that collectively are required to ensure the formation of a new centriole.

192C Asymmetric mitochondrial inheritance in the context of a *C. elegans* cell death lineage

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Mitochondria cannot be generated *de novo* and therefore are partitioned in a regulated manner during cell division to ensure that both daughter cells inherit these essential organelles. Recently, it was reported that during certain cell divisions, mitochondria are partitioned asymmetrically; however, the mechanisms and functions of this asymmetric inheritance remain to be elucidated. Asymmetric mitochondrial inheritance is best understood in the budding yeast *S. cerevisiae,* but technical challenges have so far limited progress on its analysis in higher eukaryotes. The highly reproducible development, genetic tractability and optical accessibility of *C. elegans* provide a unique opportunity to systematically study this process at single-cell resolution in a developing animal.

Through super-resolution live imaging, we discovered that mitochondria are asymmetrically partitioned by quantity during the asymmetric division of the neuroblast QL.p in L1 larvae. 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 of the mitochondria are inherited by the surviving QL.pa daughter, resulting in increased mitochondrial density in QL.pa and decreased mitochondrial density in QL.pp. We have evidence that mitochondria are asymmetrically partitioned also by morphology. Whereas mitochondria inherited by the surviving QL.pa appear to be elongated and connected, mitochondria inherited by QL.pp frequently are fragmented and not connected. Based on these observations, we propose that mechanisms exist that control mitochondrial morphology, partitioning and transport during QL.p division, thereby enabling asymmetric mitochondrial inheritance.

Using various mutants as well as photo-convertible fluorescent proteins, we are currently testing the hypothesis that mitochondrial fusion and fission, tethering and active and/or passive transport contribute to asymmetric mitochondrial inheritance during QL.p division. In addition, we are developing a protocol for the rendering and 3D segmentation of mitochondria in conjunction with live imaging. The ultimate goals of this project are to obtain a mechanistic understanding of asymmetric mitochondrial inheritance during animal development and to determine how this process can influence daughter cell fates.

193A Reciprocal interactions between the apoptosis pathway and cell size

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Caspases, a family of conserved proteases, are required for apoptosis in all metazoans but play roles in processes other than apoptosis; however, the molecular mechanisms and physiological functions of many of these 'non-canonical roles' remain largely unknown. Most of the cells that are fated to die through apoptosis during *C. elegans* development are the smaller daughter of a 'mother' cell that divides asymmetrically by size and fate. In a recent study, we reported that the loss of *C. elegans ced-3*^{caspase} compromises the asymmetric division of such mother cells and causes the daughters fated to die to increase in size. Thus, we uncovered a novel non-canonical role of *ced-3*^{caspase} in asymmetric cell division (Mishra *et al.,* 2018). To elucidate the molecular mechanism through which *ced-3*^{caspase} promotes asymmetric cell division, we screened for physical interactors of CED-3 protein and identified the conserved RhoGEF ECT-2. ECT-2 activates the small GTPase RHO-1 and thereby promotes contractility of the actomyosin network. Our genetic data suggests that *ced-3* ^{caspase} acts upstream of *ect-2*^{RhoGEF} in mother cells to promote daughter cell size asymmetry. We also provide evidence that prior to metaphase, ECT-2^{RhoGEF} is asymmetrically localised in mother cells, and that this is dependent on *ced-3*^{caspase}. Thus, we hypothesize that CED-3^{caspase} promotes local activation of ECT-2^{RhoGEF}, thereby allowing asymmetric cortical contractility, which is necessary for unequal mother cell division. Finally, it has previously been reported that increasing the size of cells fated to die compromises the ability of these cells to undergo apoptosis. We now demonstrate that, conversely, decreasing the size of cells fated to die promotes their ability to undergo apoptosis and suppresses the cell death abnormal (Ced) phenotype caused by partial *ced-3*^{caspase} loss-of-function mutations but not a putative *ced-3*^{caspase} null mutation. Based on these observations, we propose that the non-canonical role of *ced-3*^{caspase} in asymmetric mother cell division ensures that the sizes of cells fated to die are below a critical 'lethal' size, necessary for efficient apoptosis execution. Hence, we have discovered reciprocal interactions between the apoptosis pathway and cell size in the context of apoptotic cell death during *C. elegans* development.

References: Mishra, N., Wei, H. & Conradt, B. *Caenorhabditis elegans* ced-3 Caspase Is Required for Asymmetric Divisions That Generate Cells Programmed To Die. *Genetics* **210,**

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194B The SWI/SNF chromatin remodeling assemblies BAF and PBAF differentially regulate cell cycle exit and cellular invasion *in vivo*

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Chromatin remodeling complexes, such as the SWItching defective/Sucrose Non-Fermenting (SWI/SNF) ATP-dependent chromatin remodeling complex, coordinate metazoan development through broad regulation of chromatin accessibility and transcription, ensuring normal cell cycle control and cellular differentiation in a lineage-specific and temporally restricted manner. Mutations in the structural subunits of chromatin, such as histone subunits, and chromatin regulating factors (CRFs) are associated with a variety of diseases including cancer metastasis, which co-opts cellular invasion programs functioning in healthy cells during development. Here we utilize *Caenorhabditis elegans* anchor cell (AC) invasion as an *in vivo* model to identify the suite of chromatin agents and CRFs that promote cellular invasiveness. We demonstrate that the SWI/SNF ATP-dependent chromatin remodeling complex is a critical regulator of AC invasion, with pleiotropic effects on both G_0 cell cycle arrest and activation of invasive machinery. Using targeted protein degradation and RNA interference (RNAi), we show that SWI/SNF contributes to AC invasion in a dose-dependent fashion, with lower levels of activity in the AC corresponding to aberrant cell cycle entry and increased loss of invasion. Specifically, we implicate the SWI/SNF BAF assembly in the regulation of the cell cycle, whereas our data suggests that the SWI/SNF PBAF assembly promotes AC attachment to the basement membrane (BM) and promotes the activation of the invasive machinery. Together these findings demonstrate that the SWI/SNF complex is necessary for two essential components of AC invasion: arresting cell cycle progression and remodeling the BM. The work here provides valuable single-cell mechanistic insight into how the SWI/SNF assemblies differentially contribute to cellular invasion and how SWI/SNF subunit-specific disruptions may contribute to tumorigeneses and cancer metastasis.

195C Elucidating the Role of Securin in Regulating Separase during Cortical Granule Exocytosis

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Meiosis is a tightly regulated series of events leading to gamete formation. A key player in this process is the protease Separase. Known for its role in chromosome segregation, we have defined a role for Separase in vesicular trafficking during cell division. Securin is an inhibitory chaperone of Separase that is degraded at anaphase onset after ubiquitination by the Anaphase Promoting Complex/Cyclosome (APC/C). After Securin degradation, activated Separase cleaves a subunit of cohesin to allow chromosome segregation. Separase also shows dynamic localization during the cell cycle. In prometaphase, Separase localizes to kinetochores and mysterious cortical filaments. During anaphase I, Separase transfers to the midbivalent between homologous chromosomes where cohesin localizes, and concurrently appears on vesicles, called cortical granules, in the cortex. How the dynamic localization of Separase is regulated is currently unknown. Here, we investigated how APC/C and Securin regulate Separase during meiosis I. First, we observed that Securin is degraded by anaphase I and is not present on vesicles. Second, APC/C activity is required for Separase to localize to vesicles. *apc/c* RNAi results in arrested embryos with Separase and Securin remaining localized to cortical filaments. Third, partial *securin* RNAi causes precocious Separase vesicle localization. These observations suggest that Securin degradation is required for Separase to localize to vesicles during anaphase. To further test this, we made Non-Degradable Securin fused to GFP (Securin^{DM}::GFP). Securin^{DM}::GFP is stably expressed during anaphase I and causes embryonic lethality. Securin^{DM}::GFP causes chromosome segregation defects, polar body extrusion failure and inhibits cortical granule exocytosis. Importantly, expression of Securin^{DM}::GFP causes a reduced and delayed localization of Separase to vesicles during anaphase I. We conclude that degradation of Securin regulates the localization of Separase to vesicles to coordinate exocytosis with chromosome segregation. Our findings suggest a novel role for the APC/C and Securin in controlling the localization of Separase, in addition to regulating its protease activity for chromosome segregation and cortical granule exocytosis during anaphase I.

196A The role of CDK-4 in cell size and metabolism

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The cell cycle is driven by the tightly-choreographed activity of cyclins and their catalytic partners, cyclin-dependent kinases (CDKs). While the roles of these complexes in cell cycle progression have been thoroughly characterized, more recent work has identified distinct functions of cyclins and CDKs in cellular processes such as differentiation, DNA repair, and metabolism. I am interested in understanding how cell size is regulated, and how a cell knows what size to be. In mammals, cell size is largely controlled through the coordination between growth rate and time spent in the G1 phase of the cell cycle. However, we have recently shown that CDK4, acting in G1, asserts a unique influence by which it dictates the size to which a cell aims to grow, or its target size, an ability not shared by other G1 CDKs. Inhibition of CDK4 results in a population of cells with uniformly increased size, while increased CDK4 activity causes cells to be uniformly small. To investigate the physiological implications of this phenomenon, we have turned to the tractability and wealth of genetic tools offered in C. elegans.

Knockdown of the C. elegans CDK4 homolog *cdk-4* or its conjugate cyclin *cyd-1* is sufficient to increase the size of both the nucleus and nucleolus in seam cells, used here as a proxy for cell size due to the scaling of these organelles with cell size through development. Preliminary work also suggests that while overall metabolism is increased, fat stores are depleted in worms with reduced CDK-4 activity. We seek to understand the mechanisms behind this accumulation of cell mass, and the consequences of our observed changes in metabolism on the adult worm.

197B Linking centromeric factors to chromosome condensation in *C. elegans* embryos

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The centromere is a chromosomal region that recruits the kinetochore, a large multi-subunit complex that binds spindle microtubules enabling chromosome segregation. Centromeric chromatin is therefore placed on the surface of condensed chromosomes, and specifically adapted to withstand the spindle pulling forces. The cross-talk between centromeres and condensation machinery is an area of active study.

In contrast to other commonly studied model organisms, *C. elegans* is a holocentric species: its centromeres are placed along the chromosome axis, which makes it an attractive model to study centromere organisation. Depletion of the centromeric histone H3 variant CENP-A (HCP-3 in *C. elegans*) and its main loading factor KNL-2 in embryos results in defective chromosome formation. However, little is known about the mechanism of their action and the temporal regulation.

Using an immunoprecipitation followed by mass spectrometry, we identified regulatory phosphosites on KNL-2 that are targeted by CDK-1 kinase. Mutation of these residues to alanines to prevent their phosphorylation leads to defects in cell division and an increase in embryonic lethality. We show that compromised chromosome formation is underlying these phenotypes. Mutant embryos exhibit delayed prophase condensation, and the prometaphase chromosomes lack rigidity and twist around their axis. Interestingly, centromeric chromatin seems properly maintained, as evidenced by unchanged CENP-A genomic distribution and proper kinetochore recruitment in the mutant strain. We further found that condensin II levels are reduced on metaphase chromosomes in the mutant embryos, indicating that that KNL-2 may act through condensin II complex recruitment. We conclude that KNL-2 orchestrates centromeric chromatin maintenance as well as the establishment of the proper chromosome structure, and that these processes are independently regulated.

198C A polarity pathway for exocyst-dependent intracellular tube extension

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Organs are comprised of various tubes with distinct cellular compositions. The smallest of these tubes are made up of just a single cell and can form intracellularly, with a lumen extending through the center of the cytoplasm. Lumen extension in intracellular tubes can occur by the directed fusion of vesicles with an invading apical membrane domain, but the molecular events that regulate this polarized vesicle delivery remain unknown. Within the *C. elegans* excretory cell, which contains an intracellular tube, the exocyst vesicle-tethering complex is enriched at the lumenal membrane domain and is required for tube formation, suggesting that it targets vesicles needed for lumen extension. Here, we identify a polarity pathway that promotes intracellular tube formation by enriching the exocyst at the lumenal membrane. We show that the PAR polarity proteins PAR-6 and PKC-3/aPKC localize to the lumenal membrane domain and function within the excretory cell to promote lumen extension, similar to exocyst component SEC-5 and exocyst regulator RAL-1. After acute protein depletion using the ZF1 degron, we find

that PAR-6 is required to recruit the exocyst to the lumenal membrane domain, whereas PAR-3, which functions as an exocyst receptor in mammalian cells, appears to be dispensable for exocyst localization and lumen extension. Finally, we show that the Rho GTPase CDC-42 and the RhoGEF EXC-5/FGD act as upstream regulators of lumen formation by recruiting PAR-6 and PKC-3 to the lumenal membrane. Our findings reveal a molecular pathway that connects Rho GTPase signaling, cell polarity, and vesicle-tethering proteins to promote lumen extension in intracellular tubes.

199A The dynamic partnered dance between PLK-1 and MEX-5: interpreting gradient formation with computational modelling.

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In *C. elegans* embryos, PLK-1 is pivotal for cell division and it orchestrates polarity establishment together with its binding partner, MEX-5. To achieve this, their localization/activity must be precisely regulated. MEX-5 enrichment at the anterior cytoplasm results from a change in its diffusivity following uneven phosphorylation along the embryo axis. We know PLK-1 relocalization to the anterior depends on MEX-5. However, the biological and physical mechanisms behind the dynamics of this protein are still poorly described.

To address this, PLK-1 and MEX-5 gradient formation was measured in two CRISPR strains and significant discrepancies were revealed between the two proteins in terms of: 1) gradient steepness, as PLK-1 forms a less steep gradient compared to MEX-5; 2) dynamics, with PLK-1 gradient establishment delayed and slower; 3) diffusivity, as PLK-1 diffusion coefficient does not correspond to MEX-5's one from anterior to posterior.

To shed light on PLK-1 dynamics, and how it is intertwined to MEX-5, we developed a novel Monte Carlo simulation framework able to recreate the protein motions in the *C. elegans* one-cell embryo. Thanks to our computational approach, we were able to postulate on the biological mechanisms behind MEX-5 and PLK-1 dynamics during the whole cell division, from early embryos to the steady-state before cytokinesis.

The simulations succeed in reproducing PLK-1 gradient formation, in agreement with experimental measurements, if: 1) PLK-1 binds to phosphorylated MEX-5; 2) the binding is triggered after a defined time delay; 3) PLK-1 dynamically interacts with MEX-5, leading to a continuous replenishment of a pool of unbound PLK-1.

The Monte Carlo framework we propose can eventually be applied to other polarity-related factors or mutants in which polarization is perturbed, to understand if it can be traced back to a failure in PLK-1 localization. Finally, conditions where gradient formation is altered, like after stress, can be simulated.

200B A GSP-2/PKC-3 balance is required for polarity establishment in *C. Elegans*

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Cell polarity is a fundamental property of many cells and proper establishment of polarity is required for the development of organisms and for the function and integrity of cells and tissues. The one-cell *C. elegans* embryo is polarized along the anterior-posterior axis and this is essential for asymmetric cell division, a specialized division that results in two cells with a different size and fate.

PAR proteins, a network of highly dynamic proteins with scaffold and/or enzyme function, are the main actors involved in cell polarity in the one-cell *C. elegans* embryos.

Just after the fertilization, the anterior PAR proteins (aPARs), PAR-3/PAR-6/PKC-3, are uniformly distributed at the cortex, whereas the posterior PAR proteins (pPARs), PAR-1/PAR-2, are in the cytoplasm. Uniform cortical PKC-3 phosphorylates PAR-2 inhibiting its posterior localization and polarity establishment. A cortical flow, triggered by the sperm, initiates the segregation of the aPARs to the anterior side of the embryo and liberates the posterior pole, allowing the loading of PAR-2 (and PAR-1) at the posterior. How PAR-2 is relieved from PKC-3 phosphorylation to ensure PAR-2 cortical localization and hence polarity establishment is not known.

We find that the PP1 phosphatase GSP-2 antagonizes PKC-3 phosphorylation, allowing PAR-2 posterior cortical localization and polarity establishment. Depletion of GSP-2 via RNAi rescues the PAR-2 localization in the *pkc-3 (ts)* strain, suggesting that PKC-3 and GSP-2 are involved in the same process, but with opposing function. Moreover, mutation of the PP1 binding site

in PAR-2 abolishes the interaction between PAR-2 and GSP-2 in the Y2H system. We are now introducing the mutation in the endogenous PAR-2 to investigate its localization in the *C. elegans* embryo.

Our data unravel how the phosphorylation state of PAR-2 is properly balanced in the one-cell *C. elegans* embryo to ensure robust establishment and maintenance of polarity.

201C Intestinal-rectal valve cells form an epithelial bridge between two different tissues

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Epithelial cells are specialized to protect our bodies and tissues and interface with the outside environment. During both development and malignant transformation into carcinomas, a crucial yet poorly understood facet of epithelial cell biology is how different epithelial tissues connect to and align with their neighboring epithelial tissues. For circulating cancer cells, productive attachment to foreign tissues is a rate-limiting step in metastasis formation. For the developing luminal epithelia that line internal organs, forming an epithelial bridge to the epithelia on the outside of the body is necessary for viability. In particular, alignment of the apicobasal axis and cell-cell adhesion complexes between neighboring cells is essential. The development of the *C. elegans* digestive tract involves the formation of such epithelial bridges, including the two rectal valve cells that bridge between the intestinal and rectal cells that derive from two different lineages (E and ABp). This connection between the intestine and the rectum completes a toroid of epithelial cells that encases and protects the worm. Despite their importance in forming the connection between the developing intestine and the invaginating rectal epithelium, little is known about the cellular organization and morphogenesis of the rectal valve cells. My preliminary work suggests that the polarization of apicobasal proteins within the valve cells may be key to forming this epithelial bridge. During their morphogenesis, valve cells possess one anterior puncta and one posterior puncta of conserved apical proteins, which were previously unable to be visualized separately from surrounding intestinal and rectal signal. Ablation of the posterior intestinal cells disrupts the formation of the anterior valve cell apical puncta. In addition, the activity of the conserved adhesion protein HMR-1/E-cadherin, but not components of the apical PAR complex, specifically within the posterior intestinal cells is required to form the anterior valve cell apical puncta. I am further exploring the hypothesis that intestinal adhesion complexes instruct valve cell polarization. In addition, I am currently generating tools to enable better cell-type specific protein depletions within the intestine and different populations of rectal cells with the aim of testing how polarity and adhesion complexes within these cells affect their epithelial neighbors. Together, these data will improve our understanding of epithelial cell organization in different *in vivo* contexts ranging from morphogenesis to tubule formation, from metastatic colonization to wound healing. How spatial information derived from the polarization of one epithelia tissue is able to instruct the polarization of another, neighboring epithelial tissue has broad implications for explaining how epithelial neighbors meet and align to form functional organ systems.

202A Positioning of organelles during the polarization of intestinal epithelial cells

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During organogenesis of the *C. elegans* intestine, gut cells segregate the cell cortex, which includes both the plasma membrane and underlying cytoplasm, into distinct apical, lateral, and basal domains. Concomitant with this the cytoplasm becomes highly polarized; nuclei migrate and localize near the apical cell surface, while other organelles including gut granules, yolk platelets, lipid droplets, the Golgi, and mitochondria become basally polarized. By analyzing *unc-83(-)* mutants, which disrupt the apical migration of nuclei, we find that nuclear exclusion from the apical domain leads to the basal positioning of all of these organelles except for lipid droplets, whose polarization is independent of nuclear positioning. Organelles composing the conventional endosomal pathway, including early endosomes, late endosomes, and lysosomes are polarized apically via a process independent of nuclear migration. The Par polarity pathway controls the asymmetric distribution of organelles in the 1 cell embryo and the epithelial polarization of intestinal cells. However, *par-3(-)* mutants do not disrupt the apical polarization of nuclei and endosomes or the basal positioning of gut granules. We identified *wht-2(-)* in ongoing screens for mutants disrupting gut granule biogenesis, pisitionig, and morphology. In *wht-2(-)* mutants, gut granules become apically polarized, while the positioning of other organelles remains unchanged. RAB-7 function is required for the apical localization of gut granules in *wht-2(-)*, however gut granules in *wht-2(-)* mutants do not obviously take on characteristics of conventional endosomes. WHT-2 is a “half” ABCG transporter and we identify WHT-7 as a likely WHT-2 interacting protein and provide evidence that a WHT-2/WHT-7 heterodimer functions as a transporter to impact gut granule positioning. At the time that organelles become asymmetrically localized, *wht-2(-)* mutants disrupt the accumulation of GLO-1 on gut granules. Ectopic

expression of GLO-1(+) and a GLO-1(GTP-locked) point mutant, but not a GLO-1(GDP-locked) point mutant, suppresses gut granule mispositioning in *wht-2(-)* and *wht-7(-)* mutants. The targeted degradation of GLO-1 leads to the apical mislocalization of gut granules, consistent with this Rab functioning in the polarized distribution of gut granules.

203B Investigating the symmetry breaking cue and mechanism of polarity reestablishment in the *C. elegans* P1 cell.

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Asymmetric cell division is the process in which one cell divides to give rise to cells with different cell fate determinants. This process is conserved throughout animals and is important for generating different cell types. To divide asymmetrically, a cell must first polarize. In many organisms, the polarity axis is established by the PAR proteins, which accumulate in distinct mutually exclusive domains in response to a symmetry breaking cue. In the one-cell *C. elegans* embryo, the centrosome-associated Aurora A kinase, AIR-1, is the cue that specifies the posterior region of the cell. AIR-1 inhibits actomyosin contractility, resulting in cortical flow from the posterior to the anterior. This flow moves the anterior PARs (aPARs), which are initially uniform around the cortex, towards the anterior pole. Posterior PARs (pPARs) then move onto the posterior cortex. Polarity establishment and maintenance at the one-cell stage have been well studied, but how polarity is reestablished in the P1 and subsequent germline cells is still unknown. The P1 cell begins with pPARs all around the cortex, and then they must clear from the anterior of the cell. Prior work revealed a movement of the nuclear centrosome complex towards the posterior of the P1 cell and anteriorly directed actomyosin flow, suggesting that P1 polarity establishment could involve a similar mechanism as seen in the one cell. To test this possibility, we are using live imaging of fluorescently tagged proteins to determine when the pPARs clear from the anterior of the cell in relation to where the centrosome is located. Our preliminary results suggest that the pPAR domain forms before the nuclear-centrosome complex moves posteriorly. We are now comparing the kinetics of pPAR domain formation to the timing of actomyosin flow. We also plan to use a fast-inactivating temperature sensitive mutant for non-muscle myosin to directly test the role of actomyosin flow in polarity in the P1 cell. As a first step to determine if AIR-1 could be the polarity cue in the P1 cell, we examined AIR-1::GFP localization and found that AIR-1 is present on the centrosomes early in the P1 cell cycle, when the pPAR domain is forming. We are currently using temperature sensitive mutants that inhibit centrosome maturation to try to deplete AIR-1 from the centrosome and test for effects on symmetry breaking in the P1 cell.

204C Growth Cone-Localized Microtubule Organizing Center Establishes Microtubule Orientation in Dendrites

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A polarized arrangement of neuronal microtubule arrays is the foundation of membrane trafficking and subcellular compartmentalization. Conserved among both invertebrates and vertebrates, axons contain exclusively “plus-end-out” microtubules while dendrites contain a high percentage of “minus-end-out” microtubules, the origins of which have been a mystery. Here we show that in *Caenorhabditis elegans* the dendritic growth cone contains a non-centrosomal microtubule organizing center, which generates minus-end-out microtubules along outgrowing dendrites and plus-end-out microtubules in the growth cone. RAB-11-positive endosomes accumulate in this region and co-migrate with the microtubule nucleation complex γ-TuRC. The MTOC tracks the extending growth cone by kinesin-1/UNC-116-mediated endosome movements on distal plus-end-out microtubules and dynein clusters this advancing MTOC. Critically, perturbation of the function or localization of the MTOC causes reversed microtubule polarity in dendrites. These findings unveil the endosome-localized dendritic MTOC as a critical organelle for establishing axon-dendrite polarity.

205A The BAG2 co-chaperone UNC-23 regulates amphid sensory morphology

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Cell shape and cell-cell contacts are established during development and then maintained through animal life. More specifically, coordinated multi-tissue regulation is needed for proper organ function, but how this is maintained across cell types is not well understood. To study cell-shape maintenance, we focused on a single neuron in C. elegans, the AFD thermosensory neuron. The neuron receptive ending (NRE) of the AFD is made of many microvilli and a single cilium, both of which are essential for neuron activity. A forward genetic screen for mutants with defective AFD-NRE shape identified a role for UNC-23, the BAG2/Hsp70 co-chaperone. *unc-23* mutants have overgrown AFD NRE, a phenotype we term meander. These defects begin as the animal reaches adulthood and are progressive with animal age.

The AFD NRE is embedded in the surrounding AMsh glia apical end and we have found similar meander of this apical ending and other glia associated NREs. Intriguingly, cell-specific rescue studies revealed that UNC-23's function is required not in AFD or AMsh, but non-autonomously in the overlying epidermis. Thus, regulation of proteostasis in skin helps maintain sensory glia-neuron shape.

Growth of *unc-23* mutants in liquid culture and mutations that result in loss of movement both rescue the AFD NRE meander. This suggests that epidermal UNC-23 is dependent on external forces experienced by the worm. Further, *unc-23* mutants have expanded epidermal and AMsh apical domains as well as aberrant positioning of junctional markers, AJM-1 and DLG-1. Additionally, the basolateral α-integrin, INA-1, is lost from the anterior head region. Finally, mutations in the FGF receptor, EGL-15, or ligand, EGL-17, suppress *unc-23* NRE defects. Since integrins regulate apical polarity and FGF signaling, we favor the model where integrin-dependent epidermal polarity and mechanical tension dictates associated glia-neuron shape.

Intriguingly, we also found that reduced AFD neuron activity causes meanders and increasing AFD activity rescues the *unc-23* meandering defects. Thus, our working model is that skin UNC-23 dictates epidermal apicobasal polarity, causing altered neuron activity and thereby glia-neuron shape. These findings make the exciting implication that coordinated proteostasis maintenance of epidermal polarity regulates associated glia-neuron shape and sensorineural decline with aging

206B Global regulation of cell polarization by two Wnt receptors, Frizzled/MOM-5 and Ror1/CAM-1 in *C. elegans* mid-stage embryo

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Asymmetric cell division contributes to cell diversity in embryos and the maintenance of various stem cells, and hence, is critical for making multicellular organisms. Asymmetric cell division requires cellular polarization which is often regulated by extracellular signals including Wnt proteins. However, exact roles of Wnts in cell polarity remain elusive.

In *C. elegans* the mid-stage embryo, blastomeres stereotypically and sequentially divide along the anterior-posterior axis. During the divisions, TCF/POP-1 protein, the downstream transcription factor of the Wnt pathway, accumulates higher in the anterior than the posterior daughter cell nuclei. The polarization in these asymmetric divisions occurs globally, including the blastomeres far from the Wnt source cells. Although the previous study using isolated blastomeres shows the involvement of the Wnt ligand, MOM-2, and the Frizzled type receptor, MOM-5 in establishing POP-1 polarity (Park & Priess 2003). Since mom-2 and mom-5 intact embryos show normal POP-1 polarity, however, their roles in mid-stage embryos are not clear.

In this study, we found the POP-1 polarity was not established in the *mom-5* null mutant with the simultaneous knocking down of CAM-1 that is the ROR type Wnt receptor. Further, we performed the precise deletion of the Wnt-binding domain (CRD) of MOM-5 by genome editing. In the cam-1 mutant lacking CRD of MOM-5, we found that polarity orientation is randomized, unlike double receptor-deficient embryo that shows loss-of polarity. The results suggest that the intracellular domain of mom-5 facilitates the establishment of the polarity, and the Wnt ligands control polarity orientation. We will discuss how the Wnt receptors establish the cell polarity by interaction with the ligands in the mid-stage embryo.

207C PP1/SDS-22 phosphatase is required for germ plasm segregation in the one-cell *C. elegans* embryo

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We are interested in understanding the mechanisms that control the polarization of cytoplasmic factors during asymmetric cell division. During the asymmetric division of the *C. elegans* zygote, the RNA-binding protein MEX-5 recruits PLK-1 kinase to the anterior cytoplasm (Nishi, 2008). In turn PLK-1 phosphorylates the RNA-binding protein POS-1, inhibiting POS-1 retention in the anterior cytoplasm (Han, 2018). As a result, POS-1 is retained and accumulates in the posterior cytoplasm, likely reflecting the activity of a phosphatase that counteracts PLK-1 phosphorylation.

Through an RNAi screen to identify the phosphatase that enables POS-1 retention in the posterior, we identified the catalytic subunits (GSP-1 and GSP-2) and a regulatory subunit (SDS-22) of PP1 phosphatase. In *sds-22(RNAi)* embryos, MEX-5, PLK-1 and upstream PAR polarities are established normally. In contrast, POS-1 retention in the posterior cytoplasm is reduced in *sds-22(RNAi)* embryos, causing a weakening and delay in POS-1 segregation. Strikingly, we find that SDS-22 is similarly required for PIE-1 segregation and for P granule formation/stability, suggesting to a central role in germplasm organization. Whereas

PLK-1 is enriched in the anterior, endogenously-tagged SDS-22::GFP is symmetrically distributed throughout the cytoplasm. We propose that PLK-1 kinase and PP1/SDS-22 phosphatase act in opposition to control germ plasm retention and that spatial variation in their relative activities drives the segregation of factors such as POS-1 to the posterior cytoplasm.

208A PAR-3 independent mechanisms contribute to apico-basolateral polarity establishment in the embryonic *C. elegans* intestinal epithelium

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Apico-basolateral polarization is essential for epithelial cells to function as selective barriers and transporters and to provide mechanical resiliency to organs. Different epithelia use divergent mechanisms to establish polarity, which are only beginning to be understood. Here, we use the *C. elegans* embryonic intestine to investigate how epithelial cells establish apico-basolateral polarity *in vivo*. PAR-3 was previously identified as the most upstream polarity regulator in this organ (Achilleos et al 2010), but worms arrested in embryonic stages due to the essential role of PAR-3 in other tissues, precluding study of the role of intestinal PAR-3 for later developmental stages. Therefore, we depleted PAR-3 specifically from the embryonic intestine (PAR-3$^{gut(-)}$) without affecting the function of PAR-3 in other tissues. PAR-3$^{gut(-)}$ worms all arrested at the L1 larval stage, with edematous intestines that lacked continuous lumens. Consistent with prior work, we found that PAR-3 is required for the localization of the apical kinase, PKC-3, in the intestine. However, proteins associated with the apical actin brush border still localize in discreet patches in PAR-3$^{gut(-)}$ embryos. Adherens junction proteins (HMR-1, AFD-1) organize into rings in PAR-3$^{gut(-)}$ embryonic intestines, and the septate-like junctional protein, DLG-1, formed similar rings slightly later in embryogenesis. Junctional proteins often encircled the actin-associated patches, indicating that some aspects of apical-basolateral polarity are established in the absence of PAR-3. Additionally, while PAR-3 is required for the localization of the basolateral protein, LGL-1, the basolateral protein, LET-413/Scribble is still excluded from specific regions of the cell membrane in PAR-3$^{gut(-)}$ intestines. Together, these data are consistent with the existence of a PAR-3 independent apical-basolateral establishment pathway. In other tissues, adherens junction proteins play upstream roles in apico-basolateral polarity establishment. To determine if junctional proteins act in parallel with PAR-3, we are currently depleting adherens junction proteins specifically from the intestine alone or in combination with PAR-3 depletion. While aspects of apico-basolateral polarity are established in PAR-3$^{gut(-)}$ worms, a continuous apical surface is never established, suggesting that PAR-3 is required for coordinating polarity establishment across the intestinal tissue.

210C Single-embryo expression-phenotype mapping reveals highly canalized response of asymmetric division to perturbation of PAR protein balance

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C. elegans embryogenesis relies on the ability of early blastomeres to polarize and divide asymmetrically to specify distinct cell fates. Cell polarity is best understood in the zygote, where PAR proteins orchestrate a characteristic asymmetric division that results in a reproducible difference in the size and fate of daughter cells AB and P1. PAR polarity depends on the balance between two antagonistic sets of PAR proteins – anterior (a)PARs and posterior (p)PARs. These competing sets of proteins segregate into two distinct domains on the plasma membrane prior to cell division through a mutually antagonistic feedback. As predicted by this competition model, RNAi and genetic suppression experiments show that polarity is sensitive to the relative balance between aPARs and pPARs. Yet, at the same time, *par* heterozygotes are viable, suggesting that mechanisms must exist to ensure robust development in the face of gene dosage changes. We hypothesized that this robustness could arise either through the homeostatic control of PAR protein levels (i.e. dosage compensation), through an intrinsic robustness of the polarity network itself or in how PAR polarity is interpreted by downstream pathways. One challenge in testing these hypotheses is the inability to directly assess protein abundance in individual zygotes. We have developed a simple microscopy-based method for protein dosage quantification in embryos expressing fluorescently-tagged proteins, enabling direct protein abundance-to-phenotype mapping on an embryo-to-embryo basis. Our data argue against dosage compensation as an explanation for robustness to perturbations in gene expression. Rather, our data reveal a highly nonlinear dosage-phenotype relationship such that network output is robust to changes in PAR protein balance and further suggest that robustness is encoded at multiple levels of the asymmetric division programme.

211A Epithelial apical/basal polarity requires WAVE-dependent transport of E-Cadherin/HMR-1

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We are developing the adult *C. elegans* intestine as a system to study how branched actin polarizes Cadherin distribution and dynamics. Since changes in Cadherin distribution and membrane enrichment underlie morphogenesis, and the progression of diseases, including cancers, identifying mechanisms for Cadherin membrane polarization is an essential goal. Surprisingly, it is still controversial whether or not branched actin is needed to promote trafficking events at mature epithelia, and the nature of the forces driving Cadherin transport are not described. Here we investigate a mechanism for Cadherin regulation by testing our model that the branched actin regulator, WAVE, promotes polarized Cadherin protein transport. Our results show WAVE regulates Cadherin accumulation at recycling endosomes. Endogenously tagged WAVE localizes at or near to RAB-10, RAB-11, and RME-1 endosomal proteins, and in subsets of the Golgi ministacks. Further, live imaging demonstrates that recycling endosomes undergo dynamic apicobasally directed movements, and that these movements and distribution depend on branched actin. This *in vivo* analysis of Cadherin distribution and endosome association strongly suggests that the WAVE complex has an under-appreciated role in cell-cell adhesion and polarity through the promotion of Cadherin transport, including transport into and through recycling endosomes.

212B Identification of aPKC substrates and interactors in the early *C. elegans* embryo to elaborate a model for anterior PAR protein cooperation

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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 only signaling component of the anterior domain is the kinase, aPKC. Our lab has found that making an anterior domain of active 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.

To identify aPKC's substrates and interactors, we plan to use a combination of analogue sensitive kinase assays, phosphoproteomic profiling, and BioID. Once we have generated a list of candidate substrates and interactors, we shall confirm that they have roles in embryo polarization by RNAi knockdown. For candidates with roles in the embryo, phosphomimetic and non-phosphorylatable mutants will be generated for selected substrates. We will analyze these mutants for alterations to aPKC's interaction with the localization and activation complexes.

Identification of aPKC's substrates and interactors will find mechanisms responsible for the dynamic interaction between the localization and activation complexes, which is key for the polarization of the embryo. Also, we will identify aPKC substrates and interactors governing other aspects of polarity and embryonic development, providing a rich resource for future study.

213C The *bli-4*/proprotein convertase genetically interacts with *pmr-1*/calcium ATPase during cell migration in *Caenorhabditis elegans*

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pmr-1 encodes a Secretory Protein Calcium ATPase required for migration of cells on the embryo surface in *C. elegans* (Praitis, et al 2013). To identify other genes involved in cell migration, we carried out an RNAi suppressor screen. We found that *bli-4(RNAi*) significantly suppresses embryonic lethality in *pmr-(ru5)* mutant lines. BLI-4 is a calcium-dependent proprotein convertase gene that functions in cuticle development (Thacker, et al 2000). While we found neither *bli-4(e937)* nor *bli-4(RNAi)* had embryonic phenotypes, Thacker, et al (2000), showed that *bli-4(h520),* which carries a genetic change that disrupts a predicted substrate binding site, is an embryonic lethal allele. When we examined *bli-4(h520)* embryos, we found phenotypes, including displaced cells, that were similar to *pmr-1(ru5)* embryos. To further explore the genetic interaction between the genes, we crossed the *pmr-1(ru5)* into an sDp2 balanced *bli-4(h520)* line. The sDp2 balanced *bli-4(h520)* and *pmr-1(ru5)* double mutant line showed slight, but not significantly increased viability compared to the single mutant lines. While both *pmr-1(ru5)* and *bli-4(h520)* are embryonic lethal alleles, we obtained sick but viable individuals homozygous for both alleles that had lost sDp2, confirming an interaction between the two genes. To better understand how the two genes interact, we examined *bli-4* expression patterns in embryos. Fluorescence studies showed *bli-4*::GFP is expressed in leading cells during enclosure and in several other unidentified cells. We utilized the Packer, et al (2019) embryonic transcriptome database to examine the endogenous expression of *bli-4* RNA in developing embryos. While we did not observe significant changes in gene expression

in cells undergoing surface migrations, we discovered that *bli-4* is upregulated in many of these lineages once migration events are complete. Our current model is that *pmr-1* activity is critical for migratory behavior and that *bli-4* expression may be important for the transition of cells from migratory to epithelial. Because defects in *pmr-1* reduce migration rates, delays in the migratory-to-epithelial transition caused by disruption of *bli-4* could permit additional time for cells to be properly positioned during embryogenesis resulting in restored viability.

214A Identifying the In Vivo Role of Non-centrosomal Microtubule Organizing Centers During Cell Migration

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The microtubule (MT) cytoskeleton plays key roles in cell migration via intracellular signaling and modulating cell adhesions and polarity. Within a cell, MTs emanate and grow from microtubule organizing centers (MTOCs), and the localization of an MTOC helps drive specific MT functions. In cultured migratory cells, the MTOC is canonically located at the centrosome with MTs growing towards the leading edge of the cell. in vivo cell migration studies, however, have identified variations in the MTOC site and MT organization, such as MTs radiating towards the trailing edge of the cell from the centrosome, or a broad MTOC at the trailing edge with MTs radiating towards the leading edge. The molecular factors governing MTOC site, and the impact of MT organization on the migratory functions, in vivo is unclear. During C. elegans development, the Sex Myoblasts (SM) are a pair of cells that undergo a long distance migration to the gonad. Utilizing the SM cells as an in vivo model, we are investigating the presence and function of non-centrosomal MTOCs in cell migration via live confocal imaging of intact animals. Through SM-specific, temporal and spatial MT degradation, we have found an essential role for MTs in SM cell migration. Upon MT degradation, the SM cells fail to fully migrate to the gonad. In addition, directional analysis of growing MTs has identified arrays of MTs running parallel to migration and a MTOC localized near the leading edge of the cell. In contrast to the canonical MT organization, this leading edge MTOC does not appear to be associated with the centrosome. These results highlight a MTOC and pattern of MT organization not typically associated with migratory cells. Further investigation into the significance of the MTs at the leading edge in association with cell adhesions and polarity proteins will provide insights into the mechanisms governing migration in vivo.

215B Perturbed intermediate filament regulation causes aggregate toxicity

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Intermediate filaments are major components of the metazoan cytoskeleton. A long-standing debate concerns the question whether intermediate filament network organization only reflects or also determines cell and tissue function and dysfunction. This is particularly relevant for aggregate-forming diseases involving intermediate filaments. Using *C. elegans* as a genetic model organism, we have recently described mutants of signaling and stress response pathways with perturbed intermediate filament network organization. In a mutagenesis screen, we now identify the intermediate filament polypeptide IFB-2 as a highly efficient suppressor of these phenotypes restoring not only intestinal morphology but also rescuing compromised development, growth, reproduction and stress resilience. Ultrastructural analyses show that downregulation of IFB-2 leads to depletion of the aggregated intermediate filaments. The findings provide compelling evidence for the toxic function of deranged intermediate filaments and reveal novel insights into the cross talk between signaling and structural functions of the intermediate filament cytoskeleton.

216C The RGD (Arg-Gly-Asp) is a potential cell-binding motif of UNC-52/PERLECAN mediating interaction to βPAT-3 integrin

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In *C. elegans*, the *unc-52* gene encodes for the protein UNC-52/perlecan, which is a basement membrane proteoglycan that plays a role in cell attachment, organ formation, and cell motility. The null mutation of *unc-52* causes severe embryonic arrest and lethality. Within the UNC-52 protein, two RGD (Arg – Gly – Asp) motifs are located at the 748 amino acid position of exon 7 and the 2023 amino acid position of exon 19. The RGD motif is a known potential cell-binding site for β integrins. However, the complete role of RGD in *unc-52* has not previously been characterized. In this experiment, the ^{746}RGD^{748} and ^{2021}RGD^{2023} motifs had two separate mutations performed on each motif: mutation of the RGD to RGE and deletion of the entire RGD motif. Both the RGD to RGE mutation and the deletion of the RGD motif in RGD^{748} resulted in minor motility defects. However, the RGD to RGE mutation in RGD^{2023} resulted in a severe Unc phenotype, and the RGD deletion caused a Pat phenotype, which is

the same phenotype caused by *pat-3* β-integrin mutations. Further analysis revealed that the RGD to RGE mutation in RGD^{2023} also caused defective actin filaments and disrupted localization of PAT-3 β integrin and TLN-1/talin. A double mutant for the RGD to RGE mutations of RGD^{748} and RGD^{2023} was created to further examine the role of each RGD motif. Double mutants showed significantly decreased motility compared to either single mutant and also showed additive effects on the disrupted localization of *pat-3* β integrin. Since RGD^{748} mutations failed to produce severe defects, it is likely that this RGD is either not a cell-binding motif or is an alternative binding site. However, the severity of the defects caused by both mutations to RGD^{2023} suggests that this RGD motif is essential to UNC-52 function. Current work is being performed to rescue the lethal RGD deletion in RGD^{2023}. Additional work will be performed to add cell-binding domains from human RGD containing proteins to the *unc-52* RGD domains.

217A The mutation analysis of RGD (Arg-Gly-Glu) cell-binding motifs in the nematode *Caenorhabditis elegans*

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The interaction between integrin receptor and the extracellular matrix (ECM) proteins is essential for tissue organization. The mammalian system showed that RGD (Arg-Gly-Asp) motifs are the cell-binding sites of ECM proteins and known to interact with cell surface receptors of the ECM, integrins. These RGD motifs have shown to play essential roles in cell-matrix interaction, but the role of the RGD motifs beyond the mammalian system was not very well understood. The *Caenorhabditis elegans* genome contains approximately three thousand RGD motif-containing proteins, but of these proteins, ten were identified as containing RGD motifs in the basement membrane, a special ECM structure. These ten genes are DIG-1, LON-2/glypican, *B0393.5*/SNED1, CLE-1/collagen XVIII, EMB-9/collagen type IV, HIM-4/hemicentin, LAM-3/laminin α, LET-2/collagen IV, NID-1/nidogen, and UNC-52/perlecan. To study the role of the RGD motifs in the basement membrane proteins, we created mutations in the RGD motifs in these ten genes using the CRISPR-Cas9 system. To date, the RGE (Arg-Gly-Glu) mutations in *him-4*/hemicentin failed to show a discernible behavioral or morphological phenotype. The effects of deleting the RGD motifs in *lam-3*/laminin α and *nid-1*/nidogen also failed to show significant defects other than mild motility defects. A similar approach on the other genes as mentioned above is currently underway. Through the study of RGD mutations in *C. elegans* extracellular matrix binding proteins, we hope to gain further knowledge as to the role of the RGD motif in *C. elegans* and other invertebrate species

218B Nuclear lamina cooperates with inner nuclear membrane proteins to counteract LINC-mediated forces during oogenesis in *C. elegans*

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The nuclear lamina is essential to protect genome integrity from mechanical stress. This requirement is more stringent in some tissues and developmental events. During oogenesis, meiotic nuclei are situated in a stressful environment filled with cytoskeletons. The nuclear lamina, consisting of the lamin proteins, is a conserved component of the nuclear envelope that can confer mechanical rigidity to the meiotic nuclei. *C.elegans* expresses a single lamin protein, LMN-1, which is similar to mammalian B-type lamin. Loss of LMN-1 results in near-complete sterility, with hypercondensed chromatin observed in many germline nuclei. The exact functions of LMN-1 in meiotic nuclei during oogenesis, however, remains unclear.

Using the auxin-inducible degradation system, we found that acute depletion of LMN-1 in *C.elegans* germline recapitulated nuclear collapse seen in *lmn-1* homozygotes during late stages of meiotic prophase. LMN-1 depletion also led to persistent DNA double strand breaks and elevated apoptosis, but germline apoptosis is neither sufficient nor required for nuclear collapse. We further observed prolonged and excessive clustering of the LINC complex proteins SUN-1 and ZYG-12 at the nuclear envelope (NE) upon LMN-1 acute depletion. Importantly, co-depletion of SUN-1 or ZYG-12, or inhibition of dynein-mediated forces, rescued the nuclear collapse triggered by acute LMN-1 depletion. By contrast, co-depletion of the inner nuclear membrane proteins EMR-1/LEM-2 or SAMP-1 rendered nuclei susceptible to collapse even earlier, at the time of meiotic entry. Live imaging demonstrated that shrinkage of the NE preceded chromosome hypercondensation during nuclear collapse, and that before NE shrinkage happened, LINC complex asymmetrically redistribute to one side of the NE in a dynein-dependent manner. Finally, the connection between the pairing center regions of the chromosomes and the NE, albeit being important for LINC complex function during homolog pairing, is dispensable for nuclear collapse caused by LMN-1 depletion. Together our results suggest that lamin cooperates with additional inner nuclear membrane proteins to protect meiotic nuclei

from collapse by antagonizing forces exerted by dynein and transmitted through the LINC complex during oogenesis. Our work has also established an inducible system for modeling laminopathy.

219C MTOC function at the centrosome and the ciliary base is driven by specific PCM protein

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During development, the centrosome acts as a microtubule organizing center (MTOC) in mitotic cells, forming radial arrays essential to separate cellular components between daughter cells. Microtubules are organized at the centrosome by pericentriolar material (PCM) complexes. After mitosis, during cell differentiation the fate of the centrosome is going to be diverse depending of cell fate. In differentiated epithelial cells, the centrosome is inactivated, losing its PCM and having the MTOC function redirected to other cellular components, in contrast, other ciliated cells have the centrosome and its centrioles repurposed into basal bodies to ciliary structures. We are using *C. elegans* as a model to characterize MTOC recruitment and regulation at the centrosome. In *C. elegans*, the PCM is organized around the centrioles in a partial concentric overlay of protein spheres, in which the two main scaffolding protein, SPD-2/CEP192 and SPD-5 which localize the microtubule nucleating complex ɣ-TuRC to the centrosome, partially overlap delimiting two main body – an inner sphere with both partner and an outer sphere with SPD-5 (Magescas et al 2019). Upon differentiation centrosome lose their PCM, leaving 'naked' centrioles, like in intestinal cells. Interestingly, analysis of SPD-5 and ɣ-TuRC proteins revealed that ciliated sensory neurons, SPD-5 and most MTOC proteins remains at the ciliary base while centriolar protein and SPD-2 are lost. Those complexes organize the MTOC function at the base of cilia and are critical for ciliogenesis, as depletion of SPD-5 produces aberrant cilia. Interestingly, contrary to the current model, similar loss of SPD-2 in cycling intestinal cells prior to differentiation doesn't result in the loss of SPD-5 at the centrosome, nor it impairs centrosomal function. Based on our data we propose that the PCM is composed of different subcomplexes revolving around SPD-5 that are differently regulated, working in parallel to drive the MTOC function.

220A Study of the relation between molecular content, actin architectures and cell identity throught C. *elegans* early embryogenesis

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C. *elegans* embryogenesis follows a well-defined developmental pattern in which cell polarization, asymmetric or symmetric divisions or intercellular signaling events give rise to a collection of differentiated blastomeres. In the team, we rather focus on the specific role of actin architectures during cell identity acquisition. Actin is organized in a variety of functional structures such as the thin actomyosin cortex attached to the plasma membrane, cytokinesis rings or filopodia. The regulation of these actin networks is orchestrated by a plethora of Actin Binding Proteins (ABPs) that change the 3D organization and the dynamics of these specific structures. Their physical properties can affect the process of cell commitment and gene expression profiles and can therefore feedback into cell fate acquisition.

My project main objective is to reveal cell specific actin content and organization in the early *C. elegans* embryo. Published single cell transcriptomics data in the early embryos already show cell content singularities for some ABPs. On our side, we aim to verify at the protein level these observations and answer the following questions: Are ABPs equally distributed between sister cells? How does these protein imbalances impact actin architectures? Are the actin cortical properties inheritable? How is the actin nucleation machinery spatiotemporally controlled? The long-term goal is to show how these actin related differences impact cell commitment in the early embryo.

With the help of CRISPR GFP Knock-In strains, Spinning Disk Microscopy and quantitative image analysis, I am following two of the mains actin nucleators (Arp2/3 complex and the formin CYK-1) which have inter and intra cell-specific distribution patterns along the lineage. I also focus on specific subcellular structures the filopodia. One of our first goal is to question whether all cells are equal in terms of actin related content and nucleation capacity.

221B Distinct properties of broadly-expressed and tissue-specific tubulin isotypes examined by ectopic and heterologous expression

Kei Nishida[1], Kenta Tsuchiya[1,2], Yu Honda[1], Hiroyuki Obinata[1], Shizuka Onodera[1], Nami Haruta[1], Masanori Ikeda[3], Kozo Tanaka[3], Asako Sugimoto[1] 1) Laboratory of Developmental Dynamics, Graduate School of Life Sciences, Tohoku University; 2) Graduate School of Science, Nagoya University; 3) Institute of Development, Aging and Cancer, Tohoku University.

Most organisms have multiple α- and β-tubulin isotypes that likely contribute to the diversity of microtubule (MT) functions. To understand the functional differences of tubulin isotypes in *Caenorhabditis elegans*, which has nine α-tubulin isotypes and six β-tubulin isotypes, we systematically constructed null mutants and GFP-fusion strains for all tubulin isotypes with the CRISPR/Cas9 system and quantitatively analyzed their expression patterns and levels in adult hermaphrodites. Four isotypes—a-tubulins TBA-1 and TBA-2 and b-tubulins TBB-1 and TBB-2—were expressed in virtually all tissues, with a distinct tissue-specific spectrum. Other isotypes were expressed in specific tissues or cell types at significantly lower levels than the broadly expressed isotypes. Thus, MTs in *C. elegans* are mainly composed of four broadly expressed tubulin isotypes, and incorporation of a small amount of tissue-specific isotypes may contribute to tissue-specific MT properties.

Three α-tubulin isotypes (TBA-5, TBA-6, TBA-9) and one β-tubulin isotype (TBB-4) were expressed in different subsets of ciliated sensory neurons. When each of the ciliated neuron-specific isotypes was ectopically expressed in early embryos, they were less efficiently incorporated into mitotic spindle MTs than broadly-expressed isotypes and affected MT dynamics. The low incorporation efficiency was slightly improved when the ciliated neuron-specific α-tubulins were co-expressed with TBB-4, implying that some specific combinations of α- and β-tubulin isotypes form heterodimers preferably incorporated into MTs. The lower MT incorporation efficiencies of these ciliated neuron-specific tubulin isotypes were also observed by their heterologous expression in human cultured cells. Taken together, we speculate that a small amount of ciliated neuron-specific tubulins isotypes is incorporated into MTs mainly formed by broadly-expressed isotypes, which contribute to the cell-type-specific MT properties.

222C Kinetic Control of the Temporal Dynamics of a RhoA Signaling Cascade

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Cascades of activation are defined by a succession of sequential activation of signaling proteins. This leads to the activation of a downstream effector, with a precise intensity and timing, to control a specific biological function. Failure in proper timing generally leads to failure in setting up the biological function. Here, we propose to analyze the unfolding of a simple cascade in the early embryonic development of a metazoan, using the example of RhoA activation cascade as a canonical example. In this context, we can measure, at a specific location of the cell cortex, a stereotypical delay between the activation of the upstream regulator and the recruitment and activation of the downstream effector. First, we proceeded to a careful characterization of the dynamics of two sequential steps of the cascade. Using TIRF microscopy, we focused on the different steps of the RhoA activation cascade, using the Myosin as a landmark to measure the delay within the cascade at the cortex of *C. elegans* early embryos. Second, using single-molecule imaging, we focused on the last step of this cascade and measured the dynamic modulation of the binding (K_{on}) and the unbinding rate (K_{off}) of the Myosin. We then developed and, functionally challenged, a simple numerical model that takes advantage of the dynamic measurements of K_{on} and K_{off} to predict the temporal evolution of this cascade. We propose that this simple and generic model – which can in essence fit any activation cascade – offers a simple mathematical framework to understand the temporal dynamics of signaling cascades, and the delay and change in the shape of the response which can be observed between the input and the output of a cascade.

223A Probing formin FHOD-1 contributions to body-wall muscle structure and function

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In mammalian striated muscle, six actin-organizing formin proteins have been shown to influence sarcomere organization, but their precise molecular roles in this process remain obscure. In *C. elegans*, only the formin FHOD-1 works in a muscle cell-autonomous manner to influence sarcomere structure in body-wall muscle (BWM), providing a simpler system to determine the mechanism by which these highly conserved proteins influence the muscle cytoskeleton. Worms bearing a *fhod-1* allele that is a putative null for effects on actin organization display modest defects in BWM strength, cell growth and sarcomere assembly, arguing against a primary role in assembling the actin-based sarcomeric thin filaments. However, *fhod-1* muscle cells assemble fewer sarcomeres, and the muscle-specific myosin heavy chain MYO-3 is subject to elevated proteasome-dependent proteolysis. Moreover, the dense bodies that serve as thin filament-anchoring sites in BWM are malformed. Comparison of the distribution of immature and mature dense bodies in wild-type and *fhod-1* BWM cells, and the spatial relationship of FHOD-1 to those structures, suggests FHOD-1 accumulates at specific foci in the BWM cell to assist the formation of new dense bodies there.

224B Regulation of syncytial germline mechanics by the actin capping protein CAP-1

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Actin cytoskeleton remodelling is important for executing dynamic cellular processes during tissue morphogenesis and physiology. Actin filaments in the cytoskeletal network are dynamic structures that polymerise in a regulated manner. The actin filament barbed end, a hotspot of filament assembly, is regulated by competition among actin nucleators, elongation factors and capping protein, which in turn control filament length and network stability and is also capable of generating force by pushing against other structures. Although actin nucleation and elongation factors have been relatively well studied, the role of actin capping proteins *in-vivo* remains poorly understood.

We investigated the role of the actin capping protein CAP-1 in the *C. elegans* germline, a contractile tissue. Our observations show that endogenously tagged CAP-1 colocalises with the actin cytoskeleton along cell boundaries and rachis bridges in the germline. CAP-1 knockdown in the germline exhibits a disrupted structure characterised by a narrow and meandering rachis, as well as multinucleate germ cells and abnormal oocytes. Further, we observed an increase in levels of F-actin and also in the levels of the actin nucleators CYK-1 and ARX-2. In order to investigate if the structural changes observed in the rachis are accompanied by changes in contractility, we checked the levels of absolute and phosphorylated myosin II and observed their levels to be elevated in CAP-1 depleted background as compared with control, indicating an increase in contractility. The increase in contractility is likely responsible for the constricted and meandering rachis, pulling at the membranes and also resulting in multinucleate cells, in regions where the connecting membrane detached. In conclusion, we show that CAP-1 is essential for maintenance of germline function, it regulates actomyosin structure and levels to maintain the structural integrity of the germline.

225C The kinase *pig-1/MELK* is a conserved cytoskeletal regulator in *C. elegans* tubulogenesis and in human endothelial cells.

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Tubulogenesis, the process by which organisms form biological tubes, plays an integral role in blood vessel formation and function. Defects in tubulogenesis can lead to congenital vascular disease, and dysregulated tubulogenesis associated with tumor-induced angiogenesis promotes cancer progression. Kinases are critical regulators of conserved cellular functions, signal transduction pathways, and are attractive targets for therapeutic development. Therefore, finding kinases involved in vascular tubulogenesis is an important translational research goal. The *C. elegans* excretory canal (*ExCa*), a large single-celled tube, provides a tractable model to study tubulogenesis. Moreover, human orthologs of several genes that regulate *ExCa* tubulogenesis have been implicated in vascular development and disease. We performed an RNAi screen to find conserved kinase regulators of *ExCa* tubulogenesis and identified four that were not previously implicated in this process. Notably, we found that orthologs of these four kinases are expressed in human umbilical vein endothelial cells (HUVEC), a canonical model for studying angiogenesis *in vitro*, consistent with their possible role in vascular development (Socovich and Shaye, *in preparation*). We are focusing on the kinase *pig-1* and its human ortholog MELK, because despite the fact that little is known about its physiological function, MELK has been the focus of great interest due to it being highly upregulated in various aggressive cancers. Our work defines a novel mode of PIG-1/MELK regulation that is independent of canonical upstream activators like PAR-4/LKB and STRD-1/STRAD. We also discovered a new role for *pig-1* in regulating the conserved formin EXC-6/INF2 and cytoskeletal components (F-actin and microtubules) in the *ExCa*. Both MELK and INF2 are expressed in HUVEC, and shRNA-mediated knockdown of these proteins caused cell migration phenotypes consistent with cytoskeletal defects. Based on these results we propose a novel conserved PIG-1/MELK and EXC-6/INF2 pathway that regulates the cytoskeleton in tubulogenesis.

226A Nuclear deformation during P-cell nuclear migration

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Cell migration through constricted spaces is critical for developmental and disease processes, including immune cell intravasation and cancer metastasis, but is limited by nuclear deformation. During *C. elegans* development, larval P-cell nuclei migrate through a constriction between the muscle and cuticle that is ~5% of the resting diameter of the nucleus. Null mutations in the LINC pathway, consisting of SUN (*unc-84*), KASH (*unc-83*), and motors (dynein), disrupt about 50% of P-cell nuclear migrations at restrictive temperatures, but at permissive temperatures most P-cell nuclei migrate normally without LINC complexes. We hypothesized that additional pathways function parallel to the LINC pathway. In support of this

hypothesis, a forward genetic screen for *enhancers of the nuclear migration defect of unc-83/84* (*emu*) mutants identified *toca-1*, *cgef-1*, and *fln-2*. Knockdown of the Arp2/3 complex component *arx-3* in *unc-84* null mutants at the permissive temperature caused a significant nuclear migration defect. Under the same conditions, we observe a similar nuclear migration defect when *cdc-42* was knocked down, indicating that both the Arp2/3 complex and CDC-42 are required for nuclear migration in the absence of SUN-KASH. In our model, the F-bar domain protein TOCA-1 binds to the nuclear membrane and recruits CDC-42, a small G-protein which is activated by CGEF-1. CDC-42 in turn activates WAVE/WASP and Arp2/3 to generate branched actin, with the help of FLN-2 acting as either an actin bundler or crosslinker. We hypothesize that these proteins form an actin-based pathway that generates a network of branched actin to compress the nucleus. Additionally, the *cgef-1; unc-84; cec-4* triple mutant resulted in complete nuclear migration failure at the permissive temperature. 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. Altogether, these studies support a model where multiple pathways responsible for modulating nuclear deformation play important roles in nuclear migration through constricted spaces.

227B A good GEF gone GAP: investigating the mechanism that switches the Rac1/CED-10 GEF, CED-5/CED-12, into an inhibitor of F-actin formation during ventral enclosure

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Branched actin promotes cell migrations in the developing *C. elegans* epidermis that result in the movements of morphogenesis, including ventral enclosure. Our genetic screens identified the branched-actin promoting WAVE complex as the major activator of Arp2/3 to promote these movements. WAVE is activated by the small GTPase, CED-10/Rac1. Since branched actin regulators like WAVE must assemble at the correct time and place, one important question is how they are recruited. We therefore investigated the guanine exchange factors (GEFs) that activate Rac1/CED-10, which in turn activates WAVE and Arp2/3 during epidermal morphogenesis. Analysis of the proposed CED-10 GEF, CED-5/CED-12, homologous to DOCK-180/ELMO, uncovered a surprising role for this bipartite GEF. As expected, null alleles in this GEF resulted in reduced F-actin around corpses being engulfed by the embryonic epidermis. In contrast, these loss of function, null mutations resulted in elevated F-actin at the leading edge of epidermal cells during ventral enclosure. We are investigating two proposed mechanisms that would allow one GEF to have two opposing effects on F-actin enrichment, during two events, occurring in the same epidermal cells. Especially intriguing was our discovery that CED-12/ELMO appears to encode a cryptic GAP domain. CRISPR mutations that alter the key catalytic Arginine in the proposed GAP domain are being used to test the cryptic GAP inside the GEF hypothesis. In addition, live imaging and FRAP studies are being used to characterize the stability of F-actin around corpses vs. at the leading edge, and the dependence of these two populations of F-actin on CED-5/CED-12. These studies suggest that individual GEFs can acquire different, and in this particular case, opposite activities even within the same cell.

228C The proteasome is not only about degradation- using the *C. elegans* germ line to study proteasome assembly dynamics and subunit specific germ line functions *in vivo*

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The 26S proteasome is a multi-subunit protein complex that is canonically known for its ability to degrade proteins in cells and maintain protein homeostasis. It is composed of highly conserved protein subunits arranged into two 19S regulatory particles (RP) capping a 20S core catalytic particle (CP). Recent evidence shows specific proteasome subunits play tissue specific and/or non-proteolytic roles in various organisms. Historically, models such as yeast and mammalian cell lines have been used to characterize proteasome assembly and function. However, these unicellular models have limitations in comprehensively understanding the wide range of roles that various proteasome subunits might be playing in different tissues and developmental stages. We have identified specific 19S RP proteasome subunits in *C. elegans* that play previously unknown roles in the germ line. We will show that certain 19S RP subunits genetically interact with a major *C. elegans* meiotic kinase, WEE-1.3, and are required for proper germ line localization of WEE-1.3. Importantly we demonstrate that this role in WEE-1.3 localization is not related to the proteolytic function of the proteasome. To help elucidate individual proteasome subunits functions, we have endogenously tagged 19S RP lid subunits with GFP or OLLAS using CRISPR. This has revealed many novel results, including identification of subunit tissue specificity and the dynamics of proteasome assembly. We will show that the two isoforms of the essential 19S RP proteasome subunit RPN-6.1 are expressed in a tissue specific manner in the hermaphrodite. We will also demonstrate that the 19S RP subunits RPN-6.1 and RPN-7 are crucial for the nuclear localization of the lid subunits RPN-8 and RPN-9 in *C. elegans* oocytes. Collectively, our data supports the premise that certain

19S RP proteasome subunits are playing tissue specific roles, especially in the germ line. We propose *C. elegans* as a versatile multicellular model to study the diverse proteolytic and non-proteolytic roles that proteasome subunits play *in vivo*.

229A The role of ATX-2 and VPR-1 in sperm positioning within the *C. elegans* meiotic embryo

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Fertilization occurs during female meiosis in most animals, which raises the question of what prevents the sperm body (DNA, centrioles, and organelles) from interacting with the meiotic spindle. In C. elegans, the meiotic spindle and sperm body are maintained in opposite thirds of the ellipsoid zygote despite vigorous cytoplasmic streaming. In a previous study (Panzica et al. 2017. J Cell Biol 216: 2273), the sperm body was relatively stationary in control cells but moved long distances with the yolk granules when actin was depolymerized, resulting in sperm DNA within 2 µm of the meiotic spindle. This result led to the idea that the sperm body is anchored at the site of fertilization by cortical actin while maternal organelles move freely with cytoplasmic streaming. Simultaneous live imaging of paternal mitochondria, maternal ER and maternal yolk granules, however, revealed episodes with all these organelles moving together. In agreement with Kimura (2020. Mol Biol Cell 31:1765) we observed long range excursions of the sperm contents even in control embryos. These movements, however, were mostly in the short axis of the embryo. Long-distance excursions of the sperm contents toward the meiotic spindle were infrequent and followed by a return to the original spindle distal position. We are currently exploring the possibility that contacts between the maternal ER, paternal mitochondria, and cortical F-actin may play a role in restricting the movement of the sperm body to the future posterior end of the embryo. To begin, we have first defined the cell-cycle changes in ER structure by filming meiotic embryos within mKate::tubulin GFP::SP12 hermaphrodites and the cell-cycle changes in cytoplasmic streaming by filming embryos within GFP::SP12 hermaphrodites mated with mCherry::MEV-1 males. We are now using this baseline to analyze ER structure and movement of ER and paternal mitochondria in embryos depleted of maternal ATX-2, VPR-1, HSP-4, PFN-1 and other candidates predicted to disrupt sheet-like ER.

230B Characterization of sperm components required for female meiosis II in *C. elegans*

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Prior to fertilization, the *C. elegans* oocyte responds to an external, sperm-derived signal provided by major sperm protein (MSP). This signal releases the oocyte from its G2 arrest, initiating the meiosis I division (MI). In the absence of sperm entry, MSP-stimulated oocytes abort anaphase I, skip meiosis II (MII) and enter mitosis, suggesting a second signal provided upon sperm entry is required for the MII program. We discovered three similar paralogs, *memi-1*, *2,* and *3* (meiosis-to-mitosis transition), which encode oocyte components, that are required for completion of MI and entry into MII. Loss of all three paralogs results in a skipped-MII phenotype, however, a gain-of-function mutation, *memi-1(sb41ts),* results in fertilized embryos that complete MI, enter MII, but do not exit MII properly. Through whole genome RNAi screening, we previously identified a sperm-specific PP1 phosphatase, *gsp-3/4*, as a suppressor of *memi-1(sb41)*.

A subsequent EMS-based suppressor screen recovered alleles of *gsp-4* and additional genes in this pathway. One of these, *R03D7.5,* encodes a putative GSK3 protein kinase. *R03D7.5* mutants exhibit paternal-effect suppression of *memi-1(sb41)*, suggesting that this GSK3 has some role in the sperm. *R03D7.5* deletion homozygotes appear wild type, however, double-deletion analysis revealed functional redundancy with one other GSK3 member. The double-deletion hermaphrodites exhibit 45% embryonic lethality, which is completely rescued by mating to wild-type males. Approximately 35% of *GSK-3* double-deletion fertilized embryos exhibited defects in MII, including cell-cycle delays and failed polar body extrusion. Together, this suggested that these genes encode sperm-specific components that have roles in the early embryo.

Western blotting of FLAG- and Ollas-tagged worms revealed that both GSK3 homologs are detected in sperm-only *fem-3(q20gf)* worms but not in oocyte-only *fem-1(hc17lf)* females. Immunostaining for both proteins revealed a strong signal in sperm, which resembled the intracellular localization observed with anti-GSP-3/4 antibodies. Interestingly, *R03D7.5 gsp-4* double mutants exhibit synthetic embryonic lethality (28%), and a triple-deletion (both *gsk3* genes and *gsp-4*) produce 70% dead embryos, some of which skip female MII, similar to *memi-1/2/3(RNAi)*. Further characterization of the double GSK3 mutants revealed sister chromatid non-disjunction in 20% of male MII. GSK3 double-deletion sperm also exhibited reduced sperm motility *in vivo* and altered sperm morphology and slow pseudopod formation *in vitro*. Pseudopod treadmilling rates were also reduced in GSK3 double-deletion sperm. This work suggests that these GSK3s play a pivotal role during spermatogenesis and sperm motility, similar to GSP-3/4*,* and that these factors also participate in the MEMI pathway to regulate female MII.

231C Investigating the role of 5'-tyrosyl-DNA phosphodiesterase 2 (*tdpt-1)* Mediated Suppression of DNA Topoisomerase 2 (*top-2)* during meiosis in *C. elegans*

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Meiosis, a specialized cell division involving a single cycle of DNA replication followed by two cycles of chromosome segregation, is a highly coordinated event. Chromosome segregation errors during meiosis have hazardous consequences such as birth defects, genetic diseases, infertility and malignancy. DNA Topoisomerase II (TOP-2) is required to relieve the topological stress associated with the unwinding of DNA during replication, recombination and sister chromatid segregation. In addition, TOP-2 has been also found to maintain chromosome structure. The role of TOP-2 has been studied well in mitosis but is not very well defined in meiosis. However, our lab recently characterized an allele of *top-2* [*top-2(it7)*] in *C. elegans* with a male meiosis-specific phenotype. *top-2(it7)* is a temperature sensitive (ts) allele and *top-2(it7ts)* sperm that develop at the restrictive temperature of 24°C have chromosome segregation defects at anaphase I of meiosis, which results in embryonic lethality after fertilization. In a genetic suppressor screen on *top-2(it7ts* animals, we identified 11 suppressors of *top-2(it7)* induced embryonic lethality. Interestingly, seven of the 11 suppressors identified had different point mutations in the same gene 5›-tyrosyl-DNA phosphodiesterase two *(*Tdp2*, C. elegans tdpt-1)*. Tdp2 is involved in the removal of trapped Top-2-DNA complexes. Currently, our lab is focused on elucidating the mechanism of *tdpt-1* mutant-mediated suppression of *top-2(it7ts)* embryonic lethality. Our data suggest that the *tdpt-1* suppressing mutations rescue the embryonic lethality of *top-2(it7ts)* to near wild-type levels, ameliorate the chromosome segregation defects of *top-2(it7ts)* worms and restore TOP-2 protein level to various degrees. We have validated and confirmed *tdpt-1* suppressing mutation to be a loss of function mutation using RNAi and CRISPR/Cas9. Similarly, we have found that *tdpt-1* mutations do not have a phenotype independently of *top-2(it7)* in meiosis. To unravel the molecular mechanism of TDPT-1 in the context of TOP-2 mutation for ensuring proper chromosome segregation during male gamete formation, we are currently examining if TDPT-1 is required to process TOP-2 induced DNA breaks during meiosis. Further, we plan to examine biochemical properties of TDPT-1, perform TDPT-1 expression and localization studies in relation to TOP-2 in the male germ line, and investigate TOP-2 as a potential binding partner of TDPT-1 during meiosis.

232A GLH protein at the heart of P granule network

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Germ granules are cytoplasmic ribonucleoprotein assemblies conserved across the vast majority of metazoans from worms to humans. One key component of germ granules are Vasa-family DEAD box RNA helicases. Vasa mutation or knockdown compromises germ cell specification, survival and function leading to sterility in various organisms. C. elegans has four Vasa homologs, GLH-1, GLH-2, GLH-3, and GLH-4, all of which localize to germ granules called P granules. GLH proteins contribute to the integrity of P granules in the C. elegans germline (Kuznicki et al., 2000; Spike et al., 2008; Marnik et al., 2019). We asked if this function of GLH family proteins might be related to an ability to directly bind some core or transient P granule components by testing protein-protein interactions in vitro. Surprisingly, we found a diverse protein-protein interaction network for GLH-4 that includes binding to all four GLH family members, other core P granule components (PGL-1 and PGL-3) as well as P granule associated proteins FBF-2 and DLC-1. Taken as an aggregate, these results demonstrate a network of protein interaction within P granules that puts GLH-4 at the nexus for P granule organization and function.

233B Models predicting the partitioning of phosphorylated domains on *C. elegans* fusion chromosomes

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To carry out the two successive chromosome disjunction events of meiosis, the holocentric chromosomes of *C. elegans* use the position of the single off-center meiotic crossover to define two functional domains: the short arm, which loses cohesion in meiosis I, and the long arm, which loses cohesion in meiosis II. These two domains must be detected and defined on each chromosome at each meiosis, since crossovers can occur anywhere. The domains accumulate distinct sets of proteins or protein modifications, whose roles in mediating the timing of cohesion loss have recently begun to be uncovered. In contrast to the downstream mechanisms that lead to cohesin loss or protection, the mechanisms that initially sense the length difference of the two domains remains less well-understood.

We have previously shown that the accumulation of the synaptonemal complex (SC) central element protein SYP-1 phosphorylated at T452 on the short arm during the pachynema stage is one of the earliest signs of distinction between the short and long arms (Sato-Carlton et al. 2018). Phosphorylated SYP-1 localizes to the entire SC at the beginning of meiotic prophase and quickly accumulates on the short arm upon crossover designation, while departing the long arm entirely. In order to understand the mechanism of this short arm confinement, we have tagged non-phosphorylatable SYP-1 with an HA tag and compared its localization with phosphorylated SYP-1. We found that non-phosphorylatable SYP-1 also becomes enriched on the short arm, but does so more slowly than phosphorylated SYP-1. To gain further insight into the principles determining short arms, we have analyzed the formation of short arm-like domains enriched for phospho-SYP-1 on the fusion chromosome *meT7* (Hillers and Villeneuve 2003; Martinez-Perez et al. 2008), a "megasome" that combines chromosomes III, X, and IV, and which frequently receives two or three crossovers. On *meT7* chromosomes with two crossover designation sites at pachynema, domains of phospho-SYP-1 can occur in one of three modes: (1) a short arm domain at each end; (2) a single short arm domain in the middle segment, and (3) one short arm domain at one end, and one short arm domain in a small part of the middle segment. We present a model that predicts these outcomes from the strength of a putative accumulating signal emanating bidirectionally from crossover designation sites.

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Martinez-Perez E., M. Schvarzstein, C. Barroso, J. Lightfoot, A. F. Dernburg, et al., 2008 Genes Dev. 22: 2886–2901.

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234C Characterization of the transition between meiosis I and meiosis II during spermatogenesis in *Caenorhabditis elegans*

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Male meiosis exhibits two consecutive chromosome separation events in M phase. Thus, the division machinery has to re-organize between two divisions. Because proteasome-dependent degradation is important for the first division, we examined whether replenishment of cellular factors is necessary for progression into the next division event. When treated with translation inhibitors, we found that primary spermatocytes divided normally at meiosis I but failed to initiate the second division. To gain insights on how protein synthesis regulates the transition between two divisions, we first inspected the dynamics of chromosomes, kinetochore, centrosomes and microtubules during male meiosis when translation is inhibited. Perturbation of translation lead to failure of chromosome re-orientation after meiosis I. We found the levels of the outer kinetochore protein BUB-1 and HCP-1 dropped drastically at metaphase I and slowly regained before metaphase II. Inhibition of translation slightly reduced meiosis I chromosome separation rates and the departure of BUB-1, and the BUB-1 signal did not recover. Interestingly, other outer kinetochore proteins such as NDC-80, HIM-10 and ROD-1 appeared to remain associated with chromosome throughout the two male meiotic divisions, and the levels were not affected by translation inhibitors. Therefore, protein synthesis is required for reassembly of part of the kinetochore between two divisions. Furthermore, we found that although centrosome splitting took place timely during meiosis I, they failed to mature after completion of meiosis I with translation inhibition. We are currently looking for the key factors that need to be translated during meiosis I.

235A A screen to identify new genes involved in homeostatic regulation of germline stem cell proliferation

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The control of stem cell proliferation is crucial during the whole life of an organism. In fact, stem cells are at the origin of every differentiated cell types populating tissues and organs, ensuring their proper development, function, and homeostasis. Defects in stem cell regulation can lead to developmental disorders and pathologies like cancer. Two factors are known to control germline stem cell (GSC) proliferation in *C. elegans*: nutrient uptake and the quantity of mature oocytes present in the proximal gonad. In the latter, oocyte accumulation promotes GSC quiescence, to prevent oocyte hyperaccumulation. Although we have identified DAF-18/PTEN, PAR-4/LKB-1 and AAK-1/AMPK as effectors of this negative feedback loop, other effectors are likely missing to join them together in a complete molecular cascade. That cascade somehow needs to sense oocyte abundancy and signal across several cells to modulate GSC proliferation. Here, we used an *oma-1*; *oma-2* background to trigger oocyte accumulation and homeostatic inhibition of GSC proliferation. We then screened for mutants that caused oocyte hyperaccumulation, and thus phenocopied *aak-1*; *oma-1*; *oma-2* homeostatic-defective triple mutants. We screened approximately 8 000 haploid genomes over two separate screens, and isolated 17 candidates. Using whole-genome

sequencing and bioinformatics tools, we identified a list of genes that had severe mutations in two or more of the isolated mutants. Among this list was *aak-1*, which was severely disrupted in two of the candidates, demonstrating that the screening strategy was effective at isolating mutations that disrupt homeostatic signalling. We will next use RNAi to knockdown the remaining genes on our list and identify those that will cause a hyperaccumulation of oocytes upon RNAi inactivation. Positive clones will likely represent new genes involved in homeostatic regulation of GSC proliferation, and the corresponding genes will be further functionally investigated. Hence, our results will provide new insights into the mechanisms of homeostatic control of GSC proliferation.

236B Regulation of oocyte number in *C.elegans*: Counting on RAS/ERK pathway

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Oocyte numbers, a critical determinant of female reproductive fitness, are highly regulated, yet the mechanisms underlying this regulation remain largely undefined. In the *Caenorhabditis elegans* gonad, RAS/extracellular signal–regulated kinase (ERK) signaling regulates oocyte numbers; mechanisms are unknown. We show that the RAS/ERK pathway phosphorylates meiotic chromosome axis protein HTP-1 at serine-325 to control chromosome dynamics and regulate oocyte number. Phosphorylated HTP-1(S325) accumulates in vivo in an ERK-dependent manner in early-mid pachytene stage germ cells and is necessary for synaptonemal complex extension and/or maintenance. Lack of HTP-1 phosphorylation leads to asynapsis and persistence of meiotic double-strand breaks, causing delayed meiotic progression and reduced oocyte number. In contrast, early onset of ERK activation causes precocious meiotic progression, resulting in increased oocyte number, which is reversed by removal of HTP-1 phosphorylation. The RAS/ERK/HTP-1 signaling cascade thus functions to monitor formation and maintenance of synapsis for timely resolution of double-strand breaks, oocyte production, and reproductive fitness.

237C Meiosis modifications at the origin of asexuality in *Mesorhabditis* pseudogamous nematodes

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Sexuality is widespread and asexuality is a derived character. Despite the recognized costs associated with sex, asexuals remain rare, which constitutes one of the most intriguing paradox in evolutionary biology. To understand the adaptative value of sex, there is clearly a need for further exploration of the asexual world, and in particular of the transition to asexuality.

We recently characterized the nematode genus *Mesorhabditis*, in which regular sexual species are found, as well as species featuring progressive loss of males and sexuality. These pseudo-sexual species are composed of 90% females and only 10% males. Males and sperm are needed for most eggs to develop by gynogenesis (the sperm is needed to activate the oocytes but its DNA is not used, i.e pseudogamy). In the few cases where the sperm DNA is incorporated, the eggs develop as males, because only the Y-bearing sperm of males are competent to fertilize (Grosmaire & al. *Science* 2019; Launay & al. *BMC Evol Bio* 2020). In this intriguing system, females are thus produced asexually whereas males are produced sexually. *Mesorhabditis* nematodes represent an ideal system to study the evolutionary consequences of transition to asexuality in closely related species and to explore the molecular origins of asexuality.

There are many ways to lose sex. Here we asked which modifications to meiosis led to the production of diploid oocytes in asexuals. Using cytological descriptions, we found that meiosis of asexual females is similar to sexual species up to anaphase I. Chromosome pairing and crossing-over occur and bivalent forming chiasmata are present in diakinesis. Next, the first meiotic spindle forms and bivalent chromosomes initiate their segregation. However, chromosome segregation eventually stops and all univalents realign at metaphase of meiosis II. Meiosis II then proceeds normally with the segregation of sister chromatids and formation of the single polar body. Hence, meiosis in these asexuals is characterized by the maintenance of recombination and the assortment of non-sister chromatids in the diploid oocyte. In parallel, we are comparing the level of recombination in the sexual and asexual species of this genus. For that, we have sequenced the genome of a dozen strain for one sexual and one asexual species, to measure linkage desequilibrium. The theoretical consequence of such modified meiosis is genome-wide homozygosity, except maintenance of heterozygosity on chromosome centers. This will be contrasted with the actual measure of heterozygosity.

238A DAF-18/PTEN functions in the muscles and proximal somatic gonad to couple to promote oocyte arrest in the absence of sperm

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DAF-18/PTEN is an important tumour suppressor, and loss of PTEN activity is associated with various types of cancers. In *C. elegans* hermaphrodites that lack sperm, such as *fog-1* mutants, DAF-18 is required for oocytes to arrest and accumulate in the proximal gonad. As such, *fog-1; daf-18* double mutants lay unfertilised oocytes, and fail to downregulate germline stem cell (GSC) proliferation. However, the tissue(s) in which DAF-18 acts to permit oocyte arrest and accumulation in the absence of sperm, and to eventually suppress GSC proliferation, is still unknown. Here, we transgenically rescued *daf-18,* specifically in the gut, neurons, hypodermis, muscles, proximal somatic gonad, and germline, of *fog-1; daf-18* null mutants. Interestingly, we found that *daf-18* expression in the muscles, or in the proximal somatic gonad, was sufficient to allow unfertilized oocytes to arrest and accumulate in the proximal gonad of *fog-1;daf-18* double mutants. DAF-18 is also required to suppress the proliferation of germ and somatic gonadal cells during dauer formation, wherein DAF-18 acts in the somatic gonadal cells. Using our germline-specific DAF-18 transgene, we further show that maternally-provided DAF-18 is sufficient to rescue the dauer phenotype of *daf-18(-)* mutants, but not the arrest of unfertilized oocytes in spermless adults. Overall, our results demonstrate that DAF-18 can act from at least two separate tissues to non-autonomously mediate the arrest of oocytes in the absence of sperm, and allow for their accumulation in the proximal gonad. These results may provide new insights into the how PTEN prevents tumour formation.

239B Characterizing the Role of Sperm-Supplied Proteins, SPE-11 and F07A5.2, during Spermatogenesis and the Early Embryonic Development in *C.elegans*

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Haploid genomes from sperm and oocyte are required to form a diploid zygote during fertilization. Competent genes from these specialized cells are essential for the success of both molecular and physiological events from fertilization to embryogenesis. So far spe-11 is the only sperm-specific gene required for the early events of embryonic development in *C.elegans. C. elegans* embryos fertilized by spe-11 mutant gene do not complete meiosis, form a weak eggshell, and do not orient correctly at the first mitotic spindle. These embryos also do not undergo cytokinesis. *C.elegans* embryos carrying homozygous non-conditional allele hc90 show phenotypic effects of this paternal-effect embryonic lethal gene. Research has shown that SPE-11 localizes around distinct regions of the nucleus during spermatogenesis by forming a fenestrated ring around the DNA. In addition to this important sperm gene, we have found that two proteins F07A5.2 and OOPS-1 interact with SPE-11 through yeast hybrid experiment and through immunoprecipitation and mass spectrometry experiments. Like SPE-11, F07A5.2 is a potential sperm protein. We have found that a deletion of allele F07A5.2 reduces brood viability. Currently, I am performing experiments to determine if wildtype sperm can rescue deletions of the F07A5.2 gene. In addition, I will examine if the F07A5.2 protein is involved in the correct localization of the SPE-11protein in sperm meiosis. This will help determine the potential roles these sperm genes play in the events required for successful embryogenesis in C.elegans.

240C The role of MAP Kinase in modulating condensation of RNA binding proteins in the germ line

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The mitogen-activated protein kinase (MAPK) signaling pathway regulates numerous cellular processes in the *C. elegans*germ line. In young hermaphrodites, di-phosphorylated MAPK is detected at high levels in late pachytene and in the proximal oocytes; however, when meiotic maturation is arrested for extended time in *fog-2* females, activated MAPK is not detected. Interestingly, the activation of MAPK is inversely correlated with the assembly of large RNP granules in oocytes. In this study, we are addressing the hypothesis that MAPK inhibits the condensation of RNA-binding proteins (RBPs) into RNP granules. It is critical to understand how the assembly of RNP granules is regulated as they are hypothesized to maintain oocyte quality during extended meiotic arrest and other stress conditions. First, we used RNAi to knockdown *mpk-1* in GFP::MEX-3 worms, and we detected ectopic condensation of MEX-3 into granules in oocytes. Since CAR-1 condenses into large granules in response to heat stress, we next asked if activated MAPK levels decrease in proximal oocytes after 1.5 hours at 31°C. We observed reduced levels of anti-diP-MAPK and the assembly of CAR-1 granules in oocytes. In parallel, to examine the effects of decreased *mpk-1* expression on CAR-1 protein condensation, we used the temperature-sensitive allele *mpk-1(ga111)*. At the permissive temperature, MAPK levels were high in oocytes, similar to wild-type worms, and CAR-1 was diffusely distributed in the oocyte cytoplasm. At the restrictive temperature, we observed reduced MAPK levels and increased CAR-1 condensation

into granules. Wild-type worms at the restrictive temperature of 25°C had a similar phenotype as *mpk-1(ga111)* at the permissive temperature; therefore, the assembly of CAR-1 granules in *mpk-1(ga111)* is not due to mild heat stress. Current experiments are testing the effect of *mpk-1(RNAi)* in GFP::CAR-1 and GFP::CGH-1 worms. Our findings to date, support the hypothesis that MAPK activity inhibits the assembly of RNP granules; however, the mechanism by which MAPK may modulate the condensation of RNA binding proteins is not known. We plan to investigate if MAPK directly phosphorylates one or more RBPs, or if MAPK acts indirectly on RBP condensation, perhaps via the CCT chaperonin.

241A C. elegans maximize the number of euploid progeny from zim-2 parents with crossover failure on chromosome V.

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Gametes with incorrect chromosome number can lead to embryonic lethality and developmental deficiency if inherited. Parental trisomy or crossover failure are two conditions expected to yield random meiotic segregation and 25% trisomy among offspring. We previously demonstrated that parental trisomy of the X can be corrected among progeny by preferentially placing the extra chromosome in the first polar body (Cortes, eLife.06056). If 100% efficient, such a univalent elimination mechanism would generate 100% lethal monosomy among progeny of parents with crossover failure. However, high viability rates have been reported among progeny of him-8 mutants (crossover failure on X) (Hodgkin, Genetics 91:67), zim-2 mutants (crossover failure on V) and zim-1 mutants (crossover failure on II and III) (Jaramillo-Lambert, Curr Biol 20:2078), suggesting the possible existence of a distributive segregation mechanism that is more efficient during spermatogenesis than during oogenesis. Here we directly measured the frequency of monosomy, disomy and trisomy among the progeny of zim-1 mutants and zim-2 mutants mated to wild type using PCR polymorphisms that differ between three strain backgrounds. Zim-1 hermaphrodites mated to wild-type males produced 72% disomy II progeny (n=33) and 57% disomy III progeny (n=54). This difference from the 50% expected from random segregation was not significant. Zim-2 hermaphrodites mated with wild-type males produced 73% disomy V progeny (n=65) which was not significantly different than random segregation when corrected for 24% (n=42) crossovers on V observed by counting diakinesis bivalents in oocytes. In striking contrast, zim-2 males mated to wild-type hermaphrodites produced 95% disomy V progeny (n=94) which is significantly higher than random segregation. We are currently testing whether this is due to a high frequency of crossovers on V in zim-2 males, more efficient fertilization by euploid sperm, or a genuine distributive segregation system during male meiosis. A side benefit of our approach is that we can determine the phenotypes of most single chromosome aneuploidies. We have thus far observed monosomies of II, III, or V only in dead embryos and have observed trisomies of II, III, or V only among hatched larvae.

242B DAF-16/FoxO are necessary to induce Germ Cell apoptosis under starvation

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Apoptosis is a conserved processe necesary to kept the homeostasis tissues, in the adult *C. elegans* germline nearly 50% of germ cells are eliminated by apoptosis in physiological conditions. Fuetheremore different kinds of stress such as heat shock or starvation increases germ cell apoptosis. LIN-35/Rb, the closest retinoblastoma homolog in the *C. elegans* genome, regulates germ cell apoptosis during normal and starvation conditions by downregulating *ced-9*/Bcl2 expression. Upon starvation, the expression of *lin-35*/Rb is up regulated at the gene and protein level. Our main goal is to understand how *lin-35*/Rb expression is regulated during starvation. We Use MODENCODE database to identified some transcription factors that probably bind to *lin-35*/Rb´s putative promoter and we are testing if they might regulated its expression under stress. Among the transcription factors that we have found is DAF-16 the worm FoxO homolog. We found that DAF-16/FoxO is important to induce germ cell apoptosis upon fasting. By qPCR, we observed that *daf-16*/FoxO mutant animals show low levels of *lin-35* expression under stress suggesting that this transcription factor might be important to regulate its expression under this condition. Using a transgene that expresses *daf-16* specifically in the germline (*mex-3::daf-16::gfp*) in a *daf-16* mutant background, we observed that *daf-16* expression in the gonad is sufficient to induce germ cell apoptosis under starvation demonstrating that DAF-16

controls germ cell apoptosis in an autonomously manner. By immunoprecipitation, we would like to demonstrate that *daf-16* binds *lin-35* promoter. Our findings suggest an important role of DAF-16/FoxO in the gonad under stress by control the expression of LIN-35/Rb as key players of starvation-induced germ cell apoptosis.

243C Chromosome pairing and segregation during meiosis require the nuclear envelope protein MJL-1 in *C. elegans*

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During meiosis, homologous chromosomes pair, synapse, and undergo recombination to produce haploid gametes. In diverse species, telomere-led rapid movements of chromosomes promote homologous chromosome pairing during early meiotic prophase. These movements are generated by the tethering of telomeres to "LINC" (Linker of Nucleoskeleton and Cytoskeleton) complexes in the nuclear envelope (NE), which transmit cytoskeletal forces across the NE. In *C. elegans*, specialized chromosome regions known as Pairing Centers (PCs) recruit meiosis-specific zinc finger proteins, HIM-8, ZIM-1, ZIM-2, and ZIM-3, and associate with a LINC complex to generate chromosome movements instead of telomere-led movements. Loss of the zinc finger proteins abrogates the connection between PCs and LINC complex and leads to failures in homologous chromosome pairing, while mutations in an inner NE component of LINC complex, SUN-1, result in unregulated synapsis between non-homologous chromosomes. However, we do not yet understand the architecture of the connections between the zinc finger proteins and the NE, or how these connections regulate synapsis.

Through a genetic screen for meiotic defects, we identified an uncharacterized gene that encodes a small, poorly conserved protein with a single transmembrane domain. We find that the protein localizes to the NE specifically during meiosis and is strongly enriched at the "patches" where the PCs interact with the LINC complex. We have named the protein MJL-1 (Majin-Like-1), based on its similarity to Majin, an inner NE protein that bridges the interaction between telomeres and LINC complex during meiosis in most metazoans. Mutations in MJL-1 abrogate the connection of PCs and LINC complex, and result in unregulated non-homolog synapsis, similar to mutations in *sun-1*. MJL-1 is thus essential for the tethering of PCs to LINC complex and rapid chromosome movements during meiotic prophase that regulate synapsis. Further investigation of MJL-1 will help to reveal how synapsis is regulated to ensure faithful recombination and segregation of homologous chromosomes during meiosis.

244A Role of *spe-11* and *oops-1* in early embryogenesis and eggshell formation

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Gametes provide a variety of cellular and genetic materials during their fusion to form a zygote. Although the majority of proteins required for embryogenesis are maternally provided and present in the oocyte, sperm contributions are also essential to this process. In the model organism *C. elegans*, SPE-11 is a sperm protein identified to be paternal-effect embryonic lethal. It was experimentally shown that homozygous mutant sperm (from hermaphrodite or male) will always lead to non-viable embryos regardless of the source of oocyte. However, oocytes from a *spe-11* mutant can be successfully fertilized when sperm from a wild type male is provided therefore confirming SPE-11 function can only be provided by the paternal gamete. *spe-11* mutant single cell embryos are defective in a myriad of ways such as inability to complete the meiotic divisions and cytokinesis, improper spindle formation, and weak eggshell formation. Currently, little is known about the role and function of the SPE-11 protein as it has no known homology to any known domains. Recently, an interacting partner of SPE-11 was identified and given the name oocyte partner of SPE-11 (OOPS-1). As these two proteins were found to physically interact, we asked if mutant versions had similar phenotypes. Similar to *spe-11* mutants, we found that deletion mutants of *oops-1* are embryonic lethal indicating that the OOPS-1 protein is required for the embryogenesis process. We are using fluorescently labeled histones and meiotic spindles to image oocyte meiosis *in vivo* in wild-type, *spe-11(hc90)*, and *oops-1(tn1898)* strains. Interestingly, while both *spe-11* and *oops-1* mutants have defects in oocyte meiosis, the two mutants have slightly different phenotypes. As previously published, in *spe-11(hc90)* mutant embryos, the chromosomes segregate at anaphase I and II but fail to form polar bodies. In contrast, it appears that *oops-1(tn1898)* can complete meiosis I but cannot enter anaphase II. The exact timing and the analysis of other meiotic division phenotypes is still ongoing. Alongside this, we are also investigating the integrity of the embryo eggshell in these mutants.

245B An Exploration of the protein FIGL-1 in the *Caenorhabditis elegans* Germline and Insights into its role in Homologous Recombination

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Repair of damaged DNA is crucial for the survival of both the individual organism and species. Double-strand breaks (DSB) are the most cytotoxic lesions. Homologous recombination (HR) is a high fidelity DSB repair pathway that relies on an undamaged homologous template. HR is especially important during meiosis prophase I, as it is a pathway that can repair programmed DSBs as crossovers (COs). CO homeostasis is a process that prevents either too few or too many COs between homologous chromosomes and without it, genome instability may ensue. In part, CO homeostasis is achieved by the mutual antagonism between HR/CO-promoting and HR/CO-inhibiting factors. The recombinase RAD-51 is central to HR as it coats the ssDNA filament to promote strand exchange. The anti-recombinase, FIGL-1 (Fidgetin-like protein 1), was shown in humans, mice, and plants to antagonize HR by directly interacting with RAD-51. Studies of how *figl-1* functions in the germ line of *Caenorhabditis elegans* have been lacking in part because knockout of *figl-1* is embryonic lethal. To determine if the interactions between RAD-51 and FIGL-1 are conserved in worms, I have been performing a yeast two-hybrid (Y2H) with the full-length proteins. If an interaction exists, interaction motifs will be mapped through a series of amino acid deletions and substitutions. It will be particularly interesting to determine whether the partially conserved FIGL1-RAD51 binding domain (FRBD) functions similarly in *figl-1*. CRISPR-Cas9 genome editing will be employed to introduce the mapped mutations into the *figl-1* locus to observe the phenotypic effects *in vivo*. Additionally, plans to introduce targeted missense mutations into the highly conserved Walker A and B motifs, which are known to be important for ATP hydrolysis in FIGL-1, are underway. Interestingly, previous results have suggested that FIGL-1's ATP hydrolysis activity is independent of its anti-recombinase activity, suggesting that these mutations may result in separation-of-function mutants. Through these studies, specific domains within worm *figl-1* will be scrutinized to shed more light on its function in the germ line as well its role in HR.

246C The HECD-1 ubiquitin ligase acts with the STRIPAK complex to regulate MEI-1/katanin microtubule-severing in meiosis and mitosis

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Microtubule-severing plays important roles in development, including for cell structure and division. Katanin, encoded by *mei-1* and *mei-2,* is a microtubule-severing complex required for the assembly of the meiotic spindle and then must be downregulated in the span of 20 minutes, to allow for formation of the mitotic spindle. Cullin-based ubiquitin ligases (CUL-2, CUL-3) control katanin levels during this transition, while other complexes like protein phosphatase 4 (PPFR-1) and the Hect E3 ubiquitin ligase (HECD-1/HectD1) control katanin activity through non-degradative means. Interestingly, HECD-1 switches from activating to inhibiting katanin in meiosis and mitosis, respectively.

Although mammalian HECTD1 affects protein localization of its targets, mutant worm HECD-1 did not affect localization of known katanin regulators (PPFR-1, MEI-2, MEL-26), suggesting that HECD-1 is acting through novel partners. In mammals, HECD-1 interacts with the striatin-interacting phosphatase (STRIPAK) complex. This complex (without HECD-1) is known to be involved in tubule formation and endocytosis in *C. elegans*. I found that STRIPAK components genetically interacted with katanin and different components interacted with katanin differently. Generally, the STRIPAK core components (LET-92, GCK-1, CASH-1, FARL-11) acted as katanin activators in meiosis and inhibitors in mitosis, similar to the variable component HECD-1. The core component cerebral cavernous malformations (CCM-3) was an inhibitor of katanin at both divisions. In contrast, variable components (M4.1, OTUB-2) acted as activators of katanin, implying they are inhibitors of the STRIPAK complex. Other components (MOB-4, C49H3.6) were not involved in katanin microtubule-severing. I also used CRISPR flag-tagged HECD-1 and found that HECD-1 is ubiquitously expressed in wild type and *mel-26* mutants (which result in ectopic katanin in mitosis) rather than colocalizing with katanin or microtubules. Additional genetic interactions indicate that the link between STRIPAK and katanin may be through the centralspindlin component ZEN-4 and the tubulin chaperone TBCD-1. My results elucidated the interactions of nearly all of the STIRPAK complex components in a single system and revealed its role in katanin microtubule severing and cell division.

247A CCAR-1 regulates reproduction, lifespan, and apoptosis in *Caenorhabditis elegans*

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The CCAR-1 family consists of a dynamic group of genes known to regulate key physiological functions such as cancer, metabolism, apoptosis, and stress response. The dynamic nature of the CCAR family stems from the occurrence of conserved domains in its structure, which likely allow a diverse range of interactions. We use whole transcriptome RNA-sequencing to

examine CCAR-1 transcriptional regulation in *Caenorhabditis elegans.* From our dataset, we uncover a novel role of *C. elegans* CCAR-1 in the germline transcriptional regulation. We also show the involvement of CCAR-1 in key physiological processes such as reproduction, lifespan, and DNA-damage-induced apoptosis. Together, these results uncover a novel function of CCAR-1 in the *C. elegans* germline, which are relevant in the study of the CCAR-1 family of genes.

248B GRAS-1 is a conserved novel regulator of early chromosome dynamics during meiosis in *C. elegans*.

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Meiosis is a specialized cell division program resulting in the production of haploid gametes (eggs and sperm) from diploid germ cells. Errors in achieving accurate chromosome segregation during meiosis can result in infertility, miscarriages and birth defects such as Down syndrome. Therefore, understanding the mechanisms underlying accurate meiotic chromosome segregation is of tremendous importance for human reproductive health. Here, we uncovered a role for GRAS-1, the worm homolog of mammalian GRASP and CYTIP proteins, in coordinating early meiotic events with cytoskeletal forces outside the nucleus. GRAS-1/GRASP/CYTIP contains PDZ and coiled-coil domains and in mammals has been implicated in docking cytoskeleton components and in endosomal trafficking. A role for GRAS-1/GRASP/CYTIP during meiosis has not been previously demonstrated. *gras-1* expression starts upon entrance into meiosis and GRAS-1 localizes in close proximity to the nuclear envelope (NE)-associated protein SUN-1 starting at early prophase I. GRAS-1 IPs and MS analysis reveal it interacts with other NE and cytoskeleton proteins. *gras-1* mutants show an extended transition zone (leptotene/zygotene stage), a delay in achieving homologous pairing, the formation of aggregates with SC central region proteins that persist into pachytene while chromosome axes appear unaltered, and impaired DNA double-strand break repair progression. Importantly, these defects are partially rescued by expression of mammalian CYTIP in *gras-1* mutants, supporting functional conservation. These defects likely stem from a role for GRAS-1 in regulating chromosome dynamics given that *gras-1* mutants show accelerated chromosome movement during early prophase I. Moreover, in a *dhc-1* depleted background, *gras-1* mutants exhibit new and additional phenotypes, indicating that *gras-1* regulation of chromosome movement acts in parallel to the previously described LINC-controlled pathway. Finally, GRAS-1 undergoes phosphorylation, and analysis of a phosphodead mutant reveals that this post-translational modification is required for regulating GRAS-1 function during meiosis. We propose that GRAS-1 serves as a scaffold for a multi-protein complex coordinating the early steps of homolog search and licensing of SC assembly by regulating the pace of chromosome movement in early prophase I.

249C Deciphering the mechanisms of temperature-induced DNA damage in *C. elegans* spermatocytes

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Spermatogenesis and oogenesis create genetically unique haploid gametes in most sexually reproducing organisms. In comparison to oogenesis, spermatogenesis must occur in a narrow temperature range, typically 2-7°C below body temperature. In mammalian spermatocytes subjected to acute thermal stress, an increase in DNA damage occurs as well as a reduction in fertility. Despite these well documented effects on genome integrity and fertility, the exact mechanisms that cause temperature-induced heat stress in spermatogenesis remain unclear. Recent work in *Caenorhabditis elegans* revealed a large increase in DNA double-strand breaks (DSBs) marked by the recombinase protein RAD-51 when spermatocytes are exposed to a two-hour heat shock at 34°C. The temperature-induced DSBs are SPO-11 independent, suggesting other mechanisms are responsible for DSB formation. While a small portion of the temperature-induced DSBs were attributed to the transposition of the DNA transposable element Tc1, the origin of the remaining DSBs are unclear. To determine the other mechanisms that generate the temperature-induced DSBs, we will use RAD-51 chromatin immunoprecipitation followed by sequencing (ChIP-seq) on *C. elegans* males before and after heat-shock. From these experiments, we will define the wild type DSB landscape in *C. elegans* males. Also, we will determine where temperature-induced DSBs are found across the genome, therefore indicating how these DSBs are formed, including whether other transposable elements are activated upon heat shock. In addition, we will investigate the repair pathways required for resolving temperature-induced DSBs. In hermaphrodites, interhomolog recombination requires the protein RAD-50 to load RAD-51 onto DSB sites. To determine if interhomolog recombination is required to repair temperature-induced DSBs in males, we are utilizing a *rad-50* null mutation. Immunofluorescence of RAD-51 in *rad-50* mutant males before and after heat-shock will determine whether males require RAD-50 dependent RAD-51 loading during meiotic prophase I and if the RAD-50 pathway is required for repair of temperature-induced DNA damage

in spermatocytes. Together, this work will establish the DSB landscape in *C. elegans* males and provide insight into how temperature-induced DSBs are both formed and repaired.

250A Post-translational modifications of the synaptonemal complex protein SYP-4 C-terminus are involved in the regulation of crossover interference in *C. elegans* meiosis

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Meiosis is a germ cell-specialised cell division process consisting of two consecutive cell divisions, meiosis I and meiosis II, which leads to the production of gametes. Thereby, parental homologous chromosomes must segregate into different cells in meiosis I. The success of this first division depends on the accuracy of homologous chromosome pairing and in the formation of crossovers between the homologs. The latter is regulated by two mechanisms: crossover assurance, which ensures that each pair of homologous chromosomes gets at least one crossover, and crossover interference, which ensures a non-random distribution of crossovers along the chromosomes. In *C. elegans*, this give rise to the formation of a single crossover event per pair of homologous chromosomes in wild-type animals.

In most sexually reproducing organisms, the assembly of the synaptonemal complex between homologous chromosomes during meiotic prophase I is essential for the accurate segregation of the homologs by stabilising homologous pairing. In recent years, several studies have identified an additional function of the synaptonemal complex in crossover regulation. However, the question of how the synaptonemal complex regulates crossover formation still remains unsolved.

Our data now reveals that the C-terminus of SYP-4 is a critical regulator of crossover formation within the synaptonemal complex of *C. elegans*. We show that deletion of the C-terminus of SYP-4 preserves the ability to assemble the synaptonemal complex but severely impairs the regulation of crossover events along chromosomes and diminishes the viability of the progeny. We therefore hypothesised that the C-terminus of SYP-4 is the central hub for crossover regulation within the synaptonemal complex. More specifically, we hypothesised that post-translational modifications within this region are responsible for regulating this process. Indeed, our preliminary results using mass-spectrometry to identify such modifications suggest that the C-terminus of SYP-4 carries several potential phosphorylation sites. Using genome editing to generate phospho-null and phospho-mimetic SYP-4 mutants we can show that inhibiting or mimicking the phosphorylation of these sites indeed impairs crossover interference while crossover assurance and synaptonemal complex assembly remain unaffected. Thus, our results suggest that the number of crossover events along individual chromosomes is limited by post-translational modifications within the C-terminus of SYP-4. Building on these findings, we now aim to identify the regulatory network surrounding the SYP-4 C-terminus that ensures the tight regulation of crossover formation, which is essential for the production of healthy and viable progeny.

251B The CCT chaperonin selectively regulates phase transitions in the *C. elegans* germline

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When phase transitions of proteins are not properly regulated, proteins sometimes condense into aggregates that disrupt normal physiological function and lead to disease. Other times, condensation of RNA-binding proteins into ribonucleoprotein (RNP) granules is associated with homeostasis. The regulation of such phase transitions has been a topic of much inquiry but is still not completely understood. We are currently exploring the role of the Chaperone-Containing TCP1 (CCT) chaperonin in regulating RNP granule assembly in the *C. elegans* germline. Genetic screens performed by our lab and others initially identified several CCT subunits as promoters of PGL-1 granule assembly in embryos, and of MEX-3 granule assembly during extended meiotic arrest. In contrast, CCT inhibits stress granule and P-body assembly. Therefore, our goal was to carefully examine the role of CCT in regulating phase transitions of RNA-binding proteins in different developmental contexts. We used RNA interference (RNAi) to knockdown the expression of *cct* subunits in adult worms and imaged several GFP-tagged RNA-binding proteins using confocal microscopy. We find that in arrested oocytes in the *fog-2* background, CCT inhibits the condensation of the P-body protein CGH-1. However, CCT does not appear to strongly regulate the condensation of the P-granule protein MEG-3. We are in the process of analyzing effects on additional RNA-binding proteins. We also find that in young, wild-type hermaphrodites CCT inhibits the condensation of the P-body proteins CAR-1 and CGH-1 in oocytes. However, CCT does not appear to affect the condensation of MEX-3, the P-granule proteins PGL-1 and MEG-3, or the stress

granule proteins PAB-1 and TIAR-2. Taken together, we conclude that the CCT chaperonin selectively modulates RNA-binding proteins in the germline. Our results suggest that improper folding by CCT of one or more substrates in the germline, leads to aggregation for a subset of RNA-binding proteins. Possible models to explain our results include CAR-1 and CGH-1 as substrates directly folded by CCT, or alternatively, an indirect regulator of RNA-binding proteins may be a CCT substrate. We are currently testing whether subunits of Prefoldin, a co-chaperone of CCT, have similar roles as CCT subunits on CAR-1 and CGH-1 condensation.

252C Proteasome non-ATPase subunits regulate timing and polymerization of synaptonemal complex proteins in C. elegans

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The 26S proteasome is a large multi-subunit protease that plays a key role in cellular proteostasis and is ubiquitous in eukaryotic cells. This molecular machine, which consists of one 20S proteolytic core complex capped by two 19S regulatory complexes, is capable of degrading proteins in the nucleus and cytoplasm. The 19S cap complex, divided into two additional subcomplexes, the base and the lid, regulates proteasome function by identification, binding, deubiquitination, unfolding and translocation of substrates to the proteolytic chamber of the core particle. The assembly of the base complex consists of six regulatory particle AAA ATPase subunits (Rpt1-Rpt6), organized into a ring, as well as four regulatory particle non-ATPase subunits (Rpn1, Rpn2, Rpn10 and Rpn13). Rpn1, Rpn10, and Rpn13 serve as ubiquitin receptors, recognizing substrates targeted to the proteasome. The lid's main function is deubiquitination of incoming substrates and it is formed by nine different Rpn subunits (Rpn3, Rpn5-9, Rpn11, Rpn12 and Rpn15 (Dss1/Sem1)), which form a horseshoe-shaped structure.

Previous work from our group and others has shown that a structurally-compromised proteasome core complex results in severe defects in synaptonemal complex (SC) dynamics and premature reorganization of the SC prior in late pachytene. Based on these results, we wanted to interrogate how these events are affected when the 19S regulatory complex, and in particular the non-ATPase subunits, are absent. Our results reveal that RNAi knockdown of the genes rpn-1, rpn-2, rpn-3, rpn-5, rpn-6.1, rpn-7, rpn-8 or rpn-11, resulted in both the formation of SYP-1 polycomplexes and also the premature polarization of SYP onto the short arm of the chromosomes during late pachytene. In all these cases, knockdown of these proteasome subunits caused embryonic lethality and cell cycle defects, readily seen by altered nuclear sizes in the mitotic tips of the germ line. In contrast to the robust phenotypes described above, the knockdown of the rest of non-ATPase subunits (rpn-9, rpn-10, rpn-12, rpn-13 or rpn-15/dss-1) did not produce embryonic lethality and the synaptonemal complex phenotype was absent, with the fully polymerization of the SC and at the correct timing. While rpn-10 and rpn-12 have been shown to function redundantly for aspects of germ cell function (Takahashi M et al. 2002), these studies raise the possibility that the 19S proteasome may adopt a unique configuration and have unique protein partners within the C. elegans germ line.

253A Characterization of the meiotic double-strand break complex and its sensitivity to maternal age

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For most diploid organisms, the formation of haploid gametes relies on crossover (CO) recombination between homologous chromosomes for accurate chromosome segregation. Defects in CO formation can trigger errors in the correct number of chromosomes in the gametes. Recombination initiates with a double-strand break (DSB), introduced by the conserved topoisomerase like enzyme SPO11 but the timing, placement and number of COs depends on the activity of accessory factors. While these proteins have been well characterized in budding yeast, our understanding of the conserved complexes that regulate SPO11 are still poorly understood.

In *C. elegans*, *dsb-1*, *him-17*, *mre-11*, and *rad-50* are required for DSBs formation. *xnd-1* and *him-5* influence DSB formation mainly on the X chromosome. *dsb-2* impacts breaks on all chromosomes and becomes essential in older animals. *rec-1* influences CO placement and *cep-1*, *lin-35*, and *parg-1* contribute to break induction. We are investigating the relationship between these DSB factors in order to uncover the regulatory events that promote DSB formation. To this end, we are taking a multi-pronged approach using genetic epistasis with partial loss-of-function alleles, rescue with ectopically expressed transgenes and yeast-2-hybrid analyses. These studies are allowing us to construct a pathway for the recruitment and activation of SPO-11.

We are also interested in understanding why the loss of the SPO-11 accessory factors shows exacerbated phenotypes in older animals. Indeed in otherwise wild type worms, an increase of achiasmate chromosomes is seen at diakinesis starting at day 4

of adulthood, the end of their peak reproductive phase. This correlates with an increase in males and altered CO distribution. In this study, we show that both DSB formation and repair are inherently sensitive to maternal age.

254B Depletion of Cdc48 homologs during meiotic prophase results in synaptonemal complex defects in *C. elegans*

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Recombination and segregation of chromosomes in meiosis depend critically on the proper assembly and disassembly of the synaptonemal complex (SC), a protein polymer that links homologous chromosomes together. The SC is composed of axial elements localizing to each homologous chromosome, including the HORMA domain-containing protein HTP-1, and central elements localizing between the axial elements. During meiotic prophase in *Caenorhabditis elegans*, each pair of homologous chromosomes acquires a single off-centered crossover designation site. This crossover location divides the chromosome into two distinct regions of unequal length referred to as the long arm and the short arm. After crossover designation, HTP-1 disassembles from the short arm of the chromosome but persists at the long arm region, as part of a process known as chromosome partitioning (Martinez-Perez et al., 2008). Restriction of HTP-1 to the long arm of the chromosome locally antagonizes the Aurora Kinase homolog AIR-2 (Ferrandiz et al., 2018), which in turn is restricted to the short arm region of partitioned chromosomes. Improper chromosome partitioning and AIR-2 restriction defects can lead to inadequate chromosome segregation.

In other organisms, the AAA+ ATPase Pch2/TRIP13 is able to disassemble HORMA domain proteins from the synaptonemal complex during meiosis (Wojtasz et al., 2009). Another AAA+ ATPase, Cdc48, is an essential protein present in most eukaryotes. It has been reported that *cdc-48.1* and *cdc-48.2*, homologs of cdc48 in *C. elegans*, are required for appropriate AIR-2 localization prior to cell division in Meiosis I (Sasagawa et al., 2012), implying they may be involved in chromosome partitioning. We therefore decided to test the role of *cdc-48.1* and *cdc-48.2* in SC assembly and disassembly in meiotic prophase. Our results show that in *cdc-48.1* null mutants combined with *cdc-48.2 RNAi*, synapsis initiation is delayed, resulting in conglomerations of SC central elements called polycomplexes. While SC does eventually assemble correctly in these mutants, its disassembly is also perturbed, as central elements form large foci near the crossover site on each chromosome in late prophase. Our results thus show that Cdc48 homologs play critical roles in the function of *C. elegans* meiotic chromosomes.

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255C Characterizing the Sexually Dimorphic Role of Topoisomerase II During the Sister Chromatid Cohesion Release Pathway

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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 in infertility, progeny inviability, and birth defects. DNA topoisomerase II (Topo II) is a conserved enzyme crucial for chromosome fidelity by alleviating topological stresses in DNA. Topo II also plays a critical role in maintaining mitotic chromosome structure. Although the role of Topo II has been extensively characterized in mitosis, its role in meiosis has not been well defined. Previously, we identified a temperature sensitive allele of *C. elegans* Topo II, *top-2(it7)*. At the nonpermissive temperature of 24°C, homologous chromosomes fail to segregate resulting in aneuploid sperm and embryonic lethality after fertilization. Interestingly, homologous chromosome segregation during oogenesis is unaffected in this mutant even though at the nonpermissive temperature TOP-2 fails to localize to chromosomes in both spermatogenesis and oogenesis. If TOP-2 localization is disrupted in both sexes, why is chromosome segregation only disrupted during spermatogenesis? One major difference between oogenesis and spermatogenesis is the chromosomal morphology after pachytene. I hypothesize that TOP-2 is involved in chromosome remodeling during late meiotic prophase, facilitating homologous chromosome segregation during spermatogenesis. I found that REC-8, a meiosis-specific

sister chromatid cohesin and chromosome axis component, is prematurely removed from chromosomes in *top-2(it7)* during spermatogenesis. Furthermore, I discovered that AIR-2, which phosphorylates REC-8 for removal from the chromosomes, is ectopically localized in spermatogenesis, as well as phosphorylation of Histone H3 on Thr3 (H3pT3), which spatially recruits AIR-2 to the chromosomes. In addition, a lateral chromosome structural component, HTP-1/2, is prematurely removed. However, during oogenesis HTP-1/2 and H3pT3 localization is not disrupted. AIR-2 spatial localization is wild type, but its localization is delayed during oogenesis, which suggests that TOP-2 plays a temporal role in the recruitment of AIR-2. Currently, we are investigating the localization and regulation of additional proteins involved in the sister chromatid cohesion release pathway.

256A Meiotic roles of FANCM-related helicases in *C. elegans*

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Meiosis is a special type of cell division to generate haploid gametes from diploid parental cells. Crossover formation between a homologous chromosome pair is essential for proper chromosome segregation during first meiotic division. Crossover formation of *C. elegans* is regulated as only single DNA double-strand break is directed to form a crossover from nearly 10 DNA double-strand breaks along chromosomes. The location bias is also observed, that is a single crossover has a strongly tendency to locate in one of each arm region of chromosomes.

Fanconi anemia is a congenital disorder which causes defects of DNA replication and repair in born marrow. It is known that one of Fanconi anemia genes, FANCM, is required for limit crossover formation in plants and yeast to disrupt D-loop formation after the step of single-end invasion during homologous recombination. Specially FANCM homologs in plants and fission yeast are involved in inhibition of class II crossovers which are formed not through crossover designation and interference.

C. elegans has two FANCM related helicases, DRH-3 and FNCM-1. Here we present the basic phenotypes of *drh-3(fj52)* and *fncm-1(tm3148)* mutants. *drh-3* mutants are sterile and have small gonads with extended transition zone and less pachytene cells. No cross-progenies were observed by crossing *drh-3/drh-3*hermaphrodite with wild-type male and *drh-3/drh-3* male with wild-type hermaphrodite respectively. Because immunostaining of major sperm protein was positive in *drh-3* hermaphrodite gonads, spermatogenesis itself occurs in *drh-3* mutants. These results suggest that both oogenesis in hermaphrodite and spermatogenesis in male are defective in *drh-3* mutants. No obvious defects regarding plate phenotypes showed in *fncm-1(tm3148)* mutants, so far. We are trying to measur and observe the crossover type, frequency and distribution in the mutants by SNP genotyping and cytology of procrossover factors.

257B Knockdown of Bora homolog *spat-1* results in crossover and synapsis defects in *C. elegans* meiotic prophase

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Chromosomes that have undergone crossing over in meiotic prophase must maintain sister chromatid cohesion somewhere along their length between the first and second meiotic divisions. To accomplish this, the holocentric organism *Caenorhabditis elegans* creates two chromosome domains of unequal length termed the short arm and long arm, which become the first and second site of cohesion loss at meiosis I and II. The mechanisms that confer distinct functions to the short and long arm domains remain poorly understood. Previously we and others have shown that phosphorylation of SYP-1, a central element of the synaptonemal complex (SC), at Thr452 provides a binding site for a Polo-like kinase PLK-2, and phosphorylated SYP-1 and PLK-2 cooperatively localize to the short arm to guide downstream factors triggering cohesin degradation at the short arm (Sato-Carlton et al. 2017; Brandt et al. 2020). Previous studies have shown that Polo kinase is activated via phosphorylation of its activation loop by Aurora kinase, and this interaction is promoted by Bora/SPAT-1 in mitosis (Tavernier et al. 2015). To understand the mode of Polo-like kinase regulation during meiotic prophase, we examined the effect of *spat-1* knockdown during oogenesis. We found that PLK-2 failed to spread to short arms but instead was confined at crossover designation sites in *spat-1* RNAi gonads. In addition, we found that homologous chromosome synapsis and crossover formation are impaired in *spat-1* RNAi animals. Interestingly, excess crossover designation, ranging from 6 to 13 sites per nucleus, was found in *spat-1* RNAi animals. Computational tracing of three-dimensional chromosome images revealed the presence of multiple crossover designation sites on the same chromosome, suggesting that crossover interference is impaired. These observations are reminiscent of *plk-2* mutant phenotypes, and suggest the possibility that SPAT-1 regulates PLK-2 during meiotic prophase.

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258C DNA replication and chromosome decondensation occur concurrently in *C. elegans* germ cells

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The abbreviated cell cycle is a form of the mitotic cell cycle in which cells pass quickly and constitutively through the G1 phase. Rapid passage through G1 is notable because it causes increased genome instability when induced aberrantly in cells without abbreviated cell cycles, as well as in cancerous cells. How cells that normally rely on the abbreviated cell cycle avoid this increased genome instability is unclear. Here we examined passage through G1 in cells with an extreme form of the abbreviated cell cycle: *C. elegans* mitotically dividing germ cells. We report that *C. elegans* germ cells pass through G1 without decondensing their chromosomes. Chromosomes remain condensed throughout G1 and only decondense in S phase, once chromosomes begin to replicate. This pattern mirrors the cell-cycle program in the early embryo. This work suggests that *C. elegans* germ cells might avoid genome instability by retaining features of the mitotic cell cycle characteristic of the early embryo.

259A RACK-1 is required for proper GLD-1 sub-cellular localization and function

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Stem cells are central to the development of multi-cellular organisms, including *C. elegans* and humans. Key to their function is their ability to differentiate into specialized cells or proliferate to maintain their population for future use. We are focusing on identifying the molecular mechanisms that regulate the proliferation/differentiation decision of stem cells using the *C. elegans* germ line as a model. *C. elegans'* germline stem cells (GSCs) proliferate to maintain the stem cell pool and differentiate to produce gametes. The conserved STAR family, RNA binding protein, GLD-1/Quaking, promotes differentiation and is required to maintain a proper balance between proliferation and differentiation. When *gld-1* activity is lost, along with *gld-2* activity, which functions redundantly with *gld-1* to promote differentiation, a germline tumour of proliferating stem cells is formed. Previous research has shown that the pattern of GLD-1 germline accumulation is key to controlling its activity. We have found that GLD-1 subcellular localization is likely also involved in regulating GLD-1's activity. We identified *rack-1* as a regulator of GLD-1 subcellular localization. *rack-1* mutants have a severe disruption in GLD-1's subcellular localization; while wild-type GLD-1 is cytoplasmic, in *rack-1* mutants GLD-1 localizes to germ granules. This disruption in localization appears to impact GLD-1's activity, as a loss of *rack-1* phenocopies a reduction, or loss, of *gld-1* activity in various genetic backgrounds. Our research is revealing a novel mechanism where the activity of GLD-1 is regulated by its subcellular localization. This provides an additional layer of regulation in the proliferation/differentiation decision of *C. elegans* GSCs.

260B Characterization of stress-induced phase transitions in the *C. elegans* germline

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Many RNAs and RNA-binding proteins undergo condensation to form ribonucleoprotein (RNP) granules in the germline. The triggers and regulation of condensate dynamics are not yet well understood; however, stresses such as extended meiotic arrest can affect condensate size. During our investigation of regulators of RNA-binding protein (RBP) condensation, we observed unexpected dispersal of PGL-1 protein in control experiments. Using the DAF-16::GFP stress reporter, we determined that our imaging methods were inadvertently inducing a stress response. When worms were imaged immediately after slide preparation, DAF-16 remained cytosolic; however, extended time on agarose pads induced nuclear translocation of DAF-16. Our preliminary results indicate this imaging stress induces condensation of MEX-3 and CGH-1, dispersal of PGL-1 and GLH-1, and has no effect on TIAR-2, PAB-1, and MEG-3. In arrested oocytes, this stress results in dispersal of PGL-1, but not of MEX-3 and CGH-1. As a start to characterize the imaging stress, a time course study revealed that DAF-16 trans-locates to nuclei within 20 minutes; therefore, we conclude researchers studying condensation of RBPs need to be cautious and rigorous in their imaging methods. We are currently testing additional stress reporters to further characterize the type of stress inducing phase transitions. We have also begun investigating the question of why RNA binding proteins in arrested oocytes respond

differently to stress by characterizing their properties. Our preliminary results suggest the large PGL-1 granules in arrested oocytes, but not MEX-3 or CGH-1 granules, are sensitive to hexanediol, indicating hydrophobic interactions are required for PGL-1 granule integrity. In heat stress experiments, MEX-3 condensation increases in both arrested and non-arrested oocytes. However, PGL-1 becomes dispersed in non-arrested oocytes. We are also currently performing FRAP experiments to determine the mobility of these RBPs in large RNP granules. Taken together, we conclude that heat stress and our inadvertent imaging stress differentially affect the condensation of RNA-binding proteins in the germline. The differences among RBPs correlate with the degree of their liquid-like properties based on preliminary hexanediol data. Our results also suggest that the large RNP granules in arrested oocytes may contain multiple phases, similar to what has been observed for P granules in embryos.

261C Characterizing the function of the histone H3 kinase HASP-1 in the germline

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Haspin is a protein kinase that phosphorylates histone H3 at threonine 3 during mitosis and meiosis. Together with the activity of another histone kinase Bub1, haspin-dependent histone phosphorylation (H3T3ph) promotes recruitment of the Chromosomal Passenger Complex (CPC) to chromosomes, which activates the Aurora B kinase subunit of the CPC. The function and regulation of haspin outside of mitosis remains less well characterized. It is not known whether the mechanism by which haspin is activated that was established in vertebrate systems is used in a wide variety of cell types and it is unclear how the activities of Bub1 and haspin are coordinated in the context of meiotic chromosome and kinetochore structure. Haspin inhibition in mouse oocytes has been shown to reduce the recruitment of the CPC to chromosome axes but not kinetochores, suggesting that there are two pools of CPC recruited to meiotic chromosomes by distinct mechanisms (Nguyen et al. 2014). In *C. elegans*, depletion of the haspin homolog *hasp-1* has been shown to reduce the CPC to undetectable levels in diakinesis oocytes (Ferrandiz et al. 2018), suggesting that *C. elegans* BUB-1 activity may play a less important role in CPC recruitment to meiotic chromosomes in oocytes. To better understand the coordination between Bub1 and haspin, we are investigating the roles of *hasp-1* in CPC recruitment in the different types of cell divisions in the *C. elegans* germline. We generated a conditional *hasp-1* allele using the auxin-inducible degron system (Zhang et al. 2015), which allows us to distinguish the effects of *hasp-1* depletion during mitosis in germline stem cells, oocyte meiosis, and spermatogenesis. We found that phenotypes caused by *hasp-1* depletion during oocyte meiosis are much more severe than during spermatogenesis or stem cell proliferation, suggesting that *bub-1* is able to compensate for *hasp-1* loss in mitosis and in spermatocyte meiosis. We are testing this hypothesis by combining *hasp-1* depletion with mutations expected to reduce *bub-1* function and monitoring the effects on CPC recruitment and chromosome segregation in the germline.

262A Revealing hidden roles of RAD-54.B during meiotic prophase

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In meiosis, efficient repair of programmed double-strand breaks (DSBs) is crucial for genome integrity and faithful chromosome segregation. We identified RAD-54.B as factor important for normal survival following radiation exposure, and we are investigating its functions during meiotic prophase. RAD-54.B is a member of the SNF-2/SWI-2 ATPase/helicase protein family, and its paralog RAD-54.L is essential for repair of meiotic DSBs. Whereas *rad-54.L* mutants exhibit 100% progeny lethality, reflecting failure to remove recombinase RAD-51 from processed meiotic DSBs and failure to form post-strand-exchange recombination intermediates, *rad-54.B* mutants exhibit nearly wild-type (WT) progeny viability, reflecting successful formation of crossovers (COs) between homologous chromosomes. However, there are two notable abnormal features of the meiotic program in *rad-54.B* mutants. First, *rad-54.B* mutants exhibit hyperaccumulation of RAD-51 foci during the early pachytene stage. Despite highly elevated levels of chromosome-associated RAD-51, however, post-strand-exchange recombination intermediates marked by MSH-5 occur with timing and numbers comparable to WT, and in contrast to *rad-54.L* mutants, transition to the late pachytene stage occurs with normal timing in a *rad-54.B* mutant and is accompanied by disappearance of hyperaccumulated RAD-51. Second, CO distribution as assayed genetically is altered in a *rad-54.B* mutant; however, cytological measurement of positions of late pachytene COSA-1 foci along chromosome axes suggest that CO designation and CO interference are normal. Immunofluorescence analysis reveals that endogenously-tagged RAD-54.B::GFP localizes to chromosome-associated foci in early pachytene, sometimes colocalizing with markers of inter-homolog recombination intermediates; RAD-54.B::GFP foci diminish in number upon exit from early pachytene and are absent from CO-

designated sites in late pachytene. We are currently testing the hypothesis that RAD-54.B antagonizes association of RAD-51 with unbroken double-stranded DNA, a function that RAD-54.L may be able to carry out if meiotic DSBs are absent. In addition, we hypothesize that RAD-54.B also functions at meiotic DSB repair sites in cooperation and/or competition with RAD-54.L to influence the structure of DSB repair intermediates and repair outcomes.

(*KY and BW contributed equally)

263B Meiotic cell cycle progression requires adaptation to a constitutive DNA damage signal

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Defects in crossover formation lead to chromosome missegregation during meiosis. The mechanisms that ensure crossover formation and coordinate crossover designation with meiotic progression remain poorly understood. In *C. elegans*, defects in homolog pairing, synapsis, or recombination delay meiotic progression and prolong the activity of CHK-2, a meiosis-specific ortholog of the canonical DNA damage checkpoint kinase that plays essential roles during early prophase. We have now found that CHK-2 activity is both necessary and sufficient to inhibit crossover designation. CHK-2 is normally inactivated at mid-pachytene, but persists under conditions that prevent or delay the establishment of crossover precursors on one or more chromosomes. The pathway that mediates CHK-2 inactivation in wild-type or crossover-deficient conditions has not been established.

We find that CHK-2 is inactivated and destabilized through inhibitory phosphorylation by Polo-like kinases (PLKs), a mechanism previously implicated in adaptation to DNA damage checkpoint signaling in proliferating cells. Recruitment to the synaptonemal complex and establishment of crossover precursors activate PLKs to phosphorylate CHK-2 and drive meiotic progression. Additionally, we find that stepwise reduction of CHK-2 activity enables the rapid, concerted designation of crossover sites on all chromosomes at mid-pachytene.

These findings reveal that a key transition during the meiotic cell cycle occurs through adaptation to a “constitutive” DNA damage response pathway that is implemented at meiotic entry. Evidence from budding yeast suggests that the Polo-like kinase Cdc5 may similarly promote inactivation and degradation of Mek1, a CHK-2 ortholog, and data from mice are also consistent with such a mechanism. Our work further clarifies the signaling network that coordinates chromosome dynamics, double-strand break formation, and recombination during meiotic prophase.

264C Removal of cell body haze with inverse square fit

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Unwanted background fluorescence in microscopy can occur when light emitted by fluorescent structures is scattered by nearby tissues. In our *in vivo* imaging of green fluorescent protein (GFP)-tagged neurons in the roundworm *C. elegans*, scattered light produces a haze surrounding the cell body that can obscure the imaging of target structures, such as an axon or dendrite. The thin fibers appear dimmer than the much larger cell body and cannot be clearly visualized due to the low contrast between it and the bright background.

Here, we describe a method to model and remove the cell body haze utilizing an inverse square intensity distribution. Such distributions are common in nature and can describe the intensity of light that emanates away from a point or sphere, such as a fluorescent cell body. Assuming that scattering is proportional to intensity, it follows that an inverse square distribution also describes the intensity of the scattered light.

Utilizing this model, we have developed a post processing procedure to subtract background from an image. Removal of the haze surrounding the bright cell body enhances contrast of the dim axon, particularly in the region close to the cell body. Preliminary algorithms demonstrate a signal to background ratio improvement of >5x. This improvement in image quality allows us to more clearly visualize the axon.

We intend to broadly disseminate this technique via an ImageJ extension and in MATLAB. We may further release our fitting technique on other imaging platforms. We are also investigating methods to accelerate the computations for real-time image improvement.

266B Autonomous Adaptive Data Acquisition for Scanning Hyperspectral Imaging in *Caenorhabditis elegans*

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Non-invasive and label-free spectral microscopy (spectromicroscopy) techniques can provide quantitative biochemical information complementary to genomic sequencing, transcriptomic profiling, and proteomic analyses. However, spectromicroscopy techniques generate high-dimensional data; acquisition of a single spectral image can range from tens of minutes to hours, depending on the desired spatial resolution and the image size. This substantially limits the timescales of observable transient biological processes. To address this challenge and move spectromicroscopy towards efficient real-time spatiochemical imaging, we developed a grid-less autonomous adaptive sampling method. Our method substantially decreases image acquisition time while increasing sampling density in regions of steeper physico-chemical gradients. When implemented with scanning Fourier Transform infrared spectromicroscopy experiments, this grid-less adaptive sampling approach outperformed standard uniform grid sampling in a two-component chemical model system and in a complex biological sample, *Caenorhabditis elegans*. We quantitatively and qualitatively assess the efficiency of data acquisition using performance metrics and multivariate infrared spectral analysis, respectively.

267C Light-induced protein clustering to study protein-protein interactions in *C. elegans*

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Interactions among proteins are fundamental for life, and misregulation of them can lead to diseases such as cancer. We adapted the light-activated reversible inhibition by assembled trap (LARIAT) system and the light-induced co-clustering assay for use in *C. elegans.* This assay can rapidly and unambiguously identify protein-protein interactions between pairs of fluorescently tagged proteins. In the LARIAT protein-protein interaction assay, cryptochrome 2 (CRY2) and cryptochrome-interacting bHLH 1 (CIB1) bind each other in a blue-light dependent manner, forming small but densely packed protein clusters within the cell. Fusion of an anti-GFP nanobody to the CRY2 protein traps any GFP-tagged protein in the clusters. Co-clustering of a mCherry/mScarlet fluorescent protein can be monitored for inclusion in the CRY2/GFP clusters, indicating an interaction between the two fluorescently tagged proteins. We tested the system in *C. elegans* with an array of positive and negative reference protein pairs, using endogenously tagged GFP and mCherry/mScarlet alleles. CRY2/CIB1 proteins were expressed from extrachromosomal arrays since genome integration is unnecessary for the assay. Light-induced clusters form rapidly within seconds in multiple tissue types. Assay performance was extremely robust with no false positives detected in the negative reference pairs, and no lethality associated with the clustering, even with proteins essential for development and cell polarity. We have generated a toolkit containing plasmids with different promoters, an anti-mCherry nanobody variant, and a plasmid with an empty promoter module to enable one-step cloning of any particular promoter using SapTrap assembly. The light induced co-clustering assay is a powerful and rapid technique that will be valuable for the *C. elegans* community, especially due to the fact that the assay uses a universal plasmid for every protein pair, and existing fluorescently tagged strains can be used without need for additional cloning or genetic modification of the genome.

268A High-throughput phenotypic screening to identify neurotoxic chemicals causing neuro-degeneration

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Direct exposure of humans to different chemicals becomes more prevalent as society is progressing further into industrialization. In addition, an increasing number of medical drugs are being developed at different non-clinical and clinical stages aiming to treat a variety of conditions. In order to protect the public from potential deleterious effects of these chemicals, toxicology analysis is necessary to ensure early identification of toxic effects. The most common type of chemically-induced toxicity is neurotoxicity. Therefore, screening for chemical compounds, which can cause specific neuronal damage, can help identify toxic chemicals and potentially help elucidate specific neurodegeneration mechanisms, which can lead to the development of novel targeted therapeutic approaches. Current neurotoxicity assays rely mainly on mammalian models' mortality tests and are associated with high screening costs and long experimental times. To overcome these limitations, we developed a high throughput *in vivo* neurotoxicity assay using *C. elegans*. We screened animals treated with a number of well-characterized reference chemicals using Newormics' proprietary microfluidic device (*vivoChip*®), which enabled us to perform high-resolution imaging and multi-parametric structural analysis of GFP-labeled neurons in a high-throughput manner.

We characterized the chemical-induced neurotoxicity in the dopaminergic, cholinergic, GABAergic, and serotonergic neurons generating a complete set of imaging data for each reference chemical. Semi-automatic analysis of this dataset identified the cellular and sub-cellular neuronal defects and created neuron-specific degeneration metrics for the reference chemicals. We hope to gain valuable insights into potential mechanisms of action for these neurotoxic compounds while at the same time progressing towards a more complete screen through an increasing number of chemicals using our high-content and high-throughput system.

269B A simple and inexpensive add-on enables confocal imaging capacity on a widefield microscope

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Widefield microscopes are commonly used in many biological laboratories. However, their inability to reject scattered or out-of-focus light often produces images with obscured thin or dim structures when there are some bright structures nearby. Scanning microscopes like confocal microscopes and multiphoton microscopes can solve this problem, but they are comparatively slower and much more expensive. We developed an inexpensive way to empower confocal imaging capacity on a widefield microscope, by inserting a spatial light modulator (SLM) into the field stop of the widefield microscope and customizing the illumination pattern and acquisition methods. We assessed the performance of this SLM-inserted setup by comparing images taken at our widefield microscope, our widefield microscope with the SLM-inserted setup, and a commercial confocal microscope. While a widefield microscope showed no sectioning capability, our SLM-inserted setup showed 0.85 ± 0.04 µm and the commercial confocal showed 0.68 ± 0.04 µm optical sectioning capability. Additionally comparing images of the FLP neuron and the tightly bundled amphid neurons in *C. elegans* taken by the widefield, SLM-inserted setup, and confocal microscopes, we confirmed that the SLM-inserted setup greatly reduces haze from the bright cell body, allowing visualization of dim axons and dendrites nearby. Our SLM-inserted setup identified 96% of the dim neuronal fibers seen in confocal images while the widefield microscope only identified 50% of the same fibers. Our SLM-inserted setup represents a very simple (2-component) and inexpensive (<$600) approach to enable confocal capacity on a widefield microscope. This SLM-inserted setup can be broadly employed by labs that are using widefield microscopes, with minimum expense and modification.

270C The role of novel identified regulator, SFXN-1.2 in mitochondrial dynamics in neurons and establishing linked neurological disease models

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Various neurological diseases are linked to changes in mitochondrial dynamics in neurons. Thus, it is critical to understand how the dynamics of mitochondria are regulated on the molecular level. From a candidate screen (95 genes), we have identified a novel gene called sfxn-1.2 (ortholog of human Sideroflexin 1/3), a mitochondrial protein enriched in neurons associated with Alzheimer's disease and Parkinson's disease. SFXN1 also interacts with Cx32, a protein associated with Charcot-Marie-Tooth motor neuron disease. Since the function of sfxn-1.2 in mitochondrial transport at the molecular level is unknown, we aim to dissect the function and the molecular pathways of SFXN-1.2 in mitochondrial dynamics and its effects on worm behavior and neurodegeneration. Through cluster analysis and Kymograph assays, we observed that SFXN-1.2 is associated with significant changes in mitochondrial morphology and trafficking in neurons. Our results suggest a possible direct interaction between UNC-104 and mitochondria. The role of UNC-104 (KIF1A) in mitochondrial transport will have a strong impact because since decades it is thought that KIF5 and KIF1Bα are the only transporters of mitochondria. A crucial result is that the effect of sfxn-1.2 seems to be indeed specific for mitochondria such as this mutation did not affect the transport of SNB-1 (synaptobrevin-1), a common marker for "synaptic transport vesicles" and we provide clues that kinesin-1 and kinesin-3 may cooperate in transporting mitochondria. Our findings also suggest the relation between sfxn-1.2 and unc-104 at genetic and protein levels. Our results indicate that no genetic relation exists between sfnx-1.2 and fusion/fission genes as well as that sfxn-1.2 does not affect mitochondrial respiration. Neurodegeneration is common in Alzheimer's disease and Parkinson's disease; our data reveal possible motor neuron defects and sensory neuron defects in sfxn-1.2 mutants. Thus, these investigations will aid to develop novel drugs to target neurological diseases based on defects in mitochondrial transport.

271A Oxidative regulation of cholesterol transport in *Caenorhabditis elegans*

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Cholesterol is an essential metabolite present in virtually all eukaryotic organisms in which mediates highly relevant biological processes such as the regulation of membrane fluidity and the synthesis of steroid hormones and bile acids. Therefore, proper trafficking of this molecule to different subcellular locations is crucial for cell viability and correct organism functioning. In this sense, non-vesicular cholesterol transport is mediated by a multi-domain membrane protein called STARD3 that binds cholesterol through its cytosolic domain (START). While there is a significant amount of information related to the physiological processes associated with cholesterol metabolism, the regulatory events responsible for STARD3-mediated transport remain unknown. Recently, it has been reported that STARD3 co-localizes and interacts with methionine sulfoxide reductase A, an enzyme that reduces methionine sulfoxide side-chains, suggesting that methionine oxidation could modulate the sterol binding properties of the START domain and cholesterol transport. These findings have been obtained in cultured mammalian cells which lack the complex tissue context present in higher organisms and all its associated biological activities, emphasizing the need for an animal model to understand these biological processes in vivo. We are using the nematode *Caenorhabditis elegans* to study the molecular events associated with the regulation of cholesterol mobilization mediated by STARD3 and its interaction with MSRA. First, we devised an NMR-based assay to monitor methionine sulfoxide reductase activity in *C. elegans* extracts and established that these extracts have prominent MSRA activity. Next, we titrated cholesterol to 15N isotopically-enriched START and followed the interaction by 1H-15N HSQC NMR experiments. Our results clearly showed the formation of a high-affinity complex, confirming that cholesterol binding to STARD3 is conserved in *C. elegans*. We will use this set of tools to characterize with high-resolution the interaction between MSRA and START in vitro and in worm extracts. Complementary genetic and biochemical experiments will allow us to dissect the role of START and MSRA in non-vesicular cholesterol mobilization.

272B Insight into the effect of tubulin post-translational modifications on axonal transport

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A remarkable number of post-translational modifications (PTMs) on tubulin and microtubules (MTs) have been identified that affect MT dynamic, stability, organization, and its interaction with motor proteins (such as kinesin and dynein) in neurons. Dysfunctional axonal transport has a large impact on synaptic transmission affecting memory and synaptic plasticity often leading to neurological disorders. I investigate the role of three different PTMs glutamylation, tyrosination, and acetylation on axonal transport of UNC-104 (kinesin-3/KIF1A) and its cargo RAB-3, used *C. elegans* as a model organism, used motor motility assays, *in situ* immunostaining, co-immunoprecipitation assays, and *in vivo* protein-interaction assays (BiFC), etc.

For statistical analysis, I used one-way ANOVA and t-test to analyze the results. In polyglutamulase mutants (TTLL-11) worm's velocities and run lengths of both UNC-104 and its cargo RAB-3 were significantly reduced as compared to wildtype. Further, tubulin polyglutamylation in worm lysates after TTLL-11 knockout consistent with reduced fluorescence in whole-mount staining using Poly E GT335(Sigma) antibodies. On the other hand, knocking out deglutamylase CCPP-1 does neither affect motility for both motor and its cargo. Interestingly, UNC-104 interacts with glutamylase enzymes in *ccpp-1* mutant worm lysates when employing in co-IP assays. Additionally, in worms carrying a mutation in tyrosine ligase TTLL-12, kinesin-3 UNC-104 and its cargo RAB-3 exhibit reduced motility and increased pausing times compared to wildtype. Similarly, in acetylation mutant worm's mec-17 the velocity and run lengths of both UNC-104 and its cargo RAB-3 were also significantly reduced consistent with observations on kinesin-1 by others.

We hypothesize that these changes in axonal transport efficiencies are related to differentially post-translational modified tubulins. We believe that our basic research will provide important mechanistic insights important for drug design to prevent or cure neuronal diseases such as tubulinopathies.

273C A new role for the conserved G-protein regulator RIC-8/Synembryn in primary cilia biogenesis

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Primary cilia are conserved microtubule-based organelles that protrude from the surface of most metazoan cell types including neurons. They house molecular machinery of all major signaling pathways and play central roles in embryonic development, sensory signal transduction, and adult tissue homeostasis. Due to the central role of cilia in signaling, defects in cilia structure and/or function manifest in multisystemic genetic disorders called ciliopathies. Ciliopathy patients commonly present with neurological deficits that include intellectual disability, anatomical brain abnormalities, and impaired sensory functions.

Likewise, cilia and ciliary gene products have been recently associated with neurodevelopmental disorders such as autism spectrum disorder, thereby highlighting the critically important role of cilia in the nervous system.

Proteomic and genomic approaches in different models have contributed to the assembly of the ciliary proteome; however, the mechanisms, by which most of the predicted cilia genes modulate cilia function or cellular properties in neurons remain elusive. In a pilot bioinformatics screen for signaling mechanisms of neuronal cilia assembly, we identified the *resistance to inhibitors of cholinesterase-8* (*ric-8*/Synembryn) – a known regulator of G protein α biology in species ranging from fungi to vertebrates. We find that *ric-8* mutants exhibit marked defects in morphology of complex wing cilia and mild to no defects in assembly of the simple rod-like cilia. Therefore, RIC-8 likely possesses neuron-type-specific functions in ciliogenesis. In line with this hypothesis, GFP-tagged RIC-8 exhibits cell-specific differences in intra-ciliary localization. For example, in PHA/PHB neurons, RIC-8::GFP is detected in the proximal ciliary segment similarly to NPHP-2/inversin. In contrast, RIC-8::GFP appears to be distributed throughout the sensory cilium in the PQR neuron. Here, we will present our ongoing efforts to characterize the role of *ric-8* in regulating neuronal cilia morphology in *C. elegans*.

274A Identification of novel regulators involved in transport of synaptic vesicle proteins

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Neurons function to process and transmit information to target cells through the release of neurotransmitters, which are packaged into small secretory vesicles known as synaptic vesicles (SVs). Various neurological disorders, such as Charcot-Marie Tooth, Parkinson's, etc., are correlated with dysregulation of synaptic vesicle protein trafficking. It is known that pre-synaptic vesicle (pre-SV) proteins are dependent on a Kinesin-3 motor KIF1A/UNC-104 to exit from the cell body. We are attempting to identify additional players in this pathway by cloning genes that genetically interact with *unc-104*. To identify the regulator(s) involved in UNC-104 dependent pre-SV trafficking, we performed an enhancer screen in the background of mild cargo binding defective allele *unc-104(e1265tb120),* in which animals are behaviorally slightly uncoordinated, and pre-SV proteins are elevated in the cell body. After EMS-based mutagenesis, animals were isolated based on both worsened behavior and more pre-SV proteins stuck in the cell body of touch receptor neurons. A total of 16 enhancer mutants in different complementation groups have been isolated from the screen.

One such enhancer, *tb210* shows uncoordinated behavior and reduced accumulation of pre-SV proteins at the touch neuron synapse. 50% of the *tb210* animals shows the absence of the synaptic branch in the posterior touch receptor neurons. Immunostaining against UNC-104 shows reduced levels in the sublateral neuron in *tb210* animals compared to wild type. *tb210* was mapped to the X-chromosome, and whole-genome sequencing (WGS) analysis suggest some possible candidate. I will present the phenotypic characterization of this mutant and its genetic interactions with other known regulators of synaptic vesicle protein trafficking.

275B Intraflagellar transport is required for enrichment of CLHM-1 into a distinct subpopulation of extracellular vesicles released from ciliated sensory neurons

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Extracellular vesicles (EVs) are membrane-wrapped particles that mediate cell-cell communication by transporting proteins, nucleic acids, and metabolites through biological fluids. EVs play roles in many physiological and pathophysiological processes, and their precise function is dependent on molecular cargo and parent cell type. A single cell can release distinct subpopulations of EVs enriched with different molecular cargo, which adds complexity to elucidating cargo sorting and biogenesis mechanisms. In the nematode *C. elegans*, EVs bud from male sensory neuron cilia and are released into the hermaphrodite vulva during mating and the environment to mediate animal communication. We discovered that the calcium homeostasis modulator ion channel CLHM-1 localizes to cilia of EV-releasing neurons and is cargo in EVs released from males and hermaphrodites. Using total internal reflection fluorescence (TIRF) microscopy, we imaged EVs released from animals co-expressing tdTomato-tagged CLHM-1 and GFP-tagged PKD-2, a known EV cargo, in the same male sensory neurons, and observed that CLHM-1 and PKD-2 are significantly enriched in distinct subpopulations. Furthermore, release of these subpopulations is dependent on different sensory cues, as culturing males in the absence of hermaphrodites significantly increased the release of PKD-2::GFP EVs from adult males, but did not affect the number of CLHM-1::tdTomato EVs released. As CLHM-1::tdTomato and PKD-2::GFP do not completely colocalize in neuron cilia, we hypothesize that maintaining discrete separation of these proteins in the cilia is required for their enrichment into distinct EV subpopulations. Intraflagellar

transport (IFT) is required for movement of proteins along the length of the cilia. Anterograde IFT is driven by kinesin motors, including homodimeric kinesin-II OSM-3, kinesin-III KLP-6, and a heterotrimeric kinesin-II complex containing KLP-11. In *osm-3* mutants, colocalization between CLHM-1::tdTomato and PKD-2::GFP in EVs significantly increases, indicating that EV protein cargo enrichment is dependent on OSM-3. Environmental release – but not biogenesis – of both CLHM-1 and PKD-2 EVs is dependent on KLP-6. Currently, we are investigating the role of KLP-11 and the heterotrimeric kinesin-II in EV subpopulation cargo enrichment. In conclusion, our results show that IFT is required for proper packaging and release of ciliary EVs.

276C Analysis of endomembrane-resident zinc transporter mutants that suppress the systemic RNAi defects of *rsd-3* mutant

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Functional RNAs including double-stranded RNA (dsRNA) modulate gene expression in a sequence-dependent manner. In *C. elegans*, experimentally introduced dsRNA spreads over the body and leads to RNA silencing systemically. To date, several genes acting in systemic RNAi have been genetically identified. However, cellular pathways and molecules that mediate RNA transport between cells remain largely unknown. We previously reported that RSD-3/EpsinR is involved in import of silencing RNA. In this presentation, we show an analysis of suppressors for RNAi defects in the *rsd-3* mutants. Through the analysis of the suppressors, we found that a zinc transporter functions as a negative regulator for dsRNA import. Several experiments support the idea that its zinc transport activity is required for negative regulation of systemic RNAi. However, while the *C. elegans* genome contains 28 zinc transporters, mutants for all other zinc transporter genes exhibited a normal response to RNAi, suggesting a unique role of this transporter on RNAi or genetic redundancy. Since disruption of the suppressor gene itself showed enhanced RNAi (Eri) phenotype, we also tested whether this phenotype is caused by downregulation of endogenous RNAi or upregulation of exogenous RNAi, which are antagonistic. We found that certain genes targeted by the endogenous RNAi pathway were not affected in the mutants, excluding the possibility that it acts in the core endogenous RNAi pathway. Instead, its null mutants showed altered RAB-11 positive vesicle sizes in intestine and LMP-1, RAB-7 and 2xFYVE positive vesicle sizes in coelomocytes. From these observations, we speculate that this zinc transporter would be involved in the regulation of membrane trafficking, which in turn negatively regulate exogenous RNAi in parallel or downstream of RSD-3. Our data revealed the zinc transporter as a novel cellular factor functioning in systemic RNAi.

277A Regulation of vesicular trafficking by NEK family kinases

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The mammalian NEKs are a highly conserved family of protein kinases that have been primarily implicated in molecular functions linked to cell division and ciliogenesis. We have shown that two C. elegans NEK family members, NEKL-2/NEK8/9 and NEKL-3/NEK6/7, control clathrin mediated endocytosis in the major epidermis of C. elegans. Specifically, NEKL-2 and NEKL-3 (NEKLs) are required for the internalization of cargo, such as the lipoprotein receptor LRP-1, at the apical plasma membrane. Our data indicate that NEKLs control endocytosis at least in part by promoting the uncoating of newly internalized clathrin-coated vesicles. Loss of NEKL functions lead to a failure of worms to complete molting, resulting in arrested larval development. Attesting to their conserved functions, the mammalian orthologs of NEKL-3, NEK6 and NEK7, are able to rescue molting and trafficking defects when expressed in nekl-3 deficient worms.

To further broaden our understanding of NEK family functions in intracellular trafficking we have analyzed the effects of NEKL depletion on additional cargos and vesicular compartments. We find that in addition to functioning in clathrin mediated endocytosis at the apical membrane, NEKLs also promote clathrin dependent and independent endocytosis at the basolateral membrane in the worm epidermis. Specifically, we find that the class I TGF-beta receptor, SMA-6, and the class II TGF-beta receptor, DAF-4, accumulate to high levels on basolateral membranes following NEKL-3 depletion. In addition, our observations suggest that NEKLs may affect trafficking steps downstream of endocytosis, consistent with the localization of NEKL-3 and NEKL-2 to early and late endosomal compartments, respectively.

To extend our findings from worms to mammals, we have begun to characterize the potential trafficking functions of mammalian NEK6 and NEK7 kinases in cell culture. Current data indicate that NEK7 promotes clathrin mediated endocytosis including the observed reduced uptake of the transferrin receptor following NEK7 siRNA depletion. In addition, NEK7 may affect several other trafficking processes in cells including endosomal recycling, membrane fission, and caveoli-dependent

endocytosis. Collectively, our studies further support a largely overlooked but conserved function for NEK family kinases in intracellular trafficking.

278B TORC1, BORC, and ARL-8 drive tubulation of cell corpse phagolysosomes in *C. elegans* embryos

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Cells clear corpses and debris from their environment using phagocytosis. Once engulfed, cargo degradation inside phagolysosomes takes hours, an important step to provide the cell with metabolites and avoid an auto-immune response in mammals. Using *C. elegans*, we discovered that the phagolysosome that digests the second polar body, a cell that undergoes programmed necrosis after fertilization, tubulates into small vesicles to facilitate cell corpse clearance (Fazeli *et al.*, Cell Rep 2018). Tubulation occurs after the corpse membrane breaks down inside the phagolysosome, exposing the corpse cargo to lysosomal hydrolases. Here, we use time-lapse imaging of the polar body phagolysosome to reveal that tubulation depends on amino acid release by the solute transporter SLC-36.1, which in turn activates TORC1, but not TORC2. TORC1 then activates the BLOC-1-related complex (BORC), which is responsible for recruiting the Arf-like GTPase ARL-8 to the phagolysosome for tubulation. ARL-8 is thought to link the phagolysosome membrane to motor proteins on microtubules to extend tubules and release vesicles. We found that putative GDP-bound, GTP-locked, and fast exchange alleles of ARL-8 show reduced tubulation of the phagolysosome, indicating that ARL-8 GTPase needs to cycle in a regulated fashion to promote tubulation. Further, we found that altering the nucleotide state of ARL-8 with these alleles disrupts ARL-8 localization from lysosomes, mislocalizing ARL-8 to the plasma membrane, enlarged endosomes, or other compartments. Using this *in vivo* model to observe a single phagolysosome over time, we have identified the molecular pathway regulating the key steps of tubulation and vesiculation that promote the timely degradation of cargo. We demonstrate that phagolysosomes are dynamic and transient organelles and predict that TORC1-BORC-Arl8-driven vesiculation is a conserved and important step to promote the appropriate immune response after phagocytosis.

279C MEL-28-mediated regulation of microtubule motors affects oogenic fertility

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Microtubule motors are multi-molecular machines that ferry cargoes within a cell. We have been studying dynein, the minus-end directed microtubule motor, and MEL-28, a conserved protein important for nuclear envelope integrity and post-mitotic rebuilding of the nuclear pore. *dhc-1(or283ts);mel-28(t1684)* double mutants have a severely reduced brood size compared to each single mutant. This suggests that MEL-28 and dynein act in parallel to support a process necessary for fertility.

Reciprocal crosses between wild-type animals and *dhc-1;mel-28* double mutants revealed that the brood size impairment is caused by oogenic germ line defects and not sperm defects. Indeed, *dhc-1;mel-28* double mutant adults have a disorganized proximal gonad and oocyte maturity defects. To find cellular components that regulate MEL-28/dynein supported processes, we did a candidate RNAi screen searching for genes that rescue the brood size of *dhc-1;mel-28* double mutants. We found that disruption of *klc-2,* which encodes the light chain of the plus-end-directed microtubule motor kinesin-1, increases the brood size of *dhc-1;mel-28* double mutants. This suggested that simultaneous disruption of MEL-28 and dynein causes excessive plus-end-directed movement of a cargo that is rescued by impairing plus-end-directed motion via *klc-2* RNAi. Kinesin and dynein work together through physical interactions with KASH-domain protein UNC-83 to regulate nuclear positioning. We tested the idea that aberrant UNC-83-dependent nuclei positioning in *dhc-1;mel-28* double mutants contributes to the low brood size defect. Connectivity between dynein and UNC-83 is mediated via dynein adaptors NUD-2 and BICD-1. In *bicd-1;nud-2* double mutants, the connection between UNC-83 and dynein is defective. If *dhc-1;mel-28* animals are infertile because UNC-83-dependent minus-end directed nuclear movement is impaired, then we would expect that *mel-28;bicd-1;nud-2* triple mutants to have a low brood size. Indeed, the triple mutant shows a lower brood size than *bicd-1;nud-2* double mutants and *mel-28* single mutants, supporting the idea that defects to *mel-28* sensitize the oogenic gonad to defects with UNC-83-dependent minus-end-directed movement of the nuclei. These observations suggest that MEL-28 and dynein-mediated connections to UNC-83 work collaboratively to position nuclei for proper oogenic development.

280A Sorting of different dense core vesicle cargos in the same neuron

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The regulated release of monoamines and neuropeptides from dense-core vesicles (DCVs) is essential for the modulation of metabolism, behavior, emotions, memory, and cognition. DCV biogenesis begins at the trans-Golgi network and culminates in docking at the cell membrane, but the precise mechanisms of DCV cargo sorting and maturation remain elusive. Additionally, some neurons have multiple kinds of DCVs carrying different cargos and that are released at distinct sites. As a model for learning how cells sort cargos to distinct DCVs, we are using the ASI sensory neurons that modulate nociceptive behavior in *C. elegans*. The ASI neurons express over 20 different peptides that localize to different parts of the cell, including the *nlp*-9 and *nlp-18* neuropeptides which are trafficked mainly to the axon and the cell body, respectively. Prior work suggests that these two peptides are found in distinct DCVs that are released under different conditions. We crossed worms with GFP tagged *nlp-9* and *nlp-18* to worms with known mutants of DCV biogenesis such as *hid-1*, *eipr-1*, *rund-1*, and *cccp-1*, and found that the mutants had significantly decreased levels of *nlp-18* in the ASI cell body and axon compared to wildtype worms. This result is consistent with our previous observation that these mutants have decreased levels of another DCV cargo, *nlp-21*, in the dorsal nerve cord. However, the levels of *nlp-9* in both the ASI cell body and axon remained largely unchanged in all the mutants compared to the wildtype, indicating that the sorting and maturation of *nlp-9* DCVs is not affected by the genes in our known pathway. This result suggests that a different pathway might be at play in the sorting and maturation of *nlp-9* DCVs.

281B The KASH-independent role of ANC-1 in positioning organelles in *Caenorhabditis elegans*

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The LINC complexes formed by outer nuclear KASH proteins and inner nuclear SUN proteins play important roles in connecting nuclei to the cytoskeleton. ANC-1, an ortholog of Nesprin-1/2, is proposed to physically tether nuclei to the actin cytoskeleton through its KASH domain at the C-terminus and the calponin homology (CH) actin-binding domains at the N-terminus. *anc-1* null mutants show severe nuclear positioning defects in the hypodermal syncytial cells in *C. elegans*. However, we found that the CH domains were dispensable for hyp7 nuclear positioning and disruption of SUN/KASH interaction only caused mild nuclear positioning defects. In addition, in *anc-1* null mutants, the ER, mitochondria, and lipid-droplets were unanchored and sometimes moved freely in the cytoplasm. ANC-1 contains six tandem repeats predicted to form spectrin-like structures. Deletion of the spectrin-like region caused strong nuclear and ER anchorage defects. KASH proteins also contain a transmembrane domain adjacent to the luminal KASH domain, we found that the deletion of the transmembrane domain enhanced the nuclear or the ER anchorage defects of the KASH deletion mutant. In the hyp7, ANC-1 had a similar distribution pattern as the ER, and deletion of the tandem repeats strongly affected ANC-1's localization. Here, we propose a cytoplasmic integrity model where ANC-1 functions in positioning nuclei, ER, mitochondria, and likely other organelles in place, probably through the spectrin-like region.

282C Uniform mitochondrial positioning in *C. elegans* touch receptor neurons is regulated by actin and contributes both to cytosolic calcium dynamics and touch responsiveness

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Mitochondria are distributed throughout the neuron and perform important functions like ATP production, calcium buffering and regulation of apoptosis. Mitochondria are enriched at synapses and its trafficking is known to be dependent on both cytosolic and mitochondrial calcium. In the touch receptor neurons (TRNs) of adult *C. elegans*, we see that mitochondria are uniformly distributed and this uniform distribution is achieved as the animal transitions from larval stage 1 (L1) to larval stage 2 (L2). We also observe that mitochondria are positioned such that the median distance between two adjacent mitochondria is ~20 µm. How this mitochondrial distribution is established and maintained and what roles do axonal mitochondria play, both remain unknown. Prior studies demonstrated that >80% of mitochondria along the neuronal processes are localized at F-actin-rich regions suggesting that an actin dependent mechanism might play a role in axonal mitochondrial distribution. Indeed, our data reveal that the transition to uniform mitochondrial distribution correlates with a change in F-actin dynamics and the distribution of actin-rich regions in TRN processes between L1 and L2 stages. To investigate the interplay between actin and mitochondria in more detail, we assessed both the role of mitochondria on neuronal actin and the role of actin on neuronal mitochondrial distribution. In *ric-7* mutants that lack mitochondria in the neuronal process, actin dynamics is altered that is restored upon artificially driving mitochondria into *ric-7* neuronal processes. Additionally, in the absence of actin-rich regions achieved by the expression of DeAct (Disassembly-promoting, encodable Actin tool)

in TRNs

decreases the distance between adjacent mitochondria and affects mitochondrial transport properties. Upon photo stimulation of the neuron we see that mitochondria along the axonal process buffer cytosolic calcium in a MCU-1 dependent manner. Additionally, we found that the presence of mitochondria, F-actin-rich regions and MCU-1 mediated calcium uptake all modulate calcium dynamics upon photo stimulation as well as the touch responsiveness of the animal. These data suggest that axonal mitochondria plays a role in regulating actin dynamics in the neurons and both mitochondria and actin dynamics are important for modulating touch responsiveness.

283A *C. elegans* modeling and human studies of a rare *RAB5B* patient variant reveal a novel role of RAB5B in regulated secretion of pulmonary surfactant

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Many patients with severe, chronic diseases remain without a diagnosis despite extensive medical evaluation, including some cases with clinical exome sequencing. The goal of the NIH-funded Undiagnosed Diseases Network (UDN) is to provide a diagnosis for these challenging cases and to identify biological characteristics of newly discovered disease genes. The UDN uses a collaborative multidisciplinary approach that combines comprehensive medical workup, exome/genome sequencing, bioinformatic analysis, with functional studies in model organisms including zebrafish, *Drosophila*, and *C. elegans*.

Through the UDN, we evaluated a child with interstitial lung disease suggestive of surfactant deficiency. Variants in known surfactant dysfunction disorder genes were not found in trio exome sequencing. Instead, a *de novo* heterozygous variant, p.Asp136His, in the Ras/Rab GTPases family nucleotide binding domain in *RAB5B* was identified. Functional studies were performed in the *C. elegans* Model Organism Screening Center at Washington University in St. Louis. We used CRISPR/Cas9 to knock the proband variant into the conserved position (Asp135) of the ortholog, *rab-5*. Analyses of organismal phenotypes such as locomotion and size demonstrated that *rab-5*[Asp135His] is damaging. We also show data indicating that *rab-5*[Asp135His] heterozygotes were defective in endocytosis and early endosome (EE) fusion. Dosage analysis by adding extra copies of wild type *rab-5* transgene revealed that *rab-5*[D135H] has a strong dominant negative effect, requiring three wild type copies to suppress the variant's poisonous effect.

Immunostaining studies of the proband's lung biopsy revealed that RAB5B and EE marker EEA1 were significantly reduced in type II pneumocytes, and mature SP-B and SP-C were significantly reduced, while ProSP-B and ProSP-C were normal. Furthermore, staining of normal lung showed co-localization of RAB5B and EEA1 with ProSP-B and ProSP-C. These findings indicate that dominant negative-acting RAB5B Asp136His and EE dysfunction cause a defect in processing/trafficking to produce mature SP-B and SP-C, inducing interstitial lung disease, and that RAB5B and EEs normally function in the regulated surfactant secretion pathway.

Together, the data suggest a non-canonical function for RAB5B and identify *RAB5B* p.Asp136His as a genetic mechanism for surfactant dysfunction disorder.

284B Examination of P5B ATPase function *in vivo*

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P5B ATPases are evolutionarily conserved transporter proteins whose principal function is to pump polyamines from extracellular or vesicular spaces into the cytosol. The *C. elegans* genome encodes three paralogous P5B ATPase transporter proteins, CATP-5, CATP-6 and CATP-7. Each of these proteins has a distinct expression pattern and is likely to have a specific physiological function. For example, CATP-5 localizes to the intestinal brush border and is required for uptake of polyamines from the intestinal lumen. CATP-7 localizes to the basolateral membrane of the excretory cell. Since *catp-7(0)* mutants arrest when grown on polyamine-supplemented plates, CATP-7 probably pumps polyamines from the pseudocoelomic fluid into the excretory cell cytosol. CATP-6 localizes to endosomal vesicles and may be required to purge these of residual polyamines derived from the extracellular space. In order to better understand when and where the P5B ATPases are active, we are developing tagged versions that exhibit altered fluorescence properties in response to conformational changes. We are also performing screens to search for genes required for the transport of polyamines in the opposite direction, from the cytosol into the extracellular/vesicular space.

285C Interpreting human missense variants of unknown significance (VUS) in the nematode orthologue of ciliopathy-associated genes

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Ciliopathies are inherited genetic disorders caused by mutations in over two hundred cilia-associated genes. Whole genome or exome sequencing can identify the genetic cause of ciliopathies. When pathogenic variants are identified, this information can be used for genetic counselling, adjusting clinical care based on gene-specific complications, and even to qualify patients for gene-specific clinical trials. However, many missense variants are termed "variants of unknown significance" (VUS), which cannot be classified as pathogenic or benign based on standard *in silico* methods. Here, we have used the small roundworm *Caenorhabditis elegans* and CRISPR/Cas9 genome editing to model and characterize missense VUS alleles. Specifically, we have generated two benign, two pathogenic, and seven VUS alleles in *mks-3*, the nematode orthologue of the Joubert Syndrome and Meckel-Gruber syndrome (MKS) gene *TMEM67.* MKS-3 is a member of the MKS protein module at the ciliary base transition zone (TZ). Employing quantitative assays of cilium structure and function, as well as protein localisation analyses, we found that the known benign alleles phenocopy wild type controls, whereas the known pathogenic mutants display severe cilia defects that are phenotypically similar to those of a reference null deletion allele. Of the seven VUS alleles, four are strongly pathogenic, two are benign, and one displays an intermediate phenotype. We conclude that *C. elegans* is an excellent model organism for *in vivo* interpretation of VUS alleles in conserved cilia genes. Such data may be beneficial for diagnosis of genetically unresolved disease, and understanding genotype-phenotype correlations in ciliopathies.

286A A fluorescent toolkit for live analysis of mitochondrial genome maintenance in C. elegans

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Mitochondria are energy-producing organelles that are essential for eukaryotic cell function. Central to their role is mitochondrial DNA (mtDNA), a small, circular genome present in multiple copies per cell that encodes proteins and RNAs necessary for oxidative phosphorylation. In multicellular organisms, mtDNA is continuously synthesized in both proliferating and post-mitotic cells and varies in copy number between tissue types. MtDNA distribution and synthesis are linked to mitochondrial fission and fusion dynamics in budding yeast and in cancer cell models, however, whether these processes are linked in post-mitotic somatic cells remains unclear. Here, we present molecular imaging tools for the study of mtDNA maintenance within live Caenorhabditis elegans. We have coupled Mitotracker and SYBR Gold stains with Airyscan superresolution microscopy to establish "ground truth" tissue-specific patterns of mtDNA distribution. Then, using transgenic strains in which the mtDNA binding protein hmg-5/TFAM has been fluorescently tagged with wrmScarlet at the endogenous locus we identify tissue-specific regimes of transcription factor distribution within mitochondria and relate them to fusion-fission dynamics. We find that some transgenic strategies, such as the overexpression of mtDNA binding factors via extrachromosomal array, perturb normal mitochondrial DNA levels; we present a set of best practices in strain construction. These validated imaging tools lay the foundation to test whether differences in mitochondrial structure between tissues are predictive of mtDNA abundance and/or distribution. This work has the potential to inform tissue-specific pathologies of mtDNA disease in humans.

287B MEL-28 and dynactin impact male fertility in *C. elegans*

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To study the roles of dynactin and the MEL-28 protein in *C. elegans* fertility, we analyzed animals homozygous for loss-of-function mutations in genes affecting each of two proteins. Specifically, we studied *dnc-1(or 404)* a temperature-sensitive mutation impacting the p150 glued subunit of dynactin, and *mel-28(t1684)* a null mutation that destroys the MEL-28 protein product. Dynactin is a protein complex that connects microtubule motors dynein and kinesin to their cargo and is thus essential for intracellular transport. The *mel-28* gene encodes a protein that rebuilds the nuclear pore after cell division and is also important for chromosome segregation. *dnc-1* mutants have a significantly decreased brood size and produce many unfertilized oocytes, which suggests a problem with the sperm. To test the hypothesis that the *dnc-1* mutants have defective sperm, we set up sperm competition tests that found significant differences in sperm functionality between *dnc-1* mutant males and normal males. When an animal has both the *dnc-1* and *mel-28* mutations, the sperm defects are recovered. Our current goal is to further study how the sperm is impacted by the *dnc-1* mutation and why defects to the *mel-28* gene rescue

these defects. Dynactin and MEL-28 are important in all animal cells, so studying nematode fertility will eventually lead to a better understanding of how human cells work too.

288C The *C. elegans* TspanC8 tetraspanin TSP-14 exhibits isoform-specific localization and function

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Tetraspanin proteins are a unique family of four-pass transmembrane proteins that are highly conserved in metazoans. There are 33 tetraspanins in mammals, and 21 in *C. elegans*. Tetraspanins can be further classified by the number of cysteine residues in their second extracellular loops. While much work has been done to characterize the biochemical properties of tetraspanins, less is known about the in vivo functions of different tetraspanins. We have recently shown that two paralogous tetraspanins, TSP-12 and TSP-14, function redundantly to promote bone morphogenetic protein (BMP) signaling by regulating the trafficking of the type II BMP receptor DAF-4. TSP-14 is the sole member of the TspanC8 subfamily of tetraspanins that in mammals include six proteins. We have noticed that there are two isoforms of TSP-14, TSP-14A, and TSP-14B, with TSP-14B having 24 additional amino acids at its N-terminus compared to TSP-14A. By generating isoform-specific knock-ins and knock-outs using CRISPR, we found that TSP-14A and TSP-14B exhibit distinct subcellular localization patterns and functions. While TSP-14A is localized apically and on early, late, and recycling endosomes, TSP-14B is localized to the basolateral side and on the cell surface. We further identified a di-leucine motif within the N-terminal 24 amino acids of TSP-14B that serves as a basolateral and cell surface targeting sequence for TSP-14B. Using isoform-specific knockout and transgenic rescue approaches, we found that TSP-14A plays a broader and predominant role compared to TSP-14B. Specifically, TSP-14A functions redundantly with TSP-12 to regulate body size, embryonic and vulva development, while TSP-14B functions redundantly with TSP-12 to primarily regulate postembryonic mesoderm development. Moreover, proper postembryonic mesoderm development appears to require the functions of both TSP-14A and TSP-14B, as well as TSP-12. Our work highlights the diverse and intricate functions of TspanC8 tetraspanins in an intact living organism. Future work will be directed towards a molecular understanding of how TSP-14A and TSP-14B exert their distinct functions in different subcellular compartments.

289A Investigating the role of Kinesin-3 motor UNC-104 in regulating polarized distribution of synaptic vesicle proteins

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Synaptic vesicle proteins (SVps) have a polarized distribution in neurons, and are enriched at synapses. SVps are packed into precursors of synaptic vesicles (pre-SVs), that are transported anterogradely by the Kinesin-3 family motor UNC-104, in *C elegans*. In *unc-104* mutants, SVps accumulate in the cell body and mislocalize to dendrites. It is known that the dendritic mis-localization of SVps in *unc-104* mutants is dependent on Dynein. We are investigating whether UNC-104 has other roles in regulating polarized distribution of SVps as motors are also known to regulate microtubule polarity and cargo sorting. We observe that in *unc-104* mutants microtubule polarity is not changed. To test whether, the loss in polarized distribution is due to reduced motor on vesicles we overexpressed PPK-1, an enzyme that makes more $PI(4,5)P_2$, as UNC-104 is proposed to bind to vesicle membranes through $PI(4,5)P_2$. Over-expression of PPK-1 is known to suppress cargo-binding defective alleles of UNC-104. We also observed that PPK-1 overexpression supressed the loss in polarized distribution of SVps observed in *unc-104* mutants. These data are consistent with a model where having sufficient number of UNC-104 motors on pre-SVs is required to regulate the polarized distribution of SVps. UNC-104 orthologues are proposed to be clustered in $PI(4,5)P_2$ and cholesterol enriched lipid microdomains. To test whether clustering of lipids can mediate the polarized distribution and transport of pre-SVs, we depleted cholesterol from the growth media of *C elegans*. We observed that RAB-3 mislocalised to dendrites indicating that cholesterol is required for polarized distribution of SVps, although the dependence of this phenotype on $PI(4,5)P_2$ remains to be investigated.

290B *xbx-4*, a novel Joubert syndrome-related gene, acts in the CCRK/RCK kinase cascade to regulate cilia length and morphology

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Primary cilia are microtubule-based sensory organelles that are critical for sensing and transducing extracellular cues in multiple cellular contexts. The core ultrastructure of cilia is highly conserved and formed by stereotyped mechanisms. To fulfill their wide array of context-specific sensory functions, cilia exhibit diverse shapes and sizes. How cilia with specialized morphologies are generated remains unclear. In *C. elegans*, a subset of head amphid sensory neurons including the AWA olfactory neurons contains elaborate and complex cilia. I found that a highly conserved kinase cascade comprising the DYF-18 CCRK and DYF-5 RCK-related kinases plays a major role in regulating AWA cilia length and branching. Genetic and imaging

experiments suggest that these kinases destabilize axonemal microtubules to promote AWA cilia branching. Regulators of this kinase cascade are largely unknown. Using a forward genetic screen for mutants that suppress the effects of overexpressed RCK kinase, I identified XBX-4, a novel regulator of the CCRK/RCK kinase cascade. I show that XBX-4 primarily regulates CCRK activity, thereby controlling RCK function and localization. XBX-4 contains a Domain of Unknown Function, DUF3719, that it shares with just two proteins (FAM149A and FAM149B1) in the human proteome. Rare mutations in the DUF3719 domain of FAM149B1 result in the ciliopathy Joubert syndrome via unknown mechanisms. My results suggest that *xbx-4* encodes the functional ortholog of FAM149b1 and exerts its effects on cilia structure and function by controlling the activity of the CCRK/RCK kinase cascade.

291C Investigating the role of SYD-2/Liprin-α in synaptic vesicle protein trafficking

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SYD-2, the *C. elegans* homolog of Liprin-α, is a synapse active zone protein that affects distribution of synaptic vesicle proteins (SVps) and lysosomal proteins in neurons. We show that SYD-2 affects SVp trafficking, such that the composition of SVp carriers is altered. We have previously shown that LRK-1, the *C. elegans* homolog of the Parkinson's protein LRRK2, acts through the AP-3 clathrin adaptor complex to affect SVp trafficking and SVp carrier composition. Additionally, both LRRK2 and the AP-3 complex are known to affect lysosomal protein trafficking. Therefore, using *syd-2* mutants, we set out to test if there are common genetic regulators for trafficking of SVp and lysosomal proteins. We show that *lrk-1* and *apb-3* likely act upstream of *syd-2* to exclude lysosomal proteins from SVp carriers in the cell body, suggesting that separation of SVps and lysosomal proteins is a necessary step in SVp carrier biogenesis. Furthermore, we show that lysosomal proteins mislocalize to the neuronal process in *syd-2*, *lrk-1* and *apb-3* mutants, unlike in wildtype where they are restricted to the cell body. The axonal mislocalization of lysosomal proteins in the *syd-2* mutant is dependent on the AP-3 complex and independent of LRK-1, suggesting that SYD-2 acts together with AP-3 complex to retain lysosomal proteins in the cell body. Altered SVp carriers formed in *lrk-1* and *apb-3* mutants mislocalize to the dendrites via the AP-1 clathrin adaptor complex. We show that SYD-2 genetically interacts with the AP-1 complex to prevent the mis-formed SVp carriers from entering dendrites. These data suggest that SYD-2 interacts with two distinct clathrin adaptor complexes to regulate polarised distribution of SVps and lysosomal proteins. Additionally, our data suggest that early trafficking of SVps and lysosomal proteins is linked and is regulated by common genetic factors.

292A Understanding Interactions Between Microtubule-Associated Proteins And Post-Translational Modifications Of Microtubules In Sensory Neurons

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Microtubules (MTs) act as tracks upon which molecular motors, such as kinesins and dyneins, travel to transport essential materials throughout cells. However, the mechanisms that regulate MT structure and motor functions to ensure that diverse cargos are appropriately delivered to particular regions or organelles within cells is not completely understood. In neurons, regulation of MT-based transport may be especially important because they extend elongated axons and dendrites.

Although all MTs are made by assembly of α and β tubulins, they are not uniform. The Tubulin Code hypothesis suggests that MTs can be specialized by incorporating different α and β tubulins, as well as addition of reversible post-translational modifications (PTMs) such as glutamylation (addition of glutamate side chains to α or β tubulin C-terminal tails). We previously found that glutamylases that add glutamylation to MTs (TTLL-4, TTLL-5, and TTLL-11) and a deglutamylase that reduces glutamylation (CCPP-1) influence MT structure and the activity of particular motors in sensory neuronal cilia in a cell-specific manner. Particular tubulins are also essential for regulating the structure of MTs in neuronal sensory cilia. Therefore, the MT tracks themselves encode information that regulates their own structure and stability, as well as the function of kinesin motors.

Non-motor microtubule-associated proteins (MAPs) act as another mechanism to regulate the MT cytoskeleton, but the function of many MAPs is not yet known. MT binding of some MAPs, such as mammalian Tau, has been found to be sensitive to MT glutamylation *in vitro*, suggesting that these two layers of MT regulation may interact.

To understand the genetic interactions of MAPs and the Tubulin Code, we observed the localization of fluorescently-tagged MAPs, such as the *C. elegans* Tau homolog PTL-1 and the DCX family member/RP1L1 homolog F27C1.13, combined with mutations that disrupt particular tubulin genes or regulators of MT glutamylation. Both Tubulin Code PTMs and MAPs are

involved in neurodegenerative diseases in humans. Mutation of a human *ccpp-1* homolog is associated with infantile-onset neurodegeneration, Tau is implicated in Alzheimer's Disease and other Tauopathies, and mutation of RP1L1 is associated with occult macular dystrophy, resulting in progressive blindness. Both PTL-1 and F27C1.13 were expressed in ciliated sensory neurons, suggesting that *C. elegans* might be used to model the human diseases associated with these genes.

Our results support the hypothesis that PTMs and MAPs are essential cytoskeletal regulators that act in concert to regulate and specialize neuronal MTs.

293B Unraveling the role of *clk-1* in the modulation of mtDNA heteroplasmy

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The mitochondrial genome is prone to mutations and deletions that persist throughout generations despite germline selection mechanisms. Therefore, copies of wild-type and mutant mtDNA molecules can co-exist within the same cell or organism, which is termed heteroplasmy. Mitochondrial disease can manifest when a certain threshold for the levels of mutation is surpassed. Since there are multiple copies of mtDNA per cell, the mtDNA copy number can vary in different cells and tissues, according to their energetic demands. Previously, we have found that the wild-type mtDNA copy number is maintained despite an elevation in the total mtDNA levels, in the well characterized heteroplasmic *C. elegans* strain, *uaDf5*. This suggests the existence of a homeostatic copy number regulation mechanism to maintain the wild-type mtDNA levels in *C. elegans*, whereas the mutant mtDNA escapes this control. The mechanisms that dictate the persistence and propagation of deleterious mutations and the regulation of mtDNA copy number, remain to be elucidated. Previous work showed that CLK-1, a protein that participates in the ubiquinone biosynthesis pathway can bind mtDNA *in vitro*. Recently, our group found that ubiquinone deficient *clk-1(ok1247)* mutant worms display an elevated total mtDNA copy number which is not normalized to control levels in the presence of the ubiquinone precursor, 2-4-dihydroxybenzoate (DHB), suggesting an additional role for this protein. In this study, we aim to first determine if *clk-1* is capable of modulating mtDNA heteroplasmy frequency in *uaDf5* heteroplasmic worms, by crossing *clk-1(ok1247)* mutant with *uaDf5* worms and, evaluating the heteroplasmy frequency by quantitative ddPCR. These experiments were performed in the presence of 10mM DHB, to guarantee *clk-1* mutants are supplemented with ubiquinone. Our results show that *clk-1 (ok1247)* mutant; *uaDf5* worms have in average significantly lower mutant mtDNA frequency than wild-type; *uaDf5* worms. Furthermore, we have also performed the same experiments with *clk-1(e2519)* catalytic mutant worms, as another way to demonstrate our results were unrelated with *clk-1* canonical function. Our work shows CLK-1 to be involved in mtDNA heteroplasmy dynamics and possibly, mtDNA copy number regulation.

294C Identification of a potential regulator of proteasome nuclear localisation

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The proteasome is the major enzymatic machinery of the ubiquitin-proteasome system (UPS), which is required for degradation of misfolded, aged and unnecessary proteins. It is a multisubunit complex, consisting of a core particle, 20S, capped by regulatory particles, 19S. The barrel-shaped 20S particle is responsible for the proteolytic activity, whereas the 19S particles bind polypeptide substrates via polyubiquitin chains, remove the ubiquitins for recycling, and unfold the polypeptides prior to transferring them into the lumen of the core particle. Recent studies by us and others have shown that the proteasome's expression and activity are differentially regulated in specific spatial, temporal and physiological contexts in vivo to facilitate its essential function in maintenance of cellular proteostasis. Subcellularly, the proteasome is localised both to the cytoplasm and in the nucleus. The nuclear role of the proteasome is not fully understood, but it may involve also functions not related to its proteolytic activity.

In order to discover new regulators of the proteasome, we have performed a genome-wide *C. elegans* RNAi screen using a transgenic intestinal polyubiquitin-binding reporter strain previously published by our laboratory. This fluorescent reporter has been shown to respond to changes in the endogenous amount of polyubiquitinated proteins, and can be used as a tool for identifying disturbances in protein degradation mechanisms in live animals. After exclusion of hits affecting protein synthesis or having a developmental phenotype, we obtained 33 potential candidate genes. Of these, downregulation of 19 hits increased the fluorescence level of the polyubiquitin-binding reporter animals whereas 14 hits decreased the fluorescence. Particularly, one RNAi clone stood out as it not only resulted in increased reporter fluorescence but also changed the reporter's subcellular localisation from being expressed throughout the intestinal cell into a predominantly nuclear location. Immunostaining for

proteasome 20S subunits showed that the intestinal nuclei have reduced amount of the proteasome, which could cause the accumulation of polyubiquitinated substrates. Further characterization of the potential regulator will be presented.

295A Identifying novel interactors of the guanylate cyclase GCY-22 involved in NaCl chemotaxis

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C. elegans senses salts in their environment using the ASE neurons. Detection of salts occurs in sensory organelles, called primary cilia. cGMP signalling plays an important role in salt detection. 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 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 its trafficking and unique localization.

To identify proteins that physically interact with GCY-22, we performed mass spectrometry after immunoprecipitation to identify proteins bound to GFP-tagged GCY-22. Next, we study where the identified candidate interacting proteins are expressed and localized. Mutants are used to investigate their role in salt detection, GCY-22::GFP trafficking towards the cilium and localization to the ciliary tip.

The most prominent candidate interacting protein is GCY-19. GCY-19::GFP is expressed in ASER and colocalized with GCY-22. Loss-of-function of *gcy-19* resulted in lower levels of GCY-22::GFP at the ciliary tip. Similarly, *gcy-22* loss-of-function animals showed lower levels of GCY-19::GFP at the tip of the ASER cilium . We also found GCY-4 and GCY-5 as possible interactors of GCY-22. As rGCs are thought to act as dimers, these findings suggests that GCY-22 might be a common subunit for heterodimeric complexes possibly to achieve ion-selectivity.

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. Other candidate genes are currently being investigated. This work will allow us to gain insight in the molecular mechanisms that regulate ciliary tip localization of GCY-22.

296B Investigating the function of TAT proteins in lipid transport within ciliated neurons.

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Cilia have protein and lipid compositions that are distinct from that of the cells from which they protrude. Although a clear picture exists of how protein transport occurs in cilia, the mechanisms regulating lipid composition are not presently understood. Furthermore, much remains to be learned about how changes in lipid distribution affect cilium function. The major phospholipids in plasma membranes are phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylchoine (PC) and phosphatidylinositol (PI). These phospholipids are not symmetrically distributed in the leaflets that make up membrane bilayers: in particular, PC is largely confined to the outer (exofacial) leaflet while PE, PS and PI are mostly confined to the inner. These asymmetries are maintained by the movement of phospholipids through the cell membranes catalyzed by transamphipathic aminophospholipid translocases belonging to the P4 family of transmembrane ATPases. C. elegans has six translocase genes, tat-1 – tat-6. We have found that tat-6 is specifically expressed in a subset of C. elegans ciliated neurons and that TAT-6 protein accumulates in cilia. To study the function of TAT-6 and that other translocases in lipid transport in C. elegans ciliated neurons, we developed a technique to allow labelling of cilia with lipids. For the first time in C. elegans ciliary research we used fusogenic liposomes to deliver fluorescently labelled lipids to the membranes of ciliated neurons. We have used this technique to study the roles of all the TAT proteins in this organism in maintaining the distinct lipid composition and lipid asymmetry in ciliary membranes. Our experiments revealed that TAT-5 and TAT-1 translocase activities primarily promote the transport of phosphatidylethanolamine (PE) and phosphatidylserine (PS) respectively whereas TAT-6 has an overlapping function in transporting both PS and PE. Mutants lacking pad-1, which encodes a cofactor for TAT-5, also show defects in PE transport. tat-5 and tat-6 mutants display defects in behaviours mediated by ciliated neurons. Overall, our investigation indicates that the regulation of lipid asymmetry and phospholipid transport is required for cilia to function properly in C. elegans.

297C Syndapin Interacting Proteins in Recycling Endosome Function

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The regulation of membrane transport is essential for the maintenance of cell and tissue architecture. Endocytic recycling, or the selective return of internalized macromolecules to the cell surface from endosomes, is a key process in cell and tissue function, but is poorly understood compared to other aspects of the endocytic pathway. In addition to cellular physiology, endocytic recycling is important in various areas of biomedicine, such as type II diabetes and cancer. We seek a better understanding of the underlying molecular mechanisms that drive endocytic recycling. Here we describe work focusing on a trafficking model that we pioneered, the *C. elegans* intestine, a simple polarized epithelium.

Previous work in our lab identified the F-BAR protein SDPN-1/Syndapin/PACSIN as a recycling regulator associated with early and basolateral recycling endosomes in the intestine. Here we identified a physical interaction between the SH3-domain of SDPN-1 and a specific sequence in PXF-1, an exchange factor for Ras-like GTPases RAP-1/Rap1 and RAP-2/Rap2. Using interaction-defective alleles of *sdpn-1* and *pxf-1* engineered into the genome by CRISPR, we find evidence that the SDPN-1/PXF-1 interaction is important for recycling. We also find that RAP-1 is enriched on SDPN-1-positive endosomes, and find a requirement for RAP-1 in basolateral recycling. Mammalian Rap1 has been reported to act upstream of RhoA in several processes involving regulation of actin and non-muscle myosin II function, and our recent results suggest that SDPN-1 and PXF-1 affect non-muscle myosin accumulation on intestinal endosomes in the intestine. We hypothesize that SDPN-1 interacts with PXF-1 in order to activate RAP-1, which promotes recycling via RHO-1/RhoA, actin, and myosin II. Progress on this model will be discussed.

298A UNC-104 anterograde bias is regulated by ubiquitination

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UNC-104 is an ATP-dependent kinesin motor necessary for the transport of synaptic vesicle proteins from the cell body to the synapse. UNC-104 has been shown to be degraded at synapses through ubiquitin-mediated pathways depending on its ability to bind cargo. Ubiquitination is also known to regulate protein function without leading to degradation. Thus we investigated whether, in addition to degradation, ubiquitination of UNC-104 can alter motor function. To identify E3s that ubiquitinate UNC-104, we carried out a Touch Receptor Neuron-specific RNAi screen of 230 neuronally enriched E3 ubiquitin ligases. We identified F54B11.5 (an RNF141 orthologue) as a potential regulator of ubiquitination of UNC-104. We observe UNC-104 has an anterograde bias in the following three assays:- motor accumulation at the site of laser ablation, UNC-104 particle tracking, and Fluorescence Recovery after Photobleaching (FRAP). In RNAi of the E1 ubiquitin-activating enzyme, *uba-1*, and *f54b11.5*, UNC-104's anterograde bias is increased in an ATP-dependent manner. Likewise, using FRAP, we observe a reduction in UNC-104's retrograde diffusive flux in an *uba-1* mutant. These observed alterations of biased UNC-104 movement result in *uba-1* and *f54b11.5* RNAi may lead to the observed increase in anterograde net displacement of the synaptic vesicle protein RAB-3. These data suggest a hypothesis where the lack of UNC-104 degradation leads to a greater that number of motors on the cargo surface as opposed to the freely diffusing UNC-104. Together, these data suggest that ubiquitination may regulate UNC-104's ability to bind cargo and maintain an equilibrium of UNC-104's anterograde and retrograde flux.

299B Neuronal mitochondria utilize a novel fission mechanism during extrusion into exophers

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The Driscoll lab discovered a previously unknown capacity of *C. elegans* adult neurons to extrude large (~5 µm) vesicles that include cytoplasmic contents (Melentijevic, 2017). These vesicles, which we call «exophers», are a microcosm of the originating soma: they can contain almost anything from the cell, including aggregated proteins, cytoskeletal components, and organelles. Importantly, I have found mitochondria are extruded into the majority of *C. elegans* touch neuron exophers, and recent work in mouse cardiomyocytes demonstrates elimination of defective mitochondria through exopher-genesis plays a critical role in cardiac homeostasis (Nicolás-Ávila, 2020).

Mitochondrial morphology is thought to influence cell function and age-associated decline. In all animals, FZO-1/MFN1/2 and EAT-3/OPA1 respectively mediate outer and inner mitochondrial membrane fusion, while the dynamin motor DRP-1 is required for canonical mitochondrial fission. Several interesting questions arise: Are small, hyper-fragmented mitochondria (produced by inhibiting mito fusion) more amenable to trafficking into exophers? Does abolishing mitochondrial fission (knocking out DRP-1) prevent mitochondrial severance and extrusion into exophers? I have made the surprising discovery that portions of the mitochondrial network are severed and ejected into exophers *even when DRP-1-mediated mitochondrial fission is*

abolished. Furthermore, I have shown mitochondria contained within exophers are typically intact. In fact, in a small fraction of cases, exophers and their mitochondria can remain morphologically intact for weeks following separation from the soma**,**

suggesting mitochondrial fragmentation during exopher-genesis is not irreparably traumatic. Most extruded mitochondria, however, appear to be degraded by the surrounding hypodermis within 3 hours of exopher-genesis. I will be presenting my findings on the proteins, organelles, and physical factors governing this non-canonical mechanism of mitochondrial fission, as well as the biochemical and functional properties of both extruded and retained mitochondria.

This research documents a novel method of mitochondrial sculpting and distribution that likely has important implications for mitochondrial homeostasis, trafficking, and membrane repair.

Melentijevic I, *et al*. *C. elegans* neurons jettison protein aggregates and mitochondria under neurotoxic stress. *Nature*. 2017 Feb 16;542(7641):367-371. doi: 10.1038/nature21362. Epub 2017 Feb 8. PMID: 28178240; PMCID: PMC5336134.

Nicolás-Ávila JA, *et al*. A Network of Macrophages Supports Mitochondrial Homeostasis in the Heart. *Cell.* 2020 Oct 1;183(1):94-109.e23. doi: 10.1016/j.cell.2020.08.031. Epub 2020 Sep 15. PMID: 32937105.

300C RAB-10 functions opposite of the AGEF-1/Arf GTPase/AP-1 pathway to regulate vesicle trafficking

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Cell polarity is critical for the function of the many cell types. In *C. elegans*, basolateral localization of the LET-23 Epidermal Growth Factor Receptor (EGFR) in the vulval precursor cells (VPCs) is required for the development of the vulva. A class I/II Arf GTPase pathway regulated by AGEF-1, a homolog of the mammalian BIG1/2 Arf GEFs, antagonizes LET-23 EGFR signaling by promoting its apical trafficking. Loss of the AGEF-1/Arf GTPase/AP-1 pathway results in increased basolateral LET-23 EGFR and over induction of vulva tissue. Thus, this pathway is required to maintain the proper levels of LET-23 EGFR at the basolateral membrane to ensure proper vulva induction. In human, the EGFR signaling pathway is frequently over-activated in many cancers and studying the mechanisms regulating EGFR signaling is crucial to understand the development of EGFR-related diseases. We found that RAB-10 GTPase has novel antagonistic interactions with the Arf GTPase pathway. RAB-10 GTPase is required for *agef-1(vh4)* trafficking phenotypes in several tissues including increased basolateral localization of LET-23 EGFR in the VPCs, but it is not required for LET-23 EGFR localization in a wild-type background. RAB-10, AGEF-1 as well as the Arf GTPase pathway localize to Golgi and recycling endosomes to regulate polarized trafficking in epithelial cells, however, it is not known how their functions are coordinated. The focus of my project is to find how RAB-10 interacts with the Arf GTPase pathway and which effectors and regulators function with RAB-10 to regulate LET-23 EGFR localization and signaling. Our genetic epistasis indicates that *rab-10(+)* is required downstream of *agef-1(vh4)* and *arf-1(ok796)*, but upstream or in parallel with *unc-101(sy101) AP-1 m subunit*. Thus, RAB-10 might function with the AP-1 clathrin adaptor complex in the absence of Arf GTPase activity to promote basolateral transport of LET-23 EGFR. Additionally, we screened RAB-10 effectors for interactions with the AGEF-1. Two RAB-10 effectors, CNT-1, an Arf6 GAP, and SEC-15, a subunit of the exocyst complex, are suppressors of *agef-1(vh4)* embryonic phenotypes. Unexpectedly, we found that CNT-1 functions opposite of RAB-10 in the VPCs and thus might function in a distinct pathway. We are still testing the role of SEC-15 in regulation of LET-23 EGFR trafficking. These studies will determine how RAB-10 and the AGEF-1/Arf/AP-1 trafficking intersects to regulate polarized transport in epithelial cells.

301A The *C. elegans* homolog of Nucleolin, NUCL-1, contributes to nucleolar organization through its intrinsically disordered RG/RGG repeat domain

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Intrinsically disordered domains are found in 30-40% of human proteins, many of which undergo liquid-liquid phase separation (LLPS). How these domains influence LLPS-mediated membraneless organelle (MLO) formation and organization *in vivo* is unclear. One such domain, consisting of Arginine and Glycine (RG/RGG) repeats, is critical for both P-granule and nucleolar function in *C. elegans*. We have identified 551 proteins with 3 or more regularly spaced RG/RGG repeats in *C. elegans*. Gene Ontology analysis reveals that these RG/RGG repeat-containing proteins are enriched in MLOs, including the nucleolus and P-granules. MEME motif discovery was used to identify a phenylalanine-rich RG/RGG motif typical of nucleolar proteins and a tyrosine-rich RG/RGG motif typical of P-granule proteins. These motifs were then used to predict the MLO localization of a

highly abundant but uncharacterized protein, K07H8.10. The 176 amino acid-long RG/RGG repeat domain of K07H8.10 is the longest in *C. elegans* and is interspersed with phenylalanine, predicting nucleolar localization. In addition to its N terminal RG/RGG repeat domain, K07H8.10 contains a coiled-coil acidic domain and two C terminal RNA recognition motifs. Both the HHpred and the MARRVEL bioinformatics toolkits predict homology to Nucleolin, which contains these same three domains, although configured in a different arrangement in nematodes. We have fluorescently tagged K07H8.10 (now named NUCL-1) in the *C. elegans* germline and confirmed its nucleolar localization. Deleting the N terminal RG/RGG repeat domain of NUCL-1 results in fertile worms and does not impair NUCL-1 localization to the nucleolus. However, super resolution imaging of NUCL-1 in living worms reveals that sub-nucleolar compartmentalization of both NUCL-1 and Fibrillarin (FIB-1) are disrupted. Our results indicate that the NUCL-1 RG/RGG repeat domain is dispensable for localization to the nucleolus but is crucial for overall nucleolar organization.

302B Perturbation of RME-8 results in elongation of endosomes in ALM neurites.

Sierra Swords[1], Barth Grant[1] 1) Rutgers University.

Proper long-distance trafficking of membrane-associated cargo is crucial for neuronal health. Recently, RME-8, a protein known for its important role in recycling endocytic cargo in non-neuronal cells from early endosomes to the plasma membrane via the Golgi, was implicated in Parkinson's Disease (PD). A single copy of a specific allele, N855S, in human RME-8 (aka DNAJC13) was closely associated with inherited PD in a particular family, and other rare alleles of human RME-8 have been associated with a related disease, Essential Tremors. Prior to this work RME-8 function had not been studied in neurons, where membrane trafficking pathways are particularly elaborate and specialized.

Using *C. elegans* as a model, our lab has investigated the effects in neurons of perturbing RME-8 function, with a particular emphasis on the effects on endosomes traveling long-distance in neurites. Importantly, in *rme-8(b1023ts)* mutants at restrictive temperature, we find an abnormal accumulation of elongated RAB-5 and RAB-7 positive endosomes in the neurite of ALM mechanosensory neurons. These elongated vesicles are often quite dynamic, but appear to fail in long distance directional movement. We also engineered the homologous PD mutation into the worm via CRISPR (*rme-8 N861S*). We observe similar elongated endosomal structures in N861S mutant neurons, but not in young adults, only in aged animals. This appears to mimic PD pathology in that it is strongly associated with aging. Our results point to a key role in endosomal trafficking in the neurite for RME-8, and suggests that failure of such trafficking is important in understanding the etiology of PD.

303C Disruption of Golgi function induces pathogen response gene expression

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Each secretory pathway organelle has a distinct configuration of phospholipids and resident proteins in addition to separable stress pathways. These stress pathways activate restorative transcriptional programs when lipid ratios become unbalanced, or protein function fails. We and others have noticed that a common set of genes are activated by bacterial pathogens, disruption in lipid synthesis or induction o**f**

ER stress. Here, we show that these immunity-linked genes (ILGs) are also activated upon disruption of the ADP-ribosylation factor ARF-1, a key regulator of Golgi secretory function. We have previously found that ARF-1 activity is blocked by changes in membrane lipid levels, which suggests that altered lipid levels in secretory organelles other than the ER can also stimulate these ILGs as part of a stress response.

304A Determining the function of the LOV-1 polycystin-1 adhesion GPCR and TRP PKD-2 on cilia and extracellular vesicles

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Human autosomal dominant polycystic kidney disease (ADPKD) is one of the most common inherited ciliopathies and a common cause of end-stage renal failure. ADPKD is caused by mutations in PKD1 and PKD2, which encode the polycystin-1 (PC1) 11 transmembrane receptor and TRP polycystin-2 (PC2) channel. In humans and *C. elegans*, the polycystins function in a sensory capacity, localize to primary/sensory cilia, and are shed from cells in tiny extracellular vesicles (EVs), suggesting an ancient function. EVs are tiny (100-200 nm) membrane-bound vesicles that deliver lipids, nucleic acids, and proteins to neighboring cells. Cilia are important sites for EV release and interaction. The C. elegans polycystins LOV-1/PC1 and PKD-2/PC1

regulate male mating behaviors. During mating, C. elegans males transfer PKD-2::GFP-labeled EVs to the hermaphrodite vulva. Isolated EVs trigger male tail chasing behavior: PKD-2 location on ciliary EVs is essential for this EV bioactivity.

A major gap in our knowledge is the in vivo functions of the polycystins in cilia and EVs.I have developed an arsenal of CRISPR-based, LOV-1 reporters and mutants for studying endogenous localization and function of LOV-1 and PKD-2 in male-specific ciliated EV releasing neurons (CEMs, RnBs, HOB). I am testing two hypotheses: (1) LOV-1 acts as an adhesion G protein-coupled receptor (GPCR) with PKD-2-dependent and -independent functions and (2) LOV-1 autoproteolytic cleavage plays an instrumental role in LOV-1 function.

Using CRISPR-generated, N-Terminal (NTM) and C-terminal (CTM) fluorescent-protein fusions of LOV-1/PC1, I observed subcellular localization differences between NTM LOV-1 and CTM LOV-1 GPS cleavage products in dendrites, cilia, and EVs. PKD-2 and CTM LOV-1 (but not NTM LOV-1) colocalize, suggesting functional association. GPS cleavage is required for LOV-1 localization to cilia and release in EVs. In addition, preliminary data suggests the release of polycystin-containing EVs is a regulated process and our ability to detect environmental released FP-tagged EV cargoes is not the result of protein overexpression. Future efforts are aimed at determining the relationship between LOV-1 and PKD-2, the function of polycystins in EVs, the cellular targets of polycystin-carrying EVs, and the role of polycystin-carrying EVs in animal-animal communication.

305B Phagocytosis and processing of neuron-derived exophers by the *C. elegans* hypodermis.

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Previous work showed that adult *C. elegans* neurons are capable of ejecting a large portion their cell bodies as a giant vesicle (average size 3.8 micron) loaded with aggregated protein and mitochondria, a process that becomes much more frequent upon challenge by several forms of cellular stress (Melentijevic et al., 2017). Current models posit that such ejection removes toxic materials from the neuron, enhancing neuronal stress recovery. Here we have focused on the fate of exophers ejected by the mechanosensory neuron ALMR, a neuron whose soma is completely surrounded by the syncytial skin cell hyp7. By following exophers within individual animals over time we found a characteristic pattern in which a single large ALMR exopher typically moves within hyp7 in a posterior direction, away from the neuron, but initially maintains a long thin connection to the neuronal soma. Within 3 hours of ejection most intact exophers have disappeared, with remaining material derived from the exopher broken up into many smaller vesicles within the hypodermis that we have termed “starry night”. Over the next few days most starry night vesicles diminish in intensity, and in many cases fluorescent material derived from exophers appears outside of the hypodermis in coelomocyte cells. This pattern suggested that ejected exophers are phagocytosed by the much larger hypodermal cell, with the hypodermal endolysosomal machinery activated to degrade toxic exopher materials via phagosome maturation. Undigested material may then be resecreted by the hypodermis, allowing uptake and another chance at degradation by the coelomocyte scavenger cells. Work in progress supporting this model will be presented, with an emphasis on current results analyzing hypodermal uptake and phagosome processing, and the role of ARF-6, CNT-1, and RAB-35 in this process.

306C NuRD mediates mitochondrial stress–induced longevity via chromatin remodeling in response to acetyl-CoA level

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Mild mitochondrial stress experienced early in life can have beneficial effects on the life span of organisms through epigenetic regulations. Here, we report that acetyl–coenzyme A (CoA) represents a critical mitochondrial signal to regulate aging through the chromatin remodeling and histone deacetylase complex (NuRD) in Caenorhabditis elegans. Upon mitochondrial stress, the impaired tricarboxylic acid cycle results in a decreased level of citrate, which accounts for reduced production of acetyl-CoA and consequently induces nuclear accumulation of the NuRD and a homeodomain-containing transcription factor DVE-1, thereby enabling decreased histone acetylation and

chromatin reorganization. The metabolic stress response is thus established during early life and propagated into adulthood to allow transcriptional regulation for life-span extension. Furthermore, adding nutrients to restore acetyl-CoA production is sufficient to counteract the chromatin changes and diminish the longevity upon mitochondrial stress. Our findings uncover the molecular mechanism of the metabolite-mediated epigenome for the regulation of organismal aging.

307A Towards a quantitative gene network underlying robustness of seam cell fate

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Among all multicellular organisms, *Caenorhabditis elegans* offers a unique experimental system to study phenotypic robustness because of its remarkably reproducible development. In our lab, we focus on seam cells, which show stem cell-like properties being able to divide both symmetrically and asymmetrically in order to self-renew or generate daughter cells that differentiate into hypodermis and neurons, and this happens in a fairly invariant manner. To improve our understanding of the seam cell gene network, we have started focusing on key transcription factors that are thought to be its core members, such as *elt-1, egl-18* and *ceh-16.* We have used single molecule FISH to quantify the expression levels of these factors in wild-type and mutant backgrounds and characterise their putative interactions. Preliminary data suggest that individual seam cells may be subject to different quantitative relationships based on their position along the anterior-posterior axis. In order to evaluate the stability of these interactions we have built a preliminary Boolean network model based on our smFISH data. *In silico* simulations have been used to evaluate the robustness of various network architectures upon perturbation. Our aim is to integrate experimental and theoretical work to understand the structure of the seam cell fate gene network and its resilience to perturbation.

308B Positioning *sea-2* and *lin-66* in the heterochronic pathway in the context of continuous and L2d-interrupted development

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Caenorhabditis elegans larvae are born with 10 hypodermal stem cells (seam cells) along each side of their body. Seam cells execute an asymmetric (self-renewal) division before each molt, and in addition, at the L1 molt, certain seam cells divide symmetrically (doubling cell number in the corresponding lineage). The L2 stage symmetric seam cell divisions are specified by the heterochronic gene regulatory pathway and the transcription factor Hunchback-like-1 (HBL-1) is the most proximal heterochronic regulator of this L2-specific developmental event. Mutations that disrupt the timely down-regulation of HBL-1 result in abnormal seam cell numbers – too many seam cells if HBL-1 is over-expressed, or too few seam cells if HBL-1 is precociously down regulated. Previous research described two distinct and parallel HBL-1 down-regulation mechanisms: On the one hand, post-transcriptional silencing via *let*-7 family and *lin-4* microRNA complementary sites in the 3'UTR of *hbl-1* transcript and, on the other hand, post-translational regulation mediated by the *lin-28/lin-46* axis of the heterochronic pathway. Importantly, which of these HBL-1 regulatory mechanisms predominates depends upon the larva's life history (and hence dictated by the environmental conditions): during the rapid and continuous development associated with replete culture conditions, microRNA-mediated post-transcriptional regulation of HBL-1 is the primary mode, whilst LIN-46-mediated post-translational regulation dominates during L2d interrupted development. Previous genetic screens identified additional factors that affect seam cell numbers, including *sea-2*, which encodes a zinc finger protein [1] and *lin-66*, which encodes a protein of unknown function [2]. *sea-2* or *lin-66* loss-of-function mutants cause increased seam cell numbers during continuous development, and molecular analysis suggested that these genes act as negative regulators of LIN-28 expression [1, 2]. Here we employed epistasis analysis and sensitized genetic backgrounds to investigate the functional relationship of *sea-2* and *lin-66* to other genes in the heterochronic pathway, particularly with respect to *lin-28* and *hbl-1*, and during both continuous and L2d-interrupted development. Our results indicate that *sea-2* and *lin-66* (especially *sea-2*) are far more critical for the regulation of seam cell number during L2d-interrupted development than during continuous development. Also, our results suggest that *sea-2* may function by opposing a previously undefined *lin-28/hbl-1* axis that is independent of *lin-46*, and that *lin-66* may regulate *hbl-1* downstream of *lin-28* and *lin-46.* Our findings suggest additional regulation mechanisms for a developmentally critical transcription factor and expands our understanding of how robustness of animal development is achieved against adverse growth conditions.

[1] PMID: 21471153

[2] PMID: 17139256

309C Studying cell-fate convergence in the mesodermal lineage of C. elegans

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One of the main goals of developmental biology is to understand the mechanisms and principles of how cells acquire diverse identities during embryogenesis. One of the assumptions is that cells progress through embryogenesis, progressively restricting their developmental potential and gradually acquiring their terminal identities. However, sometimes this process of cell fate specification is not linear. In some cases, cells initially commit towards a specific fate, but later change their seeming commitment and veer towards a different trajectory, converging to another cell type. The C. elegans lineage reveals multiple instances of such cell-type convergence. We aim to understand the mechanisms that initiate the change in a trajectory of a cell, and whether cells with converged identities maintain differences due to their different early transcriptional histories. Using single-cell RNA sequencing and taking advantage of C. elegans's invariant development we have a unique opportunity to study how cells establish their identities at the level of gene regulatory networks with single-cell resolution. We have sequenced the whole mesodermal lineage from C. elegans, originating from the MS blastomere. This lineage gives rise to many body-wall muscles as well as part of the pharynx and contains several instances of cell fate convergence. We used the SMART-Seq2 protocol to achieve maximal sensitivity, which allowed us to detect even lowly expressed transcripts. Using these data, we have uncovered transcriptional differences along trajectories that converge on the production of muscle cells from different parts of the lineage. We are currently testing candidate transcription factors and signaling pathways that may drive this cell identity convergence. We are also exploring the transcriptional differences across terminal muscle cells and their link to the different trajectories. Our data provide new entry points for mechanistic studies to ultimately understand the principles of cell-type diversification.

310A *C. elegans* establishes germline versus soma by balancing histone methylation

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Embryos undergo extensive reprogramming at fertilization to prevent the inappropriate inheritance of histone methylation. In *C. elegans,* the H3K4me2 demethylase, SPR-5, and the H3K9 methyltransferase, MET-2, are maternally deposited into the oocyte where they cooperate to reestablish the epigenetic ground state of the newly formed zygote. Previous work from the Strome and Kelly Labs demonstrates that maternally deposited MES-4 maintains H3K36me2/3 at germline genes between generations to help re-establish the germline. To determine whether the MES-4 germline inheritance system antagonizes *spr-5; met-2* maternal reprogramming, we examined the interaction between these two systems. Here, we show that the progeny of *spr-5; met-2* mutants display a severe developmental delay that is associated with the ectopic expression of MES-4 germline genes in somatic tissues. By performing ChIP-seq on L1 progeny from *spr-5; met-2* mutants, we find that MES-4 germline genes ectopically accumulate H3K36me3 in somatic tissues. Additionally, knocking down MES-4 suppresses the ectopic expression of MES-4 germline genes and rescues the developmental delay. We also show that the developmental delay is dependent upon the H3K4 methyltransferase, SET-2. More recent data from our lab uncovered a synergistic interaction between SPR-5; MET-2 maternal reprogramming and MEC Complex member, MEP-1. This interaction links maternal reprogramming of histone methylation to the somatic mechanisms that suppress germline gene expression in somatic tissues. Together, these data suggest a model where SPR-5; MET-2 maternal reprogramming antagonizes H3K36me2/3 to enable the proper transgenerational control of germline versus somatic cell fates. Without SPR-5; MET-2 reprogramming, somatic cells struggle to specify their proper cell fate amongst the noise of inappropriate germline gene transcription, leading to developmental delay. A similar mechanism may underlie the developmental delay of Soto and Kabuki Syndrome patients who have mutations in histone modifying enzymes.

311B *ztf-16* opposes adult cell fate after dauer in *Caenorhabditis elegans*

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Diapause is an interruption in developmental progression that helps animals to survive adverse environmental conditions. However, the mechanisms that modulate developmental pathways to accommodate diapause are still unclear. In favorable conditions, *C. elegans* develops continuously through four larval stages. Alternatively, in unfavorable conditions, larvae enter

dauer diapause after the second larval molt. One cell type that is affected by dauer is the hypodermal seam cells. Seam cells are multipotent during larval development but differentiate in adults, a process that is regulated by the heterochronic genes. Interestingly, most heterochronic genes that are required during continuous development are dispensable after dauer, suggesting that a separate developmental pathway controls post-dauer seam cell development. To shed light on 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. In this screen we identified *ztf-16*, encoding a C2H2 zinc finger transcription factor. To determine how *ztf-16* interacts with the heterochronic pathway to regulate *col-19p::gfp* we did a series of epistatic experiments. The LIN-29 transcription factor directly activates *col-19 and is* in turn regulated indirectly by the *let-7* microRNA. We found that the *ztf-16* precocious phenotype was epistatic to *let-7* reiterative phenotypes. Furthermore, *ztf-16::gfp* expression was strongly upregulated in *let-7* mutants indicating that *ztf-16* acts downstream of *let-7*. Surprisingly, *col-19p::gfp* expression remained high in *lin-29(0)* mutants, suggesting that *ztf-16* regulates *col-19p::gfp* expression independently of *lin-29*. Using RNA-seq, we found >1000 genes whose expression changes in *ztf-16(-)* larvae including transcription factors that are candidate regulators of *col-19p::gfp* expression. Our work describes a novel regulator of adult cell fate that functions after dauer diapause.

312C The polarity protein PAR-4 controls intestinal cell number by regulating cell fate in *C. elegans* embryos

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Intestinal epithelial cells can absorb food through their highly specialized apical surface, the so-called brush border, which is composed of many microvilli. Two processes are crucial for proper gut function: the polarization of enterocytes as well as the regulation of their number in the tissue. The polarity protein and tumor suppressor PAR-4/LKB1 has been shown to be involved in both processes. Its ectopic activation is sufficient to induce the formation of an apical brush border with microvilli-like structures in intestinal epithelial cancer cell lines. Moreover, mutations in the *lkb1* gene are responsible for the Peutz-Jeghers syndrome in which patients develop benign intestinal polyps. However, as this master kinase acts through various signaling pathways, it is essential to better characterize its role and its downstream effectors in intestinal cells *in vivo*.

In order to do so, we used confocal and transmission electron microscopy to observe the intestinal epithelium in *C. elegans par-4* thermosensitive mutant embryos. Surprisingly, we have found that PAR-4 loss-of-function does not inhibit the formation of microvilli in intestinal cells, even if they present mild defects. However, *par-4* mutant embryos display extra intestinal cells, which lead to striking defects of the intestinal epithelium architecture, notably the appearance of strong intestinal lumen deformations. Lineage experiments revealed that PAR-4 does not control the number of enterocytes by regulating the cell cycle, but by controlling cell fate specification during embryogenesis. While the intestine exclusively arises from the E blastomere in wild type embryos, we observed that the C lineage also gives rise to intestinal cells in *par-4* mutants. We are currently investigating the molecular mechanisms underlying this novel function of PAR-4. We are in particular testing whether PAR-4 prevents intestinal specification in the C lineage by inhibiting the transcription factors SKN-1 and/or MED-1/2. Furthermore, we are also investigating the possible link between PAR-4 and two other kinases, PAR-1 and GSK-3, which control intestinal cell number and specification of the C lineage, respectively (our observations and Schlesinger *et al.*, Genes Dev., 1999; Maduro *et al.*, Mol Cell, 2001).

Altogether this work will allow us to characterize a novel signaling pathway by which PAR-4 regulates cell fate specification. This appears to be essential to prevent intestinal hyperplasia and lumen deformations.

313A The RAP-2 Small GTPase and MIG-15 MAP4 kinase promote tertiary fate in *C. elegans* VPC Patterning

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During *C. elegans* development, graded EGF signal from the anchor cell (AC) induces the six equipotent vulval precursor cells (VPCs) to assume a pattern of 3˚-3˚-2˚-1˚-2˚-3˚ cell fates. The VPC closest to the AC is induced via the Ras-Raf-MEK-ERK MAP kinase cascade to assume 1˚ fate. Presumptive 1˚ cells generate DSL ligands to induce the two neighboring cells via the Notch receptor to assume 2˚ fate. 1˚ and 2˚ developmental programs have been shown to be mutually antagonistic. Our lab showed that lower EGF dose causes Ras to switch effectors, from Raf to RalGEF-Ral, which functions to promote 2˚ fate in support of Notch. We further showed that Ral signals through GCK-2, a member of the Ste20 family of mitogen-activated protein kinase kinase kinase kinases (MAP4Ks), to trigger a p38 MAP kinase cascade to promote 2˚ fate (Shin *et al.*, 2018). 1˚ and 2˚ cells execute distinct and stereotyped division patterns to form the vulva. In contrast, 3˚

fate is typically referred to as the "ground" or "uninduced" cell fate; 3˚ cells divide once and fuse with the surrounding epithelium. We have found that a paralog of GCK-2, MIG-15, also plays a role in VPC patterning. Upon mutation or RNAi depletion of MIG-15 or RAP-2, we observed an increase in ectopic 1˚ as well as ectopic 2˚ cells. MIG-15 is also required for expression of a putative cell fate reporter in 3˚ cells. Both RAP-2 and mig-15 are necessary for full expression of the 3° biomarker. Thus, we hypothesize that, like 1˚- and 2˚-promoting signals, 3˚-promoting signals antagonize other vulval cell fates. Using CRISPR-Cas9, we engineered an insert of fluorescent protein and epitope tag into the 5' end of the endogenous *mig-15* gene, revealing ubiquitous expression in the animal, localized to the cytosol and cell-cell junctions. We also inserted auxin inducible degron (AID), which mediates conditional degradation of tagged proteins. We will use complementary degradation experiments and tissue-specific transgenic rescue to test whether MIG-15 functions in the VPCs to repress 1˚- and 2˚- signals. We will also use CRISPR to mutationally activate MIG-15, as we did previously with the paralogous GCK-2. Preliminary data suggest that RAP-2 functions similarly to MIG-15, and RAP-2 has been shown to activate MIG-15 in other systems. We hypothesize that RAP-2-MIG-15 promotes 3° fate, counter to the notion of 3˚ fate as "uninduced." Our work presents positions us to explore signals that promote "ground" developmental state and perhaps informs the relationship cancers and surrounding stromal cells.

314B Proliferation/differentiation control by the SWI/SNF nucleosome remodeler *in vivo*

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During development cells undergo well-controlled fate changes, passing through proliferating precursor states before exiting the cell cycle and permanently differentiating. Changes in gene expression underlie these cellular decisions, ensuring tight control over this dynamic process. We have combined genetics with cell type-specific next-generation sequencing and phenotypic analyses to study the divisions of the mesoblast lineage. Using low-input RNA sequencing technology we characterized changes in gene expression over time, and found that more than a quarter of transcripts change as cells exit quiescence to first proliferate, and then differentiate into muscle cells. We show that a major chromatin remodeler, the SWI/SNF complex, is required to promote cell cycle exit and cell fate changes. Tissue-specific loss of function of an essential complex component results in delayed downregulation of cell cycle genes, coinciding with cellular overproliferation. Furthermore, depletion of SWI/SNF results in a failure of mesoblast descendants to differentiate. Current work is focused on the mechanism and collaborating factors by which SWI/SNF controls the proliferation-differentiation transition. Several key transcription factors were found to be misregulated following SWI/SNF loss, and we investigated effects of loss-of function of homeobox factors *mls-2* and *lin-39*, as well as *hlh-1* CeMyoD. We show that loss of hlh-1 also causes cells to overproliferate, suggesting a potential collaboration with SWI/SNF to regulate cell cycle genes. Finally, to discover additional contributing factors, we are employing a forward and reverse genetic screening approach. We tested an RNAi library containing 350 chromatin factors, and found that depletion of putative H3K27 demethylases partially suppress the SWI/SNF overproliferation phenotype. Future experiments will be aimed at identifying the mechanism of action of SWI/SNF on the chromatin, using cell-type specific and low input genomic profiling methods.

315C Speed and fate diversity tradeoff in nematode's early embryogenesis

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Nematode species are well-known for their invariant cell lineage pattern during development. Combining knowledge about the fate specification induced by asymmetric division and the anti-correlation between cell cycle length and cell volume in *Caenorhabditis elegans*, we propose a model to simulate lineage initiation by altering cell volume segregation ratio in each division, and quantify the derived pattern's performance in proliferation speed, fate diversity and space robustness. The stereotypic pattern in *C. elegans* embryo is found to be one of the most optimal solutions taking minimum time to achieve the cell number before gastrulation, by programming asymmetric divisions as a strategy (Guan, et al. *arXiv*, 2021, 2007.05723).

316A Cell-fate decisions in dynamically perturbed signaling environments during *C. Elegans* vulval development

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During development, cells take a series of fate decisions to acquire different roles in the future organism. The decisions that make a cell specify into one role and not the others depend on complex and dynamic inter/intracellular signals. However, at each decision point, cells typically only decide between a limited repertoire of fates. This suggests that simple, intuitive regulatory biophysical principles may successfully summarize pathway complexity. Here, we propose to establish and quantitatively test such principles, using the vulval development of *C. elegans* as a model system. To do so, we propose to combine:

(i) Controlled perturbations of in-vivo signaling dynamics using Auxin-Inducible Degradation (AID) System and Temperature-Sensitive strains (Zhang *et al.*, 2015; Martinez *et al.*, 2020)

(ii) In-vivo measurements of the perturbations using a real-time biosensor for ERK activity (de la Cova *et al.*, 2017)

(iii) Large-scale 4D live imaging to capture cell-fate transformations in response to perturbed signaling in microfluidics (Keil *et al.*, 2017)

(iv) Parsimonious mathematical modeling of the underlying cell-fate acquisition dynamics (Corson and Siggia, 2012, 2017)

Our preliminary results confirm the counter-intuitive asymmetry between P4/8.p in the degree to which they are induced to 2° fates by an EGF pulse (lin-15(n765)), as predicted by (Corson and Siggia, 2017). We will also compare our model predictions to experimental measurements in a background with weak ectopic Notch activity and no Anchor Cell (lin-12(n302)). Our project promises fundamental insights into stem cell behavior by experimental consolidating a generic mathematical modeling approach, applicable to a wide range of stem-cell paradigms.

317B Uncovering highly conserved factors that contribute to phenotypic robustness of seam cell patterning in *C. elegans*

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Biological systems experience various perturbations but are able to buffer these to produce consistent phenotypes, a property known as robustness. Genes contributing to phenotypic robustness have been largely examined in unicellular organisms, such as *S. cerevisiae*, as opposed to multicellular animals. In our lab, we study phenotypic robustness using the *C. elegans* seam cells as a model. Seam cells are stem-like lateral epithelial cells, which undergo asymmetric and symmetric divisions to produce the hypodermis and neurons. Similar to other cell lineages, the number of seam cells is quite invariant between animals in the wild-type population. To this end, a forward genetic screen was performed to identify mutants with greater levels of population variance in seam cell number. Our screen has identified mutations in previously uncharacterised genes involved in the regulation seam cell pattering, some of which are highly conserved throughout the animal kingdom. Key examples are a mutation in *nath-10*, an N-acetyltransferase, and *bus-19*, an ancient transmembrane protein. Both mutations can be phenocopied by RNAi knockdown, rescued through seam cell specific expression of the wild-type gene and result in stochastic gains and losses of seam cells during development. Interestingly, both genes can be linked to the role of Wnt signalling in seam cell patterning, as revealed by studying the localisation of Wnt components and the expression of downstream targets via RNA-seq analysis, single molecule FISH and reporter gene expression. We also find that mutations in *nath-10* lead to an increase in mRNA transcript variability, which may also influence phenotypic variability. These results provide insights for novel and highly conserved regulators of seam cell patterning, which may influence phenotypic robustness through modulation of Wnt signalling.

318C Evolutionary conservation of the heterochronic pathway in *C. elegans* and *C. briggsae*.

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C. briggsae is a close relative of *C. elegans.* These species have similar morphology and share the same ecological niche. *C. briggsae* undergoes the same developmental pathway consisting of four larval stages before reaching the adulthood. It also has the same set of heterochronic genes that control the sequence and timing of cell fates during the development.

lin-28 is one of the heterochronic genes that also exists in other animals from flies to humans. It conservatively blocks the maturation of *let-7* miRNA, the proccess is generally associated with the stem cell state. *lin-28* is silenced as cells differentiate.

C. elegans mutants of *lin-28* develop a heterochronic phenotype with a reduced number of seam cells and precocious alae. *C. briggsae* develops a distinct phenotype when its *lin-28* is disrupted. Mutants have wild-type number of seam cells and rarely develop patches of precocious alae. Instead, they often arrest their development at L4 stage, become lethargic, and have gonad development problems. Oocytes often leak outside the gonad, and zero to few eggs form. It is possible, though, to maintain the homozygous strain but its reproduction rate is low.

This observation lead to the question of how conserved is the heterochronic pathway in these close species. To investigate this, I made mutations in *lin-46*, *lin-46* 5›UTR, and *lin-14* of *C. briggsae*.

All these mutants express phenotypes closely resembling those of *C. elegans* mutants of analogous genes.

Further investigation of other heterochronic genes of *C. briggsae* will determine to what extent the pathway's hierarchy is conserved.

319A Determining the role of ZEN-4/KIF23 in *C. elegans* reproductive organ development

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The kinesin-like protein ZEN-4/KIF23 and CYK-4/MgcRacGAP comprise the centralspindlin complex known to be required for cytokinesis. KIF23 is highly expressed in several human cancers [1-3], underscoring the importance of understanding its function. We have found a novel centralspindlin-independent role for *C. elegans* ZEN-4 in vulval development. During *C. elegans* larval development, the conserved EGFR/Ras/MAPK pathway relays an EGF signal from the gonadal anchor cell (AC) and induces vulval precursor cells (VPCs) to develop into the vulva. Loss of LET-23 EGFR signaling results in a Vulvaless (Vul) phenotype, while increased signaling causes a Multivulva (Muv) phenotype. Before VPC patterning, the AC is also specified through a LIN-12 Notch signaling cascade. Using temperature sensitive mutants and RNAi, I bypassed the embryonic lethality of *zen-4* and *cyk-4* loss of functions and found that loss of *zen-4* during larval development resulted in a strong (94%) Vul phenotype, while loss of *cyk-4* did not affect development. I hypothesized that ZEN-4 functions in either the VPCs or the AC to promote EGFR-mediated vulval development.

To determine ZEN-4's position relative to the LET-23 EGFR pathway, I performed genetic epistasis of *zen-4(RNAi)* with LET-23 EGFR negative regulators *lin-1* and *lin-15A/B*. I found that *zen-4(RNAi)* does not suppress the Muv phenotypes of either *lin-1(n304)* or *lin-15A/B(n765)*. This suggests that ZEN-4 is not required for VPC division and that it functions upstream of LET-23 EGFR signaling. Therefore, I investigated ZEN-4's potential role in AC specification by analyzing expressions of the *GFP::hlh-2* AC reporter [4] before and after specification. Unlike the empty vector control which specified a single *GFP::hlh-2* positive cell from 4 progenitors, *zen-4(RNAi)* and *cyk-4(RNAi)* animals often had 0 or 2 positive cells with fewer progenitors before the specification event, suggesting a possible defect in progenitor cell division. Interestingly, *zen-4(RNAi)* alone also affected the localization of *GFP::hlh-2* positive cells. I performed temperature upshifts of *zen-4(or153)* exclusively at the L2 stage and observed that it is sufficient to induce the Vul phenotype. Altogether, these results suggest that ZEN-4 promotes vulval development through its regulations of AC specification and localization.

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320B The Mechanism of LIN-42 Regulation of Temporal Patterning in C. elegans

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Throughout development, precise regulation of transcriptional timing and dosage is necessary for coordinating sequential cell fate specification and developmental checkpoints across multiple cell types and tissues. Previous reports from our lab indicate that *lin-42*, the *C. elegans* homolog of the Period gene implicated in controlling circadian rhythms, negatively regulates the transcriptional output of cyclically expressed heterochronic microRNAs (including *lin-4*, *miR-48*, *miR-241*, *miR-84* and *let-7*). Loss of *lin-42* function results in dramatic alterations in the post-embryonic cell lineages where late-staged developmental events are skipped due to the inappropriate and early down-regulation of the mRNA targets of heterochronic miRNAs. Because

C. elegans lacks the direct orthologs of the core clock transcription factors (Clock and Bmal) that Period functions through, the mechanism by which LIN-42 regulates the transcriptional output of heterochronic miRNAs, and therefore temporal patterning, are unknown. Here, we demonstrate that LIN-42 regulates heterochronic miRNA transcriptional dosage through direct physical interactions with multiple conserved nuclear hormone receptors that are expressed in the hypodermis. More specifically, through yeast-2-hybrid, biochemical, and in vivo imaging of transcriptional reporters, we demonstrate that the LIN-42 protein modulates the transcriptional activity of the REV-ERb ortholog, NHR-85, and that NHR-85 also binds to the defined regulatory regions of multiple LIN-42 target genes. Consistent with a function interaction between LIN-42 and NHR-85, loss-of-function alleles of *nhr-85* suppresses precocious developmental phenotypes of *lin-42* mutants. We present evidence that the rudimentary developmental clock composed of NHR-85 and LIN-42 functions in the hypodermis to orchestrate the cynical transcription of temporal cell fate specifying microRNAs. Specifically, we demonstrate that LIN-42 auto-regulates its own expression as the expression of and NHR-85 target, *lin-4*, in hypodermal cell types. Together our results establish the negative arm of the developmental molecular clock and demonstrate the mechanism of how LIN-42 regulates the transcriptional output of heterochronic miRNAs required to establish sequential cell fates and temporal patterning during post-embryonic development.

321C Opposing roles of DAF-16 and NHR-156 in regulation of metabolism downstream of gut specification

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We previously reported that genetic perturbations that partially compromise through the gut specification factors END-1 and END-3 result in characteristic “hypomorphic gut specification” or HGS defects, apparently resulting from variability in the timely activation of ELT-2. Even though HGS embryos may be delayed in ELT-2 activation, final ELT-2 levels are comparable to normal animals. HGS defects include accumulation of fat (dyslipidemia) seen by accumulation of large fat deposits seen by Oil Red O and a fluorescent *in vivo* fluorescent Perilipin/PLIN-1 marker for lipid droplets. The fat defects may be part of a global change in metabolism, as an ATP sensor suggests that steady-state levels of ATP are reduced in HGS strains. Such animals also display a range of pleiotropic effects on fertility and body morphology, among others, that could be downstream of these metabolic changes. From RNA-Seq studies on isolated intestinal tissue from HGS adults, we identified a small set of differentially expressed regulators, including the Nuclear Hormone Receptor gene *nhr-156.* In HGS adults, *nhr-156* is downregulated relative to the wild type. A deletion mutation in the gene results in a similar hyperlipidemia as seen in the most extreme of HGS adults, suggesting that a major component of the fat accumulation in HGS strains is the reduction of expression of this NHR. We find that restoring expression of NHR-156 to HGS strains results in partial rescue of the lipid defect, consistent with a model in which hypomorphic gut specification defects result from decreased expression of *nhr-156* in larvae and adults.

Our earlier results had also shown that accumulation and distribution of lipids in HGS adults was dependent partly on DAF-16, as simultaneous loss of DAF-16 in HGS strains caused a partial suppression of the excess of Oil Red O staining. We now find that in HGS strains, loss of DAF-16 and forced expression of NHR-156 synergistically restore fat distribution to near wild-type. The data suggest a model in which an early failure to robustly activate ELT-2 leads to a subsequent failure to activate *nhr-156* to high levels, leading to changes in fat storage and metabolism that are dependent on both repression of NHR-156 and activation of DAF-16.

These results connect intestinal specification with function of the differentiated gut, suggesting that robust embryonic specification of intestine is required to set a correct metabolic state. Unexpectedly, the apparently normal expression of ELT-2, even if delayed by only hours, misses some time window to set this state.

322A Lineage-specific paths to the same cell type

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Convergent differentation – in which the same cell type is produced by multiple unrelated developmental lineages – provides a striking exception to the classical framework of cell fate specification, in which a given cell type arises exclusively from a single lineage. Convergent differentiation has long been appreciated in *C. elegans* and is also prevalent in vertebrate development, most notably in the neural crest. However, the mechanisms that dictate whether lineages will take on divergent or convergent

transcriptional paths are not well understood. We focused on the development of six radially symmetric glial cells, called ILso glia, that are produced by distinct lineages. Through an unbiased genetic screen, we found that the Forkhead transcription factor UNC-130/Foxd3 is absolutely required for the specification of the dorsal, but not the ventral or lateral, ILso glia. Through lineaging and timed rescue experiments, we found that UNC-130 acts transiently as a transcriptional repressor in progenitors and newly-born terminal cells of the dorsal ILso lineage. UNC-130 can be functionally replaced with the neural crest determinant Foxd3, suggesting it may play a conserved role in lineages that undergo convergent differentiation. Surprisingly, in addition to loss of the dorsal ILso, we find that *unc-130* mutants also have ectopic formation of another glial type, the AMso glia. Ectopic AMso glia take on the morphology and functions of their endogenous counterparts, including dividing in males to produce an extra male-specific MCM neuron. However, they consistently arise in the wrong anatomical position, suggesting they may be produced by mis-specification of cells from the dorsal ILso lineage. We find that the neuronal determinant UNC-86/Brn3 is spuriously upregulated in the dorsal ILso lineage in *unc-130* mutants and is required for the ectopic AMso glia to form, suggesting that one role of UNC-130 may be to prevent expression of UNC-86 in this lineage. Together, these data suggest that convergent differentiation involves distinct transcriptional paths leading to the same cell type, and highlight the importance of transiently-acting transcriptional repressors that may constrain progenitor fates.

323B Tagged endogenous ERL/MPK-1 MAP Kinase provides a novel tool for examining its activation *in vivo*

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Inappropriate activation of the Ras>Raf>MEK>ERK signaling cascade is a common driver in both cancer and RASopathies, and as such represents an attractive target for development of therapeutics. The terminal MAP kinase, extracellular signal-regulated kinase (ERK), has been studied extensively *in vitro* and *ex-vivo* with cultured cells but very little *in vivo.* We utilized *C. elegans* and CRISPR genome editing to tag the endogenous *C. elegans* ERK-encoding gene, *mpk-1. We observed* endogenous MPK-1::mKate2 protein to be ubiquitously expressed, with elevated expression at L2 and L3 stages and in a distinct set of tissues. We validated our tool as a reporter of upstream activation by observing cytosol-to-nuclear translocation of MPK-1 in the most proximal maturing oocyte, a phenotype that was enhanced by deletion of the negative regulator *rskn-1*. We next examined MPK-1 nuclear recruitment during patterning of the vulval precursor cells (VPCs). MPK-1 has been shown to be necessary and sufficient for the central VPC, P6.p, to assume 1° fate during developmental patterning of the six VPCs. Unexpectedly, during VPC induction we observed MPK-1 to translocate into the nuclei of all six VPCs in a temporal and concentration gradient centered on P6.p. This observation contrasts with previous results using the ERK-nKTR reporter of substrate activation. Our reagent raises some interesting questions about mechanisms and indicators of MPK-1 activation and may provide new insights into regulation of MPK-1 activation *in vivo*.

324C Regulation of the duration of breast cancer dormancy by UNK

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Breast cancer is the most frequent malignancy diagnosed in women worldwide. After an initially successful treatment, the main cause of breast cancer related mortality remains cancer recurrence. During the time between remission and relapse to active disease (Disease-Free Survival, DFS), quiescent cancer cells persist in a state referred to as cellular dormancy. The molecular mechanisms involved in the transitions between dormancy and active disease remain obscure. Kaplan-Meier analyses of all intrinsic molecular subtypes of breast cancer revealed that increased expression of Unkempt (UNK) is associated with longer DFS periods in treated patients. UNK is a conserved zinc-finger protein that functions by binding target mRNAs in a sequence-specific manner and reducing protein production. Expression analyses of breast cancers using the cBioportal for Cancer Genomics revealed a group of genes that showed inverse expression from UNK, and further Kaplan-Meier analyses showed that increased expression of a subset of these genes was associated with reduced DFS. We hypothesized that this subset of genes may contain targets of UNK inhibition in breast cancer cells. UNK has one ortholog in *C. elegans*, *unk-1.* As many underlying molecular pathways are conserved between mammalian and nematode development, we hypothesized that a functional model for cancer cellular dormancy could be the transition of *C. elegans* into dauer diapause, and that *C. elegans* could provide a robust readout for genetic analysis of UNK targets involved in breast cancer. While in dauer, progenitor cells remain multipotent and quiescent, similar to dormant cancer stem cells. Previous work from the Karp laboratory has shown that *unk-1* is involved in determination of cell fate, and that *unk-1(0)* dauer larvae aberrantly express the adult cell-fate marker *col-19p::gfp.* We are using RNAi of *C. elegans* orthologs of the inversely expressed putative UNK targets to screen *unk-1(0)* dauer larvae for suppression of the *col-19p::gfp* expression phenotype. By identifying potential targets of UNK inhibition, surrogate pharmacological inhibitors could be developed that would improve DFS when used as combination therapeutics in breast cancer.

325A Analyzing the spatiotemporal structure of heterochronic miRNA transcription using microfluidics live-imaging of nascent miRNA dynamics

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The expression of *C. elegans* heterochronic miRNAs during larval development, among them *lin-4* and *let-7*, has previously been shown to be highly pulsatile, with expression peaking once per larval stage, phase-locked with the molting cycle (Hendriks et al., 2014; Kim et al., 2013, Perales et. al.,2014; Wynsberghe et. al.,2014). While the molecular mechanisms driving this pulsatile expression are unknown, our labs have previously identified two regulators of pulse amplitude. LIN-42/Period is also shown to dampen transcriptional output of miRNAs by negatively regulating the overall duration of transcription within each larval stage (Perales et. al.,2014; Wynsberghe et. al.,2014). Antagonistically, *blmp-1* and *elt-3* null mutants completely suppress *lin-42(lf)* phenotypes and BLMP-1 acts as pioneer factor to enhance miRNAs transcription output (Stec et al., 2021). All of the previous approaches to measure the transcriptional dynamics of miRNAs in-vivo have relied on fluorescent transcriptional reporters, limiting temporal and spatial resolution. Here, we present our ongoing efforts to overcome this limitation by monitoring miRNA transcription using MS2/MCP-GFP based RNA-localization. By combining high-resolution long-term imaging with microfluidics (Keil et al., 2017) with extensive image registration, segmentation, tracking and image analysis, we reveal intriguing wave-like spatiotemporal transcriptional patterns of *lin-4* in hypodermal cells as well as vulval precursor cells within larval stages. We also characterize the relationship between lineage descendancy, cell cycle and transcriptional timing among hypodermal and vulval precursor cells. Finally, we measure how these spatiotemporal features of transcription are altered in *lin-42* and *blmp-1* mutants. Our results establish a new approach to 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* epidermis.

326B Identifying genes regulating cell fate and multipotency in the SGP/hmc cell fate decision

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Pluripotency is the potential for a cell to give rise to any cell type in an organism. Multipotency is the potential for a cell to give rise to multiple different cell fates, but the scope of possible cell fates for a multipotent cell is narrower than that of a pluripotent cell. While the determinants of pluripotency are being heavily studied, not as much research is being done on the determinants of multipotency. In *C. elegans*, there are a pair of precursor cells that divide into two daughter cells with distinct fates. One daughter cell becomes a multipotent progenitor known as the somatic gonadal precursor (SGP) while the other becomes a terminally differentiated head mesodermal cell (hmc). The SGP gives rise to all of the tissues of the somatic gonad. This suggests that the factors specifying multipotency are segregated into the SGP daughters and away from the hmc daughters. Our goal is to identify these determinants of multipotency vs. terminal differentiation. We used fluorescence-activated cell sorting to isolate SGPs and hmcs from the same worms and performed RNA sequencing to identify ~6000 genes that are differentially expressed between the two cell types (Mathies *et al.,* 2019). Of these 6,000 genes, 175 were transcription factors that were more highly expressed in SGPs than in hmcs. The goal of the project is to identify transcription factors that regulate multipotency in this lineage. We have taken the approach of inactivating the transcription factors by RNAi and observing if there is any change in the expression of SGP or hmc markers. The hmc is marked with *arg-1::*GFP, while the SGPs are marked with *ehn-3::tdTomato*. We are also examining L4 staged worms for changes in gonadal morphology indicative of a loss of multipotency. Each SGP generates one of the two gonadal arms; therefore, changes in the fate or potency of the SGPs could be reflected in gonadal shape. To date, we have screened 44 of the 175 transcription factor genes and found two genes in which expression of GFP is higher in SGPs compared to our negative control and three genes for which gonadal shape is altered. These genes are good candidate transcription factors that may play a role in determining cell fate and/or multipotency. Our plan going forward is to continue screening the list of 175 transcription factors to identify genes with differentiated expression for further analysis. Ultimately, we aim to identify the downstream targets of these transcription factors; among these are the direct mediators of multipotency. This project will contribute to our understanding of multipotency, and it opens the door for research into homologous genes in other organisms that may be conserved regulators of multipotency.

327C Quantitative model formation of the heterochronic pathway in *C. elegans*

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Development of multicellular organisms requires appropriate timing of cell division, differentiation and morphogenesis. In *C. elegans*, and likely other animals, temporal organization is explicitly provided by a genetic regulatory system called the heterochronic pathway. Mutations in heterochronic genes cause abnormal timing of developmental events. Although central to temporal control, the dynamics of the pathway are not well described, and therefore it is not clear how this pathway times the different developmental events, and how mutations in these genes are leading to the observed phenotypes. To gain a better understanding of the dynamics in the heterochronic pathway, we use a confocal imaging technique that allows us to track multiple individual worms simultaneously at high temporal resolution from hatch to adulthood, while the worms are able to freely move around. We are developing an automated imaging analysis workflow in Python where the endogenous levels of the core heterochronic proteins are quantified, while the program keeps track of the developmental stage of each specific worm. This will result in an atlas which describes the detailed expression patterns of heterochronic genes in wild-type worms. Based on this information, and known molecular interactions, we plan to build mathematical models to get a deeper understanding of the observed dynamics and how they relate to the timing of development. To study the effect of altered expression patterns caused by mutations, we plan to zoom in on the dynamics of expression in epidermal cells, and correlate this with more detailed developmental events e.g., timing of cell division and alae formation. We expect that these results will give us a better mechanistic understanding of the heterochronic pathway, and how the passage of time is recorded and acted upon.

328A Sub-toxic concentrations of perfluoroalkyl substances (PFAS) dose-dependently delay *C. elegans* larval development and population growth

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Perfluorooctanoic acid and perfluorooctanesulfonic acid, collectively referred to as per- and polyfluoroalkyl substances (PFAS), are man-made organic chemicals used in the production of many industrial items. Environmental exposure to PFAS is correlated with health complications ranging from cancer to infertility in humans and animals. However, the mechanisms underlying these effects are still unclear. Here, we explore the effects of PFAS on *C. elegans* development and reproduction. We exposed *C. elegans* to a range of PFAS concentrations (~0.1 - 4 mM) and examined aspects of developmental and reproductive toxicity including population growth, timing of larval development, and brood size. While the highest concentrations of PFAS led to acute toxicity, lower concentrations had more subtle effects. Exposure of worms to PFAS dose-dependently delayed population growth without impairing food access. This was, in part, due to a lengthening of larval development as PFAS-exposed worms took longer to reach sexual maturity. In the future, we plan to take advantage of the short generation time of *C. elegans* to examine transgenerational effects of PFAS exposure. We will also exploit their ease of genetic manipulation to identify genes important for PFAS susceptibility or resistance. This will provide insight into cellular pathways that are affected by PFAS exposure and will help to elucidate mechanisms underlying related health effects.

329B Temporal scaling in C. elegans larval development

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It is essential that correct temporal order of cellular events is maintained during animal development. During post-embryonic development, the duration of development depends on external conditions, such as food availability, diet and temperature. How timing of cellular events is impacted when the rate of development is changed is not known. We used a novel time-lapse microscopy approach to simultaneously measure the timing of oscillatory gene expression, seam cell divisions and cuticle shedding in individual animals during *C. elegans* larval development. We then studied how timing of these events was impacted by changes in temperature or diet, and in *lin-42/Period* mutants that show strongly perturbed and heterogeneous timing of larval development. We uncovered significant variability in timing between individuals under the same conditions. However, we found that changes in timing between individuals were fully explained by temporal scaling, meaning that each event occurred at the same relative time, when rescaled by the total duration of development in each individual. Upon changing conditions, we found that larval development separated into distinct epochs that differed in developmental rate. Changes in timing of individual events were fully captured by temporal scaling for events occurring within each epoch, but not for events from different epochs. We further studied the impact on timing of shifts in food abundance and environmental conditions during larval development. Overall, our results reveal a surprisingly simple structure that governs changes in timing of development in response to environmental conditions. The unexpected observation of continued development and

accurate temporal scaling in growth-arrested *lin-42* mutants rules out a mechanism that explains temporal scaling by linking developmental timing to body size.

330C Recursive Transcriptional Feedforward Loops Ensure Robust Endoderm Development in *C. elegans*

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The interface between specification and differentiation is a pivotal phase in animal development. What is the underlying architecture of developmental regulatory networks that directs the transition from specification to differentiation of progenitor cells during embryogenesis? How do these mechanisms provoke a restricted pattern of differentiation and how is differentiation established and maintained? By studying the *C. elegans* endoderm gene regulatory network, we found that GATA transcription factors (MED-1/2, END-1/3, and ELT-2/7) act through a series of feedforward loops, leading to robust and rapid lockdown of cell fate. We further found that END-1 is poised at the interface between specification and differentiation: END-1, acting with END-3, regulates *specification* of the endoderm E lineage, whereas END-1, acting with ELT-7 and ELT-2 controls *differentiation* of the intestine. Finally, we observed ectopic characteristics of pharyngeal-intestinal valve cell differentiation evident in the anterior gut when the functions of END-1 and ELT-7 are eliminated, suggesting a role of END-1 and ELT-7 in repressing valve cell fate in the intestine and/or regulating valve development cell non-autonomously.

331A Coordinating proliferative and invasive cellular fates: insights from C. elegans somatic gonad development

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The decision between proliferation and invasive differentiation is a key part of several developmental events as well as cancer; however, how cells switch between these states is not well understood. Two cell types in the *C. elegans* somatic gonad, the anchor cell (AC) and ventral uterine (VU) cells, both specified from initially equipotent cells through a Notch-mediated cell fate decision, serve as an excellent model of this dichotomy in cellular behavior. The post-mitotic AC, the default state of the AC/VU decision, goes on to invade the underlying basement membrane during development of the reproductive system, while the VUs remain proliferative. We have previously shown that four transcription factors, *fos-1* (Fos), *egl-43* (EVI1/MEL), *hlh-2* (E/Daughterless), and *nhr-67* (NR2E1/Tailless/TLX), are necessary for AC invasion, with the latter three playing a role in regulating cell cycle arrest. We next examined whether any of these transcription factors are sufficient for invasion and find that ectopic expression of NHR-67, which is enriched in the AC compared to its neighbors, results in transdifferentiation of VUs into invasive ACs. We hypothesize that post-translational degradation of HLH-2 in the VU results in lower levels of NHR-67 and that a transcriptional repressive mechanism functions in the VU to prevent remaining NHR-67 activity from inappropriately activating AC-specific targets. We find that knockdown of *unc-37* and *lsy-22*, homologs of the transcriptional repressor Groucho, results in a low penetrance of animals with multiple ACs. Others have previously demonstrated that Groucho forms a repressive complex with histone deacetylase (*hda-1*) and TCF/LEF (*pop-1*) to restrict endoderm differentiation in the early embryo. Unlike *hda-1* or Groucho, which are not differentially expressed between the two cell types, *pop-1* is enriched in the VUs compared to the AC, but does not appear to activate transcription based on quantification of a transgenic POP-1 activity reporter. Furthermore, we find that depletion of the pro-invasive TFs associated with cell cycle arrest results in ectopic expression of *pop-1* in the AC. Here, we investigate whether differential *pop-1* levels are necessary for AC/VU fate maintenance as well as whether this process is downstream or synergistic with Notch signaling and/or cell cycle dynamics. Together, these results provide new insights into how cells can establish and maintain cellular programs necessary for growth and morphogenesis.

332B Y-to-PDA transdifferentiation occurs through an epithelial cell intermediate and requires *ngn-1, hlh-16, unc-44, unc-119,* and *unc-33*

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In *C. elegans*, the rectal epithelial Y cell transdifferentiates into the PDA motor neuron (Jarriault *et al.*, 2008). Transdifferentiation (Td) has been suggested to occur in discrete steps: the Y cell loses rectal contacts and de-differentiates, migrates away from the rectum, and re-differentiates into a motoneuron. Using a membrane-tagged fluorescent reporter, we now observe a more complex pattern of events. While the cell body migrates away from the rectal slit, the Y cell maintains rectal tube contacts, marked with the apical junction protein AJM-1. From the rectal contact site, the cell then extends a process that matures into the PDA axon. Rectal apical junctions are lost later. Previous studies revealed that SEM-4 and

EGL-5 transcription factors are required for erasure of Y cell epithelial identity and acquisition of neuronal identity. We identified two novel Td transcriptional regulators, the bHLH proteins NGN-1 and HLH-16, also required for these steps. *ngn-1* expression increases as the Y cell migrates away from the rectum and changes cellular morphology. From reporter fusion studies, we found that SEM-4 represses *ngn-1* expression, whereas EGL-5 and HLH-16 promote *ngn-1* expression. Although *ngn-1* induction correlates with the timing of Y cell migration, precocious expression of *ngn-1* in *sem-4* mutants does not change migration timing, suggesting involvement of other regulators. We also identified three cytoskeletal organizing proteins required for acquisition of neuronal identity: *unc-44, unc-119,* and *unc-33.* These proteins appear to act downstream of NGN-1 and HLH-16 and are required for PDA axon extension. Y-to-PDA Td bears striking morphological and molecular similarities to vertebrate spinal-cord pMN-domain motoneuron formation, and to islet cell formation in the pancreas. During development, motoneuron and pancreatic beta cell precursors originate from an epithelium. These precursors then lose their junctions and delaminate from the epithelium. As they migrate and mature, the cells initiate transcription of motoneuron-associated genes, become innervated, and secrete neurotransmitters or insulin, respectively. In both the spinal cord and pancreas, NGN-1 and HLH-16 homologs (Ngn2/Olig2 and Ngn3/bHLH4, respectively) are required for epithelial identity erasure. Furthermore, *unc-44/*Ankyrin promotes neuronal and beta cell maturation. Thus, our findings may provide mechanistic insight into the differentiation of these important vertebrate cell types.

333C Sexually dimorphic glia-neuron reprogramming in *Caernorhabditis elegans.*

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How fully differentiated cells can retain their neurogenic potential remains a central question in developmental biology. Previously, we described a glia-to-neuron cell fate switch during sexual maturation in the nervous system of male *Caenorhabditis elegans*; whereby stably differentiated amphid socket (AMso) glial cells in the head undergo asymmetric cell division to self-renew and give rise to the MCM (Mystery Cells of the Male) neurons[1].

Using forward genetic screening and candidate gene approaches we have identified a particularly exciting class of mutants (Class III) in which the AMso divides but fails to adopt MCM neuronal identity, instead retaining glial marker expression. We also find that mutations in the zinc finger transcription factor *lin-48*, an ortholog of vertebrate OVO1 and in the proneural bHLH transcription factor *hlh-14,* a homolog of vertebrate ASCL1 generate a Class III phenotype. In addition, we have isolated two novel Class III mutants; *nom-7* and *nom-9* and we are currently performing mapping-by-sequencing to identify the causal locus and will present our most recent findings here. Our analysis of Class III genes will help us to elucidate the molecular mechanisms regulating the plasticity underlying this sexually dimorphic AMso-to-MCM transdifferentiation event.

[1]Sammut, M. *et al.* Glia-derived neurons are required for sex-specific learning in C. elegans. *Nature* **526**

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334A Characterization of two evolutionarily conserved *C. elegans* Ceh-6/Oct and Sox-2/Sox2 transcriptional factors during a natural Y-to PDA transdifferentiation event

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During trans-differentiation, a differentiated cell changes its identity and becomes another different specialized cell. This process is also known as direct cell conversion or direct reprogramming and plays an important role in development, tissue regeneration and diseases. In *C. elegans,* our lab has pioneered the study of natural trans-differentiation, several examples of which are now documented, starting from embryonic to larval development and including Y-to-PDA transdifferentiation (Jarriault et al., 2008, Rothman & Jarriault &, 2019). Initially, the Y cell, together with the other five cells forms the functional rectum of *C. elegans* and harbor specialized epithelial features. During larval development, it naturally transdifferentiates, acquires neuronal cell identity, and becomes a PDA motoneuron. This process is governed by various molecular players such as chromatin remodelers and transcription factors (Kagias et al 2012, Zuryn et al 2015). Previous work from the lab has shown that the *C. elegans* POU transcription factor CEH-6 and SOX-2 form, with other genes, a NODE-like complex (NODE mammalian counterpart) and are important for the initiation of this reprogramming event. Here, through genetics and biochemical studies, we have analyzed the characteristics of *C. elegans sox-2* and *ceh-6* and have compared them to their mammalian counterparts. We have dissected their function over time and which domains are important for Y-to-PDA Td. We have further tested putative

functional redundancy with the other two Pou paralogs *ceh-18* and *unc-86*, and mammalian Oct4. Our work highlights both conserved and divergent molecular mechanisms underlying different reprogramming processes.

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335B *daf-16*/FOXO blocks adult cell fate in *C. elegans* dauer larvae via a branched pathway involving *lin-41*/TRIM71

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Tissue-specific stem cells maintain the ability to produce multiple cell types during long periods of non-division, or quiescence. Similarly, in *C. elegans* dauer larvae, progenitor cells are quiescent and maintain the ability to produce all normal cell types after dauer. Indeed, a process involving *daf-16* actively re-establishes multipotent cell fate in vulval precursor cells during dauer. Here, we examine the role of *daf-16* in a different progenitor cell type, lateral hypodermal seam cells. Seam cells are multipotent in larvae but differentiate at adulthood. We found that *daf-16(0)* dauer larvae expressed multiple endogenous adult-specific collagens as well as the adult cell-fate marker, *col-19p::gfp*, thus linking *daf-16* to seam cell multipotency. During continuous development, *col-19* expression is directly activated by the LIN-29 transcription factor. *lin-29* is in turn directly repressed by the LIN-41 RNA-binding protein. We found that *lin-41* also regulates *col-19* during dauer because *lin-41(RNAi)* dauer larvae expressed *col-19p::gfp*. *daf-16* appears to act upstream of *lin-41* because *lin-41* expression was reduced in *daf-16(0)* dauer larvae. Furthermore, a *lin-41* gain-of-function allele suppressed the *col-19p::gfp* phenotype in *daf-16(RNAi)* dauer larvae. Surprisingly, our data suggest that *lin-29* plays a minor role in regulating *col-19p::gfp* expression during dauer. Loss of *lin-29* did not completely suppress the precocious *col-19p::gfp* phenotype observed in *lin-41(-)* dauer larvae. In addition, loss of *lin-29* had no effect on the *col-19p::gfp* phenotype in *daf-16(0)* dauers, and expression of an endogenously tagged *lin-29::gfp* was unaffected in *daf-16(0)* dauer larvae. Taken together, our data suggest that *col-19p::gfp* expression during dauer is regulated at least partially independently of *lin-29*. We used RNA-seq to identify other potential regulators of *col-19p::gfp* during dauer and found over 3000 genes that are differentially expressed at least 2-fold (FDR < 0.05) in *daf-16(0)* vs. control dauers, including over 200 transcription factors. This work demonstrates that *daf-16* coordinates dauer formation with seam cell fate via a novel mechanism. This mechanism may be conserved in mammals where the *daf-16* ortholog, FOXO is essential for both quiescence and stem cell maintenance.

337A The 3'UTR is required for MEX-3 expression pattern and contributes to animal fecundity

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In early embryonic development prior to zygotic transcription, post-transcriptional regulation of mRNA is key to cell fate specification and patterning. In *C. elegans*, there are several RNA-binding proteins (RBPs) that coordinate oogenesis, spermatogenesis, and embryogenesis through 3'UTR-mediated post-transcriptional mechanisms. How these RBPs are themselves regulated is largely unknown. Here, we assess the role of the 3'UTR in both patterning the expression and coordinating the function of the highly conserved RBP MEX-3. Null *mex-3* mutants are maternal-effect embryonic lethal with anterior cell fate specification defects (Draper et al., 1996). Previous studies demonstrate that the 3'UTR of *mex-3* is sufficient to establish its germline expression pattern (Merritt et al., 2008, Kaymak et al., 2016). To test whether the UTR is necessary for patterning and reproductive viability, we used CRISPR/Cas9 to make an allelic series of *mex-3* 3'UTR deletions in a strain where the endogenous MEX-3 is tagged with GFP (GFP::MEX-3). Among the 3'UTR deletion mutants, two variants displayed increased expression of GFP::MEX-3, with the biggest deletion showing ectopic de-repression throughout the germline indicating that the 3'UTR is required for MEX-3's expression pattern. Surprisingly, both variants were homozygous fertile but exhibited reduced fecundity. To identify the RBPs that mediate MEX-3 expression and activity through its 3'UTR, we used RNAi to knock down several candidate RBPs in the GFP::MEX-3 strain and a *mex-3* 3'UTR transgenic reporter strain (GFP::*mex-3* 3'UTR). We confirm that GLD-1, LIN-41, and OMA-1/2 regulate MEX-3 expression, and show that this repression is mediated through its

3'UTR. We also found that an additional RBP, DAZ-1, regulates MEX-3 expression but not through the 3›UTR. To determine the mechanisms involved in MEX-3 patterning, we used RNAi to systemically knock down components of the adenylation and de-adenylation complexes and translation initiation factors. Our results revealed that both poly(A) tail length and translational control orchestrate the unique expression pattern of MEX-3, with different mechanisms predominating in different regions of the germline. Taken together, our results demonstrate that the *mex-3* 3'UTR is essential for patterning its expression, but not required for fertility. Our results describe the complex network of pathways required to coordinate the MEX-3 expression pattern, but also suggest that precise spatiotemporal control is not as critical as previously thought.

338B Modeling the *C. elegans* Germline Stem Cell Genetic Network using Automated Reasoning

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Computational methods and tools are a powerful complementary approach to experimental work for studying regulatory interactions in living cells and systems. We demonstrate the use of formal reasoning methods as applied to the *Caenorhabditis elegans* germ line, which is an accessible model system for stem cell research. The dynamics of the underlying genetic networks and their potential regulatory interactions are key for understanding mechanisms that control cellular decision-making between stem cells and differentiation. We model the "stem cell fate" versus entry into the "meiotic development" pathway decision circuit in the young adult germ line based on an extensive study of published experimental data and known/hypothesized genetic interactions. We apply the reasoning engine for interaction networks tool (RE:IN) to derive predictive networks for control of differentiation. Using RE:IN we simultaneously specify many possible scenarios and experiments together with potential genetic interactions, and synthesize genetic networks consistent with all encoded experimental observations. In silico analysis of knock-down and overexpression experiments within our model recapitulate published phenotypes of mutant animals and can be applied to make predictions on cellular decision making. This work lays a foundation for developing realistic whole tissue models of the *C.elegans* germline where each cell in the model will execute a synthesized genetic network.

339C Cytokinesis incompletion drives the initial expansion of the *C. elegans* syncytial germline

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While syncytial architectures are common among animal germlines and required for fertility, the mechanisms leading to syncytium formation and expansion remain largely unknown. *C. elegans* constitutes a powerful *in vivo* model to study syncytium regulation throughout development. The adult germline is organized as a syncytium in which each germ cell possesses an intercellular bridge that is maintained by a stable actomyosin ring and that connects it to a common pool of cytoplasm, termed the rachis. How germ cells undergo cytokinesis while maintaining this syncytial architecture is not completely understood. We characterized the organization of the primordial germ cells (PGCs) in *C. elegans* first stage larvae and studied the first PGC division to better understand how the syncytial structure expands. Using confocal and electron microscopy, we found that each PGCs possesses a stable intercellular bridge that connects it to a common pool of cytoplasm, which we term the proto-rachis. We found that as in the adult syncytium these intercellular bridges are stabilized by actomyosin rings and enable cytoplasmic exchange between the germ cells. These results indicate that the organization of the primordial germline in first stage larvae is fundamentally the same as in adult animals. Using live imaging and fluorescence photobleaching approaches, we further found that the first PGC cytokinesis is incomplete and that the stabilized cytokinetic ring progressively moves toward the proto-rachis and eventually integrates into it. In addition, we found that cytoplasmic exchange maintained as PGCs undergo cytokinesis, indicating that the connection to the proto-rachis remains effective during PGC division. Our results support a model in which the initial expansion of the *C. elegans* syncytial germline occurs by incomplete cytokinesis, where one daughter germ cell inherits the actomyosin ring that was newly formed by stabilization of the cytokinetic ring, while the other inherits the pre-existing stable actomyosin ring. We propose that such mechanism of iterative cytokinesis incompletion underpins *C. elegans* germline expansion and maintenance.

340A GLP-1 Notch - LAG-1 CSL control of the germline stem cell fate is mediated by transcriptional targets *lst-1* and *sygl-1*

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Stem cell systems are essential for the development and maintenance of polarized tissues. Intercellular signaling pathways control stem cell systems, where niche cells signal stem cells to maintain the stem cell fate/self-renewal and inhibit differentiation. In *C. elegans*, GLP-1 Notch signaling specifies the germline stem cell fate and inhibits meiotic development, employing the sequence-specific DNA binding protein LAG-1 to initiate the transcriptional response. We undertook a comprehensive genome-wide approach to identify transcriptional targets of GLP-1 signaling. We expected primary targets to (a) be directly bound by LAG-1 in the germline observed through ChIP-seq experiments, and (b) require GLP-1 signaling for their RNA accumulation in dissected germlines based on RNA-seq analysis. Furthermore, we have shown that all stem cells switch to meiotic development in response to germline autonomous auxin inducible degradation of LAG-1 or GLP-1. We therefore performed a time-course transcriptomics analysis, following auxin inducible degradation of LAG-1, to distinguish between genes whose RNA level was a primary or secondary response of GLP-1 signaling. Thus far, we have identified *lst-1* and *sygl-1* as two genes that fulfilled these criteria, consistent with their known function to promote the stem cell fate. In addition, three secondary response genes were identified based on their timing following loss of LAG-1, their lack of a LAG-1 ChIP-seq peak and that their *glp-1* dependent mRNA accumulation could be explained by a requirement for *lst-1* and *sygl-1* activity. Moreover, our analysis also suggests that the function of the primary response genes *lst-1* and *sygl-1* can account for the *glp-1* dependent peak protein accumulation of FBF-2, which promotes the stem cell fate and, in part, for the spatial restriction of elevated LAG-1 accumulation to the stem cell region.

341B Significance of RNA Binding Motif Protein (RBM-39) in developmental processes in *C. elegans*

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RNA Binding Motif Protein 39 (or Caperα) is a conserved RBP that is known to act as an alternative splicing activator. Caper (RBM39 homolog in *Drosophila*) has been studied in the development and maintenance of the nervous system, and in cancers. Deficiency in Caper/RBM-39 compromises dendrite morphology, sensory neuron development, neuromuscular junction morphogenesis, and lifespan, mainly in *Drosophila*. However, the full phenotypic effect of RBM-39 remains obscured due to the absence of a knockout mutant. Using *C. elegans* as a model, we have successfully generated an *rbm-39* knockout mutant *(cnj4)* via CRISPR. Homozygous deletion of *rbm-39* leads to severe developmental defects, including sterility, early death, and larval arrest. We used a genetic balancer *tmC25* to maintain the recessive sterile allele in heterozygotes. To investigate the potential causes of mutant sterility, a phenotypic analysis of germline development defects in *rbm-39* mutants was conducted. Our results show that *rbm-39(cnj4)* animals have either delayed or failed in oogenesis. We found that the mitotic zone length of the germline was shorter in the homozygous *rbm-39(cnj4)* than N2 (wild type) and heterozygotes, suggesting disruptions in proliferation and/or maintenance of the germline stem cells. Gonad visualization also revealed abnormal oocytes that were seemingly endomitotic in *rbm-39(cnj4)* animals. Taken together, RBM-39 is essential for proper germline development and oogenesis. Since RBM-39 is predominantly an alternative splicing factor, genes that control aspects of germline development may be mis-spliced in the mutant. In general, our study highlights the importance of this conserved splicing factor, RBM-39, in reproductive and early development. We have established a novel mutant strain for future investigations while also expanding the knowledge of this protein›s functions in *C. elegans*. Given that germline development in *C. elegans* is well studied and the processes are highly coordinated with defined signaling pathways, the mechanism of RBM-39 in germline development can be studied using the *rbm-39(cnj4)* strain. Since *rbm-39(cnj4)* mutants exhibited mild uncoordinated locomotion, which is strongly associated with neuronal or muscular defects, future studies should examine the RBM-39 knockout phenotype in neurons and dendritic branches.

342C 3’ UTR mediated post-transcriptional regulation of *glp-1* in the germline of *Caenorhabditis elegans*

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Translational control of maternal mRNAs is a major form of gene regulation during germline development and embryogenesis. In *Caenorhabditis elegans*, the maternal gene *glp-1* encodes a homolog of the Notch transmembrane protein required for cell proliferation in the germline and cell fate specification in the embryo. The RNA binding proteins POS-1 and GLD-1 directly regulate the translation of GLP-1 protein by binding to the specific elements within the *glp-1* 3’ untranslated region (3’ UTR). When POS-1 or GLD-1 binding is disrupted by mutation of their respective elements, the expression pattern of a *glp-1* 3’ UTR transgene changes in both the germline and in embryos. The mechanism by which POS-1 and GLD-1 mediate translation repression is not well understood. Previous work showed that loss of *pos-1* increases the average polyA tail length

of endogenous *glp-1* transcripts in embryos. Here, we show that mutation of either the GLD-1 or POS-1 binding motifs in transgenic reporters does not change

polyA site selection. This result rules out alternative polyA site usage as a mechanism of regulation. We also show that wild-type *glp-1* transgenic reporter embryos have a shorter average polyA tail length compared to mutant transgenic reporters in GLD-1 or POS-1 binding motifs. We further used our reporters to measure the effect of cytoplasmic polyA polymerases, deadenylases and translation initiation factors on the *glp-1* expression level and pattern. The results reveal that POS-1 and GLD-1 exert their effects through different pathways. Our studies provide insights of into the mechanism of *glp-1* post-transcriptional regulation in the germline and embryo.

343A FBF binding elements in the *gld-1* 3'UTR and their role in germline regulation

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PUF RNA binding proteins (for Pumilio and FBF) are key regulators of self-renewal in the *C. elegans* germline. Two nearly identical PUF proteins, FBF-1 and FBF-2 (collectively FBF), have long been known as self-renewal regulators of late larval and adult germline stem cells (GSCs) (1). Genomic studies using iCLIP identified a battery of FBF target RNAs as well as FBF binding elements (FBEs) within them (2). That knowledge of *in vivo* FBEs provides a powerful inroad for studying the direct effects of FBF binding on regulation of individual target RNAs and germ cell fate. A major target of FBF regulation is *gld-1*, which promotes meiotic progression and oogenesis (3). Strong evidence has accumulated for FBF repression of *gld-1* in GSCs (1), but FBF had also been proposed to activate *gld-1* to promote meiotic entry. (1,4). We have now begun to focus on FBEs in the *gld-1* 3'UTR to assess their molecular and biological roles. To this end, we used CRISPR/Cas9 gene editing to mutate the two canonical *gld-1* FBEs, FBEa and FBEb, in the 3'UTR of endogenous *gld-1*. Phenotypically, FBEa and FBEb single mutants both maintain GSCs and are fertile; an FBEa FBEb double mutant was just recovered and is being characterized. Using GLD-1 immunostaining, we find that the FBEa single mutant makes higher than normal levels of GLD-1 protein in GSCs, while the FBEb single mutant makes wild-type levels in GSCs. We are currently examining *gld-1* mRNA levels in these single mutants using smFISH, and soon will start analyzing the double mutant.

1 Crittenden et al.(2002).*Nature*, *417*,660. **2** Porter, Prasad et al.(2019).*G3*,*9*,153. **3**Francis et al (1995) *Genetics* 139, 579 **4** Suh et al.(2009).*Genetics*,*181*,1249.

344B DLC-1 promotes germ granule integrity in *C. elegans* embryo

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P granules are RNA-protein complexes located in the germline of *Caenorhabditis elegans* that are important for RNA regulation, germ cell identity and fertility. P granules are present throughout the *C. elegans* germline lifecycle, during which they undergo several dynamic transitions. For example, P granules are perinuclear during most of germline development but become cytoplasmic during oocyte maturation and during embryogenesis P granules segregate asymmetrically with the P cell lineage that produces the primordial germ cells. Here we identify dynein light chain (DLC-1) as an important determinant of P granule formation and subcellular localization in the *C. elegans* embryo. DLC-1 is a bimolecular hub that interacts with a variety of cellular proteins and functions in diverse processes from dynein-dependent transport to allosteric regulation of ribonucleoprotein complexes. We used an *in silico* motif scanning approach to search for new DLC-1 binding partners, identifying several P granule components as a result. Direct interaction between DLC-1 and PGL-1, PGL-3, GLH-4 and MEG-4 was established using a biochemical binding assay *in vitro*. We confirmed that DLC-1 is in complex with P granule components PGL-1 and PGL-3 in the germline and embryo *in vivo* using proximity ligation assay. Loss of *dlc-1* disrupts assembly of embryonic P granules as assessed by localization of multiple P granule components but does not impact the levels of PGL-1 or PGL-3 protein expression. Analysis of a temperature sensitive dynein heavy chain mutant *C. elegans* suggests that the dynein motor related function of DLC-1 is important for P granule segregation during asymmetric cell divisions and contributes to phase separation of core P granule components at select stages of embryonic development. DLC-1 is a member of the conserved LC8 protein family, whose members contribute to germ granule dynamics in other organisms such as zebrafish. Our findings may shed light on general mechanisms behind LC8 contribution to germ granule phase separation.

345C Investigating the basis for the *aak-1*-specific requirement in homeostatic regulation of GSC proliferation

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The AMP-activated protein kinase (AMPK) is a metabolic master switch that is activated under energy stress and acts to restore that balance. It is a heterotrimeric complex composed of an alpha catalytic subunit, and two regulatory subunits (beta and gamma). In *C. elegans*, the catalytic subunit is encoded by the *aak-1* and *aak-2* genes, which have 53% amino acid identity, and 68% similarity. Both genes are required and act additively, to inhibit germline stem cell (GSC) proliferation during dauer development. That functional equivalence between the two genes however does not hold true for other defects. For example, AAK-2 is largely responsible for promoting the animal's longevity and dauer survival. On the other hand, AAK-1 is specifically required for inducing oocyte and GSC quiescence in animals that lack sperm. As such, feminized *aak-1* mutants, but not feminized *aak-2* mutant adults, lay activated unfertilized oocytes, and have ongoing GSC proliferation. Here, we ask what is the basis for this *aak-1*-specific requirement in homeostatic regulation of GSC proliferation. We first fluorescently tagged endogenous *aak-1* with mNG and compared its expression with that of tagged endogenous *aak-2* (a kind gift from Shaolin Li). We found that aak-1 is weakly expressed in the gut and germline, and more strongly in the gonadal sheath cells. We then asked in which of these tissues *aak-1* was required for homeostatic GSC regulation. Our preliminary results suggest that AAK-1 functions in the gonadal sheath cells. Interestingly, *aak-2* is also expressed in the sheath cells, although at a lower level. As such, we consider two possible scenarios to explain the specific requirement for *aak-1*. Either the two alpha subunits are functionally equivalent and the difference in expression levels in the sheath cells is what explains the respective mutant phenotypes. Alternatively, amino acid sequence divergences between the two subunits must translate into functional differences. To test this, we will overexpress *aak-2* in the gonadal sheath cells to verify whether that could compensate for *aak-1* function. In parallel, we will attempt to rescue *aak-1* null mutants with various *AAK-1::AAK-2* chimeras to identify whether there are functional differences in their slightly divergent domains. Such analysis will help understanding the basis for the functional divergence among the catalytic subunits of AMPK.

Keywords:

Germline, *aak-1*, *aak-2*, AMPK, GSC

346A Regulation and function of the "PUF hub" governing *C. elegans* germline stem cells

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The "PUF hub" is responsible for self-renewal of germline stem cells (GSCs)[1]. This hub consists of four PUF (Pumilio and FBF) RNA-binding proteins – FBF-1, FBF-2, PUF-3 & PUF-11 — and two novel proteins, LST-1 and SYGL-1. The molecular elucidation of both regulation and function of the PUF hub is essential to understand how GSCs are maintained in the face of challenges from physiology and the environment. GLP-1/Notch signaling activates *lst-1* and *sygl-1* transcription to launch and maintain hub activity[2,3]. We now find that point mutations at one or more of three LAG-1 binding sites (LBS) in the *sygl-1* promoter tune its response to niche signaling. Single LBS mutants substantially lower the number of s*ygl-1* active transcription sites (ATS) and double mutants nearly eliminate them; importantly, the GSC pool shrinks in single mutants and is lost in double mutants, when assayed without LST-1. We also find that LST-1 has an unexpected role in *sygl-1* transcription: s*ygl-1* ATS number increases in *lst-1(ø)* mutants with a corresponding increase in SYGl-1 protein. The converse is not true: *lst-1* expression is unaffected in *sygl-1(ø)* mutants. Therefore, LST-1, but not SYGL-1, affects transcription. The primary PUF hub function is regulation of target RNAs. We proposed earlier that each hub PUF protein forms a complex with either LST-1 or SYGL-1 to repress differentiation RNAs[4]. We have investigated this model. We first used co-immunoprecipitation (coIP) to explore the proposed battery of complexes. Though the matrix is only about half done, all coIPs so far support the model. We next identified "PUF-interaction sites" (PIS) in both LST-1 and SYGL-1. Intriguingly, each harbors two redundant PIS: single PIS mutants of either protein retain self-renewal activity but double mutants abolish it. We finally tethered LST-1 and SYGL-1 to a reporter RNA with the λN-boxB system, and found that both λN-LST-1 and λN-SYGL-1 repressed a GFP::H2B reporter with boxB sites in its 3'UTR. These results provide strong evidence that PUF:LST-1 and PUF:SYGL-1 partnerships are essential for GSC maintenance and that LST-1 and SYGL-1 are RNA repressors. In sum, both hub regulation and function are intensively buffered, and our mutants provide new tools for analyses of the hub when poised in an intermediate state.

[1] Haupt et al 2020 *Genetics 214,*147; [2] Kershner et al 2014 PNAS *111*, 3739; [3] Lee et al 2016 eLife 5, e18370; 4 Shin et al 2017 *PLoS genetics 13,* e1007121.

347B Two eIF4E isoforms regulate distinct mRNAs and effect one another in germ cells

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mRNA translational regulation is an important step in protein synthesis in cell differentiation processes. During gametogenesis undifferentiated germ cells progress through multiple developmental stages as they differentiate into mature gametes. Translational control of mRNAs plays an essential role in germ cell gene regulation, particularly as mRNAs move from nucleus to perinuclear granules and eventually to ribosomes. Understanding the biochemistry of germline mRNA interactions with the cap binding protein eukaryotic Initiation Factor 4E (eIF4E) and RNA-binding proteins will help us understand both positive and negative translational control modes that drive germ cell fates by modulating new protein expression. Two major eIF4E isoforms are abundant in the C. elegans germline. IFE-1 and IFE-3 are known to interact selectively with subsets of mRNAs and each appears to have a different role in germ cell fate. IFEs are modulated by interaction with repressive binding 4E-interacting proteins (4E-IPs); IFE-1 by PGL-1 and IFE-3 by IFET-1. Each localize as a complex on adjacent, but distinct, perinuclear granules.

The IFE-3 isoform plays a role in switch from spermatogenesis to oogenesis. Here we describe changes in localization of fluorescently tagged IFEs in the complete absence of the other isoform. Gonads of *ife-3* mutant hermaphrodites adopt a masculinized phenotype, and the IFE-1-expression pattern becomes similar to males. However, its normal association with P granules is diminished. Gonads are narrow and the rachis less evident. IFE-1 is known to have an important role in spermatogenesis; null mutant worms are unable to produce mature sperm. Hermaphrodite gonads lacking *ife-1* show pronounced enlargement in the transition zone. IFE-3 is more in a dispersed pattern in the rachis rather than the tight centralized association with lattice-like structures in control oogenic gonads. We do not have an immediate explanation for the marked changes in gonad morphology by reciprocal loss of each IFE isoform. We will explore the structure of their respective germ granules using mutations in the VASA/GLH-1 helicase found more centrally in P granules as well as the 4E-IP themselves. We will follow the routing of mRNAs held dormant by these granules through their activation and binding of ribosomes.

348C Regulation of GLP-1/Notch signaling in *C. elegans* Germline Stem Cells by Protein Interactions

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Reproductive fitness requires a balance between stem cell self-renewal and differentiation. In the *C. elegans* germline, GLP-1/Notch signaling controls this balance. In the distal region of the germline, high GLP-1/Notch levels promote proliferation. GLP-1 signaling decreases moving proximally, allowing the GLD-1/NOS-3, GLD-2/GLD-3 and SCF^{PROM-1} redundant pathways to be gradually activated, directing cells to enter meiosis/differentiation. Therefore, a stem cell's decision to self-renew or differentiate is governed by the establishment of an opposing gradient 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 that protein-protein interactions between GLP-1 and GLD-1/GLD2 could result in the repression of their normal regulatory roles. The possibility of a direct physical interaction reveals a new level of regulation. We hypothesize that GLP-1 physically interacts with GLD-1/GLD-2 to rapidly establish opposing domains. 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. Current work focuses on how this protein interaction may negatively regulate GLP-1/Notch or the GLD-1/GLD-2 pathways. One approach is to analyze GLP-1 signaling in germlines with ectopic expression of the N termini of GLD-1/2 domains. While GLD-2N was able to be ubiquitously expressed, GLD-1N localization largely resembles endogenous GLD-1, suggesting a novel mechanism of controlling GLD-1 accumulation. More work is underway to express GLD-1N in the distal germline. This research will contribute to a better understanding of the precise regulatory mechanisms governing stem cell proliferation.

349A PAR-CLIP experiments used to identify parallel pathways to the core germline development pathway

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The *C. elegans* germline is a model system for studying the intricate pathway interactions that drive cell fate decisions. The hermaphrodite gonad is spatially separated with a population of stem cells at the distal end, while cells at the proximal end have switched to meiosis and form both spermatocytes and oocytes. The regulation of germline stem cell fates in *C. elegans* is similar to other higher eukaryotes, in that the canonical network seems to involve many RNA-binding proteins and multiple points of post-transcriptional control. It certainly seems like there needs to be a carefully controlled and coordinated process to regulate all the gene products that influence the ultimate entry to meiosis.

There are several studies that suggest additional regulators are required for normal germline proliferation, beyond the core GLP-1 Notch pathway. These regulators could represent one or more pathways that function parallel to GLP-1 Notch signaling and the meiotic switch. The fact that many of the regulators identified so far are proteins involved in RNA metabolism bolsters the hypothesis that the core pathway for sex determination is incomplete, and there are more branches yet to be identified in the control of the meiotic switch.

In this research we have utilized PAR-CLIP experiments to identify potential RNA targets of regulatory proteins found in germline developmental pathways. The central theme to this research is to tag RNA-binding proteins, and then crosslink these hybrid proteins with potential target RNAs. By identifying the RNAs, we can begin to flesh out how these proteins function to influence germline fates. Example research results will be discussed further.

350B The PAF1 complex cell-autonomously regulates oogenesis in *Caenorhabditis elegans*

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During animal development, spatiotemporal gene transcription regulation is essential to regulate cell behavior. To precisely regulate gene transcription, regulation of the targeting of RNA polymerase II (pol II) to specific genes is required.

RNA polymerase II-associated factor 1 complex (PAF1C) is a protein complex that consists of PAF1, CDC73, CTR9, LEO1, and RTF1, and has been shown to be involved in regulating pol II-mediated transcription. Although, it has been shown to regulate a variety of biological processes including cell differentiation and tumor suppression, a precise role for PAF1C during germ line development has not been clarified.

The *C. elegans* hermaphrodite gonad produces sperm during late larval stage, which is then reserved in spermatheca. Subsequently, the gonad produces oocytes during the adult stage. Finally, gametogenesis is achieved via self-fertilization. At present, the mechanism of oogenesis is not fully understood.

In this work, we found that although penetrance of *leo-1 (RNAi)* is lower than that for knockdown of the other four PAF1C component genes, oogenesis was not occurring in RNAi fed day1 adult stage escapers. Similar results were observed in *leo-1 (gk1081), rtfo-1 (tm5670),* and *pafo-1 (tm13347)* deletion mutants. We next checked the average number of oocyte maturation marker-positive oocytes using the *bkcSi11[oma-1::GFP]* genomic transgene driven by its ~2.9 kbp 5' region and its ~2.9 kbp 3'region. We observed about five OMA-1::GFP-positive oocytes per posterior gonad in both wild-type and *control (RNAi)* animals. By contrast, the number of OMA-1::GFP-positive oocytes was decreased in *leo-1 (RNAi), cdc-73 (RNAi), pafo-1 (RNAi)*, and *pafo-1 (tm13347)* animals. We also found that functional PAFO-1::mCherry, which is expressed from an integrated genomic transgene, *tjIs280[pafo-1p::pafo-1::mCherry]* rescued the oogenesis defective phenotype in the *pafo-1(tm13347)* mutant. Moreover, functional PAFO-1::mCherry expressed by a regulatory element of the germ cell-specific gene *pie-1* significantly rescued the oogenesis-defective phenotype. Thus, germ cell expression of PAF1C might be essential for oogenesis. Finally, we found *bkcSi11[oma-1::GFP]* partially rescued the oogenesis defective phenotype.

Taken together, these results suggest that PAF1C cell-autonomously regulates oogenesis by regulating oocyte maturation.

351C MIG-6 PLAC domain affects Notch signaling and the extracellular matrix composition.

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In a forward genetic screen aimed at isolating mutations that disrupt germline stem cell (GSC) regulation, we found a new allele of *mig-6*, which we called *qz2*. MIG-6 is homologous to mammalian Papilin, a component of the extracellular matrix (ECM), and exists as two isoforms. The *mig-6(qz2)* mutation disrupts the long isoform, which is known to cell-autonomously promote DTC migration (Kawano et al. 2009). More specifically, it affects its C-terminal well-conserved protease and lacunin (PLAC) domain, thought to be important in matrix metalloproteases and proprotein convertases. The *mig-6(qz2)* mutants lay 26% less eggs than wild-type and have a slight increase in embryonic (+5.9%) and larval lethality (+1,8%). In addition, the distal half of their gonad arms are 30% shorter and 43% wider than in wild type. By comparing gonad morphology in *mig-6(qz2)* with *mig-6l(Ø)/+*, *mig-6(qz2)/+* and wild type animals, we found the *qz2* mutation to have semidominant features regarding this morphological defect. Consistent with its altered distal gonad morphology, the distal tip cell (DTC) cytonemes are shorter and spread wider in *mig-6(qz2)* mutants than in wild-type. Interestingly, the rate of GSC proliferation is normal in *mig-*

6(qz2) mutants, indicating that the morphology of the distal gonad, including niche morphology, can be dramatically altered without affecting the rate of GSC proliferation. On the other hand, we noticed that *mig-6(qz2)* greatly aggravates the *glp-1(gf)* thermosensitive defects at a permissive temperature. Namely, *glp-1(gf); mig-6(qz2)* double mutants develop 3.8 times more tumors than *glp-1(gf)* single mutants, while they lay 57% less eggs that show drastic increases in embryonic (+38.7%) and larval lethality (+9.1%). Despite the changes in DTC and gonadal morphology in *mig-6(qz2)* animals, we failed to detect a difference in both *lag-2* expression and GLP-1 levels. However, we found that the fraction of intracellular GLP-1 (cleaved NICD) was higher. Indeed, using Imaris 3D reconstructions of confocal z-stacks acquisitions of GLP-1::GFP animals (kind gift from David Greenstein), we found that there was significantly more GLP-1::GFP in the rachis of *mig-6(qz2)* mutants. In parallel, we noticed that the ECM in the distal gonad of *mig-6(qz2)* mutants was enriched in one of its main components, Collagen IV (EMB-9). Our results are thus compatible with the notion that MIG-6's PLAC domain may modify the ECM composition in a way that dampens Notch activation.

352A Determining the mechanism of attachment of the *C. elegans* germline stem cell niche, the distal tip cell

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The balance between stem cell proliferation and differentiation is important for growth and development in many organisms. This balance is highly regulated, and requires an interaction between the niche and the stem cell pool in order to be maintained. In *C. elegans*, the somatic distal tip cell (DTC) acts as the germline stem cell niche by providing cues to the stem cell pool via GLP-1/Notch signalling. We have identified the Derlin protein DER-1 (CUP-2) as having a role in regulating the DTC. The DTC is normally located at the distal most end of the gonad. It can move several cells away from the distal most end as the worms age (Kocsisova et al., 2019). As *cup-2* mutant worms age, the DTC moves further proximally down the gonad arm than what is seen with wild type. In some more extreme cases, the stem cell pool moves along with the DTC, which suggests the DTC remains functional at the new location. We are characterizing *cup-2's* expression along with its displacement phenotype to determine where *cup-2* is required such that the DTC will remain in place. Additionally, we are exploring *cup-2*'s known functions, endoplasmic reticulum associated degradation (ERAD) and endocytosis, to determine if they play a role in the placement of the DTC. Finally, we are looking at the surrounding basement membrane to determine if it may play a novel role in holding the DTC in place. This investigation of CUP-2 may help us understand how the DTC and potentially other niches are held in place, and how this displacement affects germline stem cells and the balance between stem cell proliferation and differentiation.

References

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353B A secreted immunoglobulin domain-containing protein, SPE-51, is required for sperm function at fertilization

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Successful fertilization requires the sperm and egg to recognize and bind to each other in order to fuse and form a zygote. The molecular basis of fertilization is not well understood. In mammals, several cell-surface proteins were shown to be required for the interactions between the sperm and egg, with IZUMO1, an immunoglobulin (Ig) superfamily protein on the sperm, and JUNO, a GPI-anchored protein on the egg being the only known receptor pair (Bianchi, 2014). In *C. elegans* sperm, interactions with oocytes are mediated by the *spe-9* class of proteins, which consist of transmembrane proteins on the sperm surface. The non-redundant roles of these molecules suggest that they function with one another to form a complex, which we refer to as the fertilization synapse (Krauchunas, 2016). Here, we report the first secreted molecule in the *spe-9* class, SPE-51. *spe-51* mutant sperm display normal morphology, can activate, migrate and even outcompete self sperm, but fail to fertilize the oocytes. The predicted protein contains an Ig-like fold and a stretch of hydrophobic amino acids. Surprisingly, SPE-51 expressed in the body-wall muscle was taken up by coelomocytes, suggesting the protein is secreted rather than membrane-bound. The *spe-51* mutant sperm display cell-autonomous behavior, suggesting that this secreted protein stays associated with the sperm cell surface and functions either in cis on the sperm surface or in trans with the egg cell surface to complete fertilization. Consistently, endogenously tagged SPE-51 localizes to the surface of the spermatozoa. Our work presents the first examples of a secreted protein required for the interactions between the sperm and egg. We further show evidence that

one of the molecules required for mammalian sperm-egg interactions, SOF1, is also secreted. Together, our work could serve as a paradigm for other mammalian sperm-secreted or reproductive tract-secreted proteins that coat the sperm surface and influence their survival, motility, and/or the ability to fertilize the egg.

354C The *C. elegans* spermiogenesis-inducing compound DDI-4 can trigger the acrosome reaction in mouse spermatozoa

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In *Caenorhabditis elegans*, spermiogenesis undergoes two pivotal events; sperm formation and activation. Pseudopods extend from round spermatids to form motile spermatozoa, whereas membranous organelles (MOs) in spermatids fuse with the plasma membrane (PM) to activate spermatozoa. During the latter process, MOs release their contents extracellularly, and some proteins that are essential for fertilization relocate from the MO membrane onto the sperm surface, resulting in the acquisition of sperm fertility. These cytological features of MO fusion are similar to those of the acrosome reaction in mouse spermatozoa, representing one event for sperm activation. Thus, we hypothesized that *C. elegans* and the mouse might share a common mechanism for sperm activation. To explore this, we first screened a chemical library to obtain compounds that trigger *C. elegans* spermiogenesis. Of 480 entries contained in the library, we got several compounds as *C. elegans* spermiogenesis activators and chose one of the positive agents, named DDI-4, for further analyses. Intriguingly, 100 µM DDI-4 could induce the acrosome reaction in ~85% of mouse cauda epididymal spermatozoa, while ~70% became acrosome-reacted with 10 µM the calcium ionophore A23187. Moreover, DDI-4 promoted tyrosine phosphorylation of mouse sperm proteins, a typical capacitation signature, at least *in vitro*. These results indicate that DDI-4 can activate both *C. elegans* and mouse spermatozoa *in vitro*. In other words, these two species presumably possess targets of DDI-4 that function in sperm activation. To obtain clues regarding the DDI-4 targets or DDI-4-related factors, we screened mutants whose spermatids were resistant to DDI-4 in ethyl methanesulfonate-treated worms. Since two mutant strains were eventually isolated, we are currently investigating those strains by next-generation sequencing to identify mutated genes by which spermatids will become incapable of being activated with DDI-4. Information of such genes might contribute to elucidating the common mechanism for sperm activation in *C. elegans* and the mouse.

355A EGGD-1 and EGGD-2 are novel LOTUS domain proteins that promote perinuclear localization of P granules

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Across the animal kingdom, ribonucleoprotein condensates called germ granules are required to maintain the immortality and totipotency of the germ lineage. Since their discovery, *C. elegans* germ granules, called P granules, have been extensively characterized to understand the role of germ granules in metazoan development. However, studies to-date have been impeded by incomplete knowledge of the P granule proteome. To broadly define the proteins localizing to P granules, we employed a proximity-based labeling approach by tagging known P granule proteins with a promiscuous biotin ligase. In our biotinylated protein fraction, we uncovered over 150 novel P granule proteins. Notably, we identified a pair of previously uncharacterized proteins, which we named EGGD-1 and EGGD-2 for **E**mbryonic and **G**ermline P **G**ranules **D**etached. We find that EGGD-1 is enriched at the base of P granules, and its depletion causes P granules to separate from the nuclear envelope in the adult germline and embryonic primordial germ cells. Further, loss of *eggd-2* exacerbates the P granule mislocalization in *eggd-1* mutant animals. EGGD-1/2 harbor four domains: two intrinsically disordered regions and two putative LOTUS domains. Previous work in *Drosophila* and mice showed LOTUS domain proteins directly recruit Vasa RNA helicases to organize germ granules, yet the role of LOTUS domain proteins in *C. elegans* is uncharacterized. Our ongoing work examines the interactions between GLH proteins (*C. elegans* Vasa orthologs) and EGGD proteins using mutational analysis to determine contributions of individual domains in P granule perinuclear localization. Together, our work defines the P granule proteome and provides novel insights into principles of germ granule formation and perinuclear attachment.

356B A male pheromone that improves quality of the oogenic germline uncovers a strategy to counteract reproductive aging

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Conspecific males and females communicate with potential mating partners via sex pheromones to promote reproductive success, but the underlying mechanisms remain largely enigmatic. We discovered how a *C. elegans* male pheromone, ascr#10, improves the oogenic germline and in the process identified an apparently conserved strategy to improve oocyte quality using commonly available pharmaceuticals. As they age, *C. elegans* hermaphrodites start producing lower quality oocytes characterized by abnormal morphology, increased rates of chromosomal nondisjunction, and higher penetrance of deleterious alleles. We showed that exposure to the male pheromone substantially ameliorates all of these defects and reduces embryonic lethality. ascr#10 stimulates proliferation of germline precursor cells in adult hermaphrodites. Greater precursor supply increases physiological germline cell death, which is required to improve oocyte quality in older mothers. Because ascr#10 effects on the germline require serotonergic signaling, we tested whether pharmaceuticals, including serotonin reuptake inhibitors, could improve germline quality in the absence of the pheromone. We found that compounds that potentiate serotonin signaling do indeed improve oocyte quality in *C. elegans* as well as in *Drosophila*. Together, our results suggest the male pheromone improves oocyte quality, but shortens organismal longevity because of the competition over resource allocation between soma and the germline. Practically, our findings reveal a class of therapeutic interventions using available compounds that could forestall reproductive aging.

357C Understanding the Role of Scaffold Protein Activated C Kinase 1 (RACK-1) in Germ Line Stem Cells of *Caenorhabditis elegans*

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Stem cells are unspecialized cells that are able to proliferate to produce more stem cells (mitosis) or differentiate to produce specialized cells. The balance between proliferation and differentiation is controlled by highly regulated signaling pathway(s). The *C. elegans* germ line is a powerful model that allows genes and pathways that play a crucial role in establishing and maintaining this balance to be identified. The *C. elegans* gonad contains two gonad arms, where proliferating germline stem cells are located at the distal ends of each arm, while differentiating cells such as sperm and oocyte are located more proximally. The balance between proliferation and differentiation allows this spatial patterning to be maintained in the germline. Recently, our lab has identified the influence of the scaffold protein RACK-1, which is conserved between humans and *C. elegans*, on the proliferation vs. differentiation pathway. My research is investigating the role of this scaffold protein on maintaining the balance between proliferation and differentiation. A mutation that eliminates *rack-1* results in lowering and mis-localization of the translational repressor protein GLD-1 (Germ Line Defective-1) in the differentiation pathway. Furthermore, loss of *rack-1* results in lower brood size and sterility at higher temperatures. My research seeks to identify the mechanism (either direct or indirect) by which RACK-1 is impacting GLD-1 and furthermore, the differentiation and proliferation decision. My research will provide insight into how stem cell behaviour is regulated in the *C. elegans* germline.

358A Investigating the germline function of the RNA-binding protein *cfim-1*

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Post-transcriptional regulation is crucial to proper organismal and tissue-specific development. An abundance of work has uncovered the essentiality of the core repertoire of factors and complexes constituting the cleavage and polyadenylation (CPA) machinery in post-embryonic development. Despite these findings, there is a need to define the precise relationship between these factors and tissue-specific development. Here, we demonstrate a novel role for the RNA-binding protein *cfim-1* in germline function. *cfim-1* functions in the CFIm complex, which is associated with preferential utilization of distal cleavage sites of pre-mRNAs bound for maturation by the core CPA machinery. Ablation of *cfim-1* function results in reduced brood sizes. This effect is exacerbated at higher temperatures where a completely penetrant sterility phenotype is observed. We employ a combination of genetic and imaging analysis tools to characterize the organization and morphology of *cfim-1* ablated germlines, demonstrating a precocious organization of meiotic-proliferating cells along the distal-proximal axis of the germline at the sterility-inducing temperature. We further provide genetic evidence to suggest that these effects are specific to the CFIm complex and not a general effect of antagonizing the function of the core CPA machinery. Data mining of worm 3'-sequencing data reveals an enrichment of the UGUA motif that is canonically bound by the CFIm complex upstream of the proximal cleavage sites of genes undergoing preferential transcript isoform utilization in response to ablation of *cfim-1*. These data, in conjunction with our previous findings, may suggest a novel model of regulation in the worm whereby recruitment of the CFIm complex to proximal cleavage sites in *cfim-1* regulated genes antagonizes usage of those sites by the core CPA machinery.

359B Combinatorial analysis of human PAF1 complex-interacting proteins using *in silico* phylogenetic profiling and RNAi knockdown screening

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RNA polymerase-Ⅱ associated factor-1 complex (PAF1C) is a heptameric protein complex consisting of PAF1, CTR9, LEO1, CDC73, and RTF1. PAF1C regulates gene transcription by interacting with RNA polymerase-II. PAF1C is evolutionarily conserved within multicellular organisms, and has been shown to regulate various important processes, including cell proliferation, cell differentiation, and tumor suppression. To date, the functional role of PAF1C in germ cell development remains unexplored.

We previously reported that RNAi knockdown of PAF1C components renders animals oogenesis defective, as are the *rtfo-1(tm5670)* and *leo-1(gk1081)* mutants (International *C. elegans* conference 2019). In this work, we attempted to identify the molecular network underlying PAF1C's role in oogenesis by comprehensively analyzing proteins that interact physically with each human PAF1C component *in silico*.

Phylogenetic profiles were generated for 542 species for which the whole genome sequence is available. These profiles allowed identification of 458 proteins that directly interact with PAF1C components in humans based on the presence or absence of orthologs. Further clustering analysis revealed a gene cluster comprising 192 conserved proteins in animals , and Blastp search revealed *C. elegans*homologues (170 of 192). Among these we selected 12 *C. elegans* homologues that displayed all three of the following features: proteins that share similar expression with PAF1C in the gonad (55 of 170), proteins that produced an embryonic lethal phenotype in previous RNAi screened data (32 of 55), and proteins that localize to the nucleus (12 of 32). To examine the role of these 12 proteins in germ cell development, we compared each proteins RNAi knockdown phenotype with that of *leo-1*, which encodes one component of PAF1C. We found that 3 ubiquitin-related genes exhibit an oocyte-defective phenotype, as seen in *leo-1(RNAi)* animals. These results imply ubiquitin-mediated regulation of gene expression may play a role in PAF1C- dependent regulation of oogenesis. We plan to assess functional relationships between PAF1C and these factors in *C. elegans* oogenesis. Based on the results of these studies, we expect to elucidate a mechanism of oogenesis regulation that will aid in understanding and treating PAF1C-related reproductive organ pathologies in multicellular organism.

360C Analysis of Class I histone deacetylase in the regulation of oocyte size and embryonic development in *Caenorhabditis elegans*

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In multicellular organisms, germ cell formation and differentiation are essential for gamete formation and propagation of genomic information to the next generation. Regulation of mature oocyte size is essential for precisely regulating the chromosome segregation. Therefore, this regulation play an essential role in lineage segregation by precisely regulating cell division. At present, the oocyte size regulatory mechanism is not fully understood.

Histone modifying enzymes positively and negatively regulate gene expression by modulating nucleosome structure. Among them histone deacetylases (HADCs) limit transcriptionally active regions by inhibiting transcription factor-mediated gene expression.

HDACs are divided into four classes (Class I ~ Class IV). Among the four members of Class I, HDAC-1 and HDAC-2 act redundantly to regulate cell survival in mammals. By contrast, *C. elegans* has three Class I HDACs, HDA-1, HDA-2 and HDA-3. Embryos exposed to *hda-1 (RNAi)* exhibit fully penetrant embryonic lethality. However, the functional role of these HDACs in germ cell development has not been explored.

In this study, we analyzed the role of HDACs in oocyte size regulation and embryonic development. We analyzed oocyte size by calculating the area of its central cross section, and found that this parameter's variance in *hda-2 (ok1479)* mutants was significantly larger than in wild-type animals. By contrast, an increase in oocyte size variance was not observed in *hda-3 (ok1991)* mutants, or *control (RNAi)* or *hda-1 (RNAi)* treated animals. Embryonic lethalities in *hda-2 (ok1479)* mutants, *hda-3 (ok1991)* mutants, and wild-type animals were 25.5%, 5.5%, and 4.6%, respectively. Finally, introduction of an extrachromosomal array including a *GFP::hda-2* genomic fragment controlled by an ~6.8 kbp *hda-2* 5' cis regulatory region rescued oocyte size and embryonic lethality phenotypes.

Taken together, these results suggest that among the three Class I HDACs, only *hda-2* is involved in regulating oocyte size and embryogenesis. The embryonic lethality caused by *hda-1 (RNAi)* does not correlate with oocyte size variance.

We plan to perform phenotypic analyses of the effect of *GFP::hda-2*-overexpression and tissue-specific roles for HDA-2 to elucidate its mechanism of oocyte size regulation that impacts embryonic survival and development.

361A Temperature stress effects cytoplasmic streaming during oogenesis

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Apoptosis during oogenesis is highly conserved in animals. During *Caenorhabditis elegans* oogenesis, roughly fifty percent of germ cells undergo apoptosis. While *C. elegans* oogenesis is widely studied, it is unclear why this mass cell death occurs. Under moderate temperature stress, there is a decrease in worm brood size but an increase germline apoptosis. There are two proposed hypotheses for the function of increased germline apoptosis that would aid in developing fit progeny under temperature stress. The first is that apoptotic cells serve as nurse cells, donating their cytoplasmic materials to developing oocytes. An increase in apoptosis during temperature stress may help ensure that developing oocytes receive sufficient cytoplasmic materials when an embryo is born under stress conditions. The second hypothesis is that apoptosis serves to remove defective cells that are made due to problems with oogenesis under moderate temperature stress. If the first hypothesis is correct and apoptotic cells act as nurse cells, an increase in apoptosis and thus an increase in cytoplasmic donations under temperature stress would also increase the rate of cytoplasmic streaming in the germline. Additionally, in mutants that have reduced apoptosis compared to wild type worms, the rate of cytoplasmic streaming would decrease compared to wild type animals at the same temperature. To test if increased apoptosis contributes increased amounts of cytoplasmic materials to developing oocytes, DIC microscopy was used to video tape live anesthetized young adult N2 worms raised at 20°C as well as worms upshifted to 26°C for 24 hours. Six worms were taped under both temperature conditions. Using ImageJ, five particles within the rachis of the germline were tracked in each worm over a two-minute period and each particles average velocity was calculated (n=30). Our analysis showed a significant increase in the rate of cytoplasmic streaming in worms upshifted to 26°C compared to worms raised at 20°C. Additional imaging is currently being performed on *lin-35* and *lin-54* mutants, which have been shown to have decreased apoptosis at high temperatures. Because these mutants do not show an increase in apoptosis under temperature stress, it is expected that their analysis will not show a significant increase in rate of cytoplasmic streaming as seen in the wildtype.

362B A multi-organism genetic model for microbiota-driven parasite burden

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Parasitic nematodes infect over one billion people worldwide and although the majority of them live in the digestive tract, a possible role for the microbiota in determining successful host infection is underexplored. The *Trichuris* nematode parasite lives in the microbiota-rich mammalian cecum for years, and it has an enormous reproductive capacity that contributes to its infectious spread. We previously determined that *C. elegans* germline development is sensitive to the microbial environment. Though the two nematode species are not closely related, we predicted that some aspects of reproduction in the well-characterized, model organism *C. elegans* may be conserved in *Trichuris*.

To assess systematically which components of the bacterial diet are most important for *C. elegans* reproduction, we screened an *E. coli* library for mutants that, when fed to *C. elegans*, interfered with timely fertility. We identified ten *E. coli* mutants. Focusing on two that function in fatty acid biosynthesis (*fabF, fabH*) and two that function in ethanolamine utilization (*eutD, eutN*), we found that *fabF* and *eutN* delayed *C. elegans* germline development relative to somatic development, though none of the four reduced germline progenitor numbers and none were dependent upon DAF-2 insulin or DAF-7 TGFß signaling pathways. Metabolomics and functional analysis unexpectedly revealed that *fabH E. coli* mutants provide insufficient arginine, such that arginine supplementation rescued the *C. elegans* fertility delay. We further investigated whether *E. coli* mutants might interfere with development or reproduction of the *Trichuris* species that infects mice*, Trichuris muris.* Remarkably, *fabH* and *eutN* mutant *E. coli* were associated with *T. muris* hatching defects, the former also being arginine-dependent, while *eutN* likely acts by producing a toxic substance. Both were also associated with aberrant reproduction of *T. muris in vivo*. Overall,

these findings establish *C. elegans* as a novel system for investigating the effects of specific microbial genes and pathways in supporting the parasitic nematode life cycle.

363C The Role of the RNA-Induced Silencing Complex (RISC) Component VIG-1 in *C. elegans* Germline Stem Cell Regulation

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Stem cells are undifferentiated cells that can give rise to a wide variety of specialized cell types. In development and tissue maintenance, these cells play crucial roles in maintaining homeostasis and reproductive fitness. The *C. elegans* germline is set up in an assembly line-like format, in which mitotically dividing proliferative cells, or stem cells, reside in a distal niche. As cells move away from this niche, they gradually progress through meiosis until ultimately becoming fully differentiated sperm or oocytes. Notch signaling in the distal end of the germline provides the proliferative signal, while downstream post-transcriptional regulatory pathways promote differentiation. Although this main pathway has been well characterized, there exist various components that serve to fine-tune this balance in subtle but important ways. Previous work has identified small RNA molecules, such as miRNAs, as having a role in germline stem cell regulation. Here, the role of *vig-1*, a gene involved in the RNA-induced silencing complex (RISC) pathway, in *C. elegans* germline stem cell regulation is investigated. Although *vig-1* performs multiple functions in a variety of tissues, it was initially implicated in the germline through its physical protein interaction with another RISC component, *teg-1*, that was known to modulate germline stem cell balance. We show that *vig-1* likely functions to repress the activity of Notch signaling. Genetic null mutants for *vig-1* and GLD-1/GLD-2 pathway components do not cause any changes to germline phenotype, suggesting that *vig-1* does not function downstream of Notch. However, in worms that lack *vig-1* activity, Notch signaling is seen to be enhanced, manifesting through ectopic mitotic activity and over-proliferation of stem cells. In addition, *vig-1* can partially suppress Notch loss-of-function mutant phenotypes. In addition, we show that *vig-1* may act directly on Notch signaling through increasing the expression of its direct transcriptional targets, LST-1 and SYGL-1. Finally, we propose that *vig-1* acts through modulating miRNA levels, as germline miRNA levels are reduced in *vig-1* mutants.

364A Distal tip cell-specific mRNA profiling sheds light on the molecular mechanism of gonad morphogenesis

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Cell motility is essential for the normal development and physiology of an organism. In *C. elegans*, the stereotypical U-shaped gonad is formed by the directional chemotactic movement of two somatic cells, known as Distal-Tip Cells (DTCs), whose movement is divided into three phases. In phase I (early L3 larval stage) they move along the ventral surface. Phase II (late L3 stage) involves two 90° turns, and finally in phase III (early L4 larval stage) the DTCs move along the dorsal surface towards the midbody of the worm. Migration halts during the late L4 stage. While multiple genetic screens have identified general regulators of gonad morphogenesis, a DTC-specific role has remained largely unexplored. To address this, we isolated GFP-labelled DTCs from different stages of development and performed RNA-sequencing, to characterize and compare the transcriptome of migratory DTCs (late L3 and early L4 stage) with non-migratory DTCs (late L4 and adult stage). We identified between 1700 to 3000 genes that are upregulated in each of the larval and adult stage DTC relative to other cell types. We confirmed the identification of the few DTC-specific transcripts known in the literature. The overlap between the genes we found upregulated in DTCs and the published germline-enriched genes (Reinke et al., 2004) is only 0.9 to 6.4%. Furthermore, we found that among the 99 genes identified in a genome-wide RNAi screen (Cram et al., 2006) to have a role in DTC migration, 43 genes are enriched in the migratory early L4 stage DTC transcriptome, while only 7 genes are present in the immobile adult stage DTCs. Taken together, it appears our dataset of cell-specific and stage-specific DTC transcripts is highly accurate. A bioinformatic functional analysis of the migratory DTC transcripts revealed enrichment of genes related to neuronal guidance, cytoskeleton, signaling, and membrane trafficking. Currently, we are performing a DTC-specific RNAi screen to identify the novel cell-autonomous regulators of DTC migration which in turn guides gonad morphogenesis. Identifying these regulators will help us to decipher the molecular mechanisms of DTC migration deployed in a three-dimensional microenvironment, which will eventually give insight into the mechanism of gonadogenesis.

365B Multiple lipocalins are required for apical extracellular matrix organization

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All exposed epithelial surfaces are lined by some type of lipid and glycoprotein-rich apical extracellular matrix (aECM). The contents, trafficking, and assembly mechanisms of the aECM are not well understood. In C. elegans, an early pre-cuticular aECM lines and shapes developing (or molting) external epithelia such as the hypodermis, vulva, rectum, and excretory duct and pore tubes. We hypothesize that lipocalins transport lipids or lipoproteins into this early aECM and are important to it's formation.

Lipocalins (fat cups) are small secreted proteins that transport lipids or other hydrophobic cargos throughout the body. C. elegans has seven lipocalin-related (lpr) genes. We found that mutants for at least five of these genes have aECM abnormalities such as alae defects, cuticle barrier defects, or molting defects. Most also affect shaping of the narrow excretory duct tube. All five of these lpr genes show oscillatory expression patterns that track with those of pre-cuticular aECM factors, and at least one LPR protein (LPR-3) transiently incorporates into specific regions of the pre-cuticle aECM. We are currently tagging the remaining LPR proteins using CRISPR/Cas9 and plan to interrogate lipid content in lpr mutants and study several lpr suppressors to understand how these lipocalins affect aECM assembly. One of the less studied lipocalin genes that I am particularly interested in is lpr-6. A frameshift deletion in the first three exons of lpr-6 has shown defects in the formation of adult alae and excretory defects. We hypothesize that lpr-6, like previously investigated lipocalins, has a critical role in the formation of the aECM. Understanding the role of LPR-6 and the remaining lipocalins will contribute to a greater knowledge of basic aECM structure and assembly.

366C Characterizing a Matrix Protease important for epithelial tissue shaping in C. elegans

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The apical extracellular matrix (aECM) is a lipid-and glycoprotein-rich protective layer that lines epithelial surfaces exposed to the environment. Despite its importance in tissue shaping and protection, the components and organization of aECM are not well understood. C. eleganshas a pre-cuticular aECM that lines and shapes developing epithelia such as the hypodermis, vulva, rectum, and excretory duct and pore tubes. Our lab has identified proteins in the C. eleganspre-cuticular aECM, many of which share domains with mammalian matrix proteins. We are working towards characterizing these aECM components to understand function and hierarchy within the matrix.

Proteases in the matrix often regulate assembly, disassembly and proper function of aECM components; we are interested in identifying proteases that cleave some of our matrix components. One candidate is BLI-4, a relative of the mammalian proprotein convertase subtilisin/kexin (PCSK) family. We generated bli-4 knock-out mutants using CRISPR and found its phenotypes were very similar to those of some of our aECM mutants. We are conducting further experiments to investigate endogenous BLI-4 expression and which BLI-4 isoform(s) is necessary for pre-cuticle development/embryonic viability. Understanding the role of BLI-4 will contribute to a greater knowledge of basic aECM structure and assembly.

367A *FRK*$^{src\text{-}2}$ is a Novel Candidate as a Hemifacial Microsomia and Mandibular Dysplasia Gene that Exhibits Developmental Defects in Zebrafish (*D. rerio*) and *C. elegans*

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Hemifacial microsomia (HFM), also known as lateral facial dysplasia, is a common birth defect involving the first and second branchial arch derivatives. Several chromosomal abnormalities (like trisomy 10p and *OTX2* duplication) and gene variants (like *PLCD3* and *MYT1*) were identified in a few cases with HFM; however, the genetic etiologies in a majority of cases with HFM remain unknown. Using whole-exome sequencing, we identified a homozygous missense mutation (c.484G>A; p.V162I) in a SH2 domain) of the *FRK* gene in an affected individual with HFM. *FRK* encodes the Fyn-related Src family Tyrosine Kinase and is highly expressed in the Meckel's cartilage during embryonic development in zebrafish and mouse. Knockdown of *FRK* in zebrafish with the fynrk-specific morpholinos showed several defects, including a shorter ratio of length and width of Meckel's cartilage, a larger angle of ceratohyal, disorganized ceratobranchial, and smaller otoliths. Using CRISPR/Cas9, we generated the patient-specific allele (p.V162I) in the *C. elegans* ortholog gene *src-2*, which is named *src-2(V170I)*. The homozygous *src-2(V170I*) mutant as well as a null mutant *src-2*(*ok819*) were found to have ~ 25% reduction in the brood size

when compared with wildtype ($P < 0.0001$). Given that *src-2* has a paralogous gene *src-1*, which is an essential kinase during embryogenesis, we tested the genetic interactions between the *src-2* mutants and *src-1*with RNAi interference (RNAi). Indeed, the embryonic lethality and the defective morphogenesis in pharyngeal and intestinal tissues in the *src-1* RNAi treated worms were synergistically enhanced in either *src-2(V170I)* or *src-2(ok819)* (*P*£0.0001). Using a 4-D high-content imaging approach, irregular neuronal and hypodermal patterning as well as protrusive rupture phenotypes were observed in the *src-1(RNAi)* arrested embryos. These defective embryonic phenotypes were increased by 23%, 23%, and 30%, respectively, when the *src-2(V170I)* mutants were combined with the *src-1(RNAi)* (*P* £ 0.0296 by one tailed chi-square test), suggesting a synergistically interaction between these paralogous genes. Taken together, we identified the Fyn-related Src family Tyrosine Kinase as a novel candidate gene for human hemifacial microsomia or lateral facial dysplasia and revealed its effects on brood size and craniofacial development in the models of zebrafish and *C. elegans*.

368B BAR-1/β-catenin and PRY-1/Axin show asymmetric and complementary expression in neuroblasts during *C. elegans* ventral nerve cord assembly

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During embryogenesis DD, DA, and DB neuroblasts arise from left and right lineages, move towards the midline and intercalate into a single tract to form the ventral nerve cord (VNC). VNC assembly involves rosette-mediated convergent extension and planar cell polarity (PCP) and Robo pathways [1]. Disruption of this process results in the mispositioning, usually manifested as an anterior displacement, of embryonically-derived motor neurons in the VNC. A more recent analysis of canonical Wnt pathway components also revealed motor neuron position defects. The six DD neurons are evenly spaced along the anterior-posterior (AP) axis and therefore a convenient marker of VNC assembly. We found that mutations in *bar-1*/β-catenin and *pop-1*/TCF display DD spacing defects. For example, in *bar-1* and *pop-1* mutants, DD2 is displaced anteriorly and therefore in closer proximity to DD1. In contrast, disruption of the negative Wnt signaling regulator PRY-1/Axin results in the posterior displacement of DD1 such that it is in closer proximity to DD2.

To begin to understand the roles of BAR-1 and PRY-1, we examined where endogenously-tagged proteins were expressed during VNC assembly. Using CRISPR/Cas9, we generated *bar-1(zy97[GFP::bar-1])* and found that BAR-1 is expressed in right but not left side-derived VNC neuroblasts. In contrast, PRY-1::mNG (*cp383*) is expressed in left but not right side-derived neuroblasts. In *pry-1* mutants, BAR-1 is expressed symmetrically in right and left neuroblasts indicating a left-sided repression of BAR-1 signaling. We are examining how neuroblasts intercalate into a single VNC tract in *bar-1* and *pry-1* mutants to understand the role of this asymmetric expression. Preliminary observations suggest that motor neuron positioning defects begin to manifest around the 1.5-fold stage and later. We hypothesize that BAR-1 asymmetry in right side neuroblasts may help contribute to the proper ordering of DD, DA and DB neurons along the VNC.

[1] Shah et al. Dev Cell 2017.

369C A partial nuclear atlas of the post-twitching *Caenorhabditis elegans* embryo

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The limited number of cells and invariant cell lineage of the *Caenorhabditis elegans* embryo make it an excellent system for examining complex developmental events, such as tissue movement and neurodevelopment. Prior work from the WormGUIDES project has digitized the position of all nuclei for the first half of embryogenesis, creating a computational map of the embryo that can be used to overlay developmentally relevant information like gene expression or neurite outgrowth. Creating a similar map for the second half of embryogenesis is difficult due to embryo elongation and movement. We have developed software to computationally untwist the moving embryo, allowing for analysis of cell position during this period

of development, and have begun expanding our computational map into the second half of embryogenesis. Our current map includes 202 nuclei across the embryo, including 32 neuronal nuclei, 81 body wall muscle nuclei, 20 intestinal, and 20 seam cell nuclei. We also include a tract-based model of the nerve ring, showing how it is positioned relative to neuronal and body wall muscle nuclei as the embryo elongates. In addition to our partial nuclear atlas, we describe improvements to our untwisting and tracking workflow, including a deep-learning image restoration capability which improves image quality during rapid embryo movements, and a semi-automated tracking upgrade to our untwisting software which improves tracking throughput. As we continue to add nuclei and neuronal morphology to the atlas, we plan to integrate our post twitching model with previous pre-twitching work to develop a digital atlas spanning the entirety of embryogenesis.

370A Discerning the temporal organization of development

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Understanding temporal regulation of development remains an important challenge. Whereas average, species-typical timing of many developmental processes has been established, less is known about inter-individual variability and correlations in timing of specific events. We are studying these questions in the context of development in *Caenorhabditis elegans*. In one study, based on patterns of locomotor activity of freely moving animals, we inferred durations of four larval stages (L1-L4) in over 100 individuals. Analysis of these data supports several conclusions. Individuals have consistently faster or slower rates of development because durations of L1 through L3 stages are positively correlated. The last larval stage, the L4, is less variable than the earlier stages and its duration is largely independent of the rate of early larval development, implying existence of two distinct larval epochs. Interestingly, stage durations tend to scale relative to total developmental time. Fractional stage durations (calculated as L1 duration/total time to adulthood, etc.) are indistinguishable across several independent studies that relied on different methodologies and were conducted at different temperatures. This scaling relationship suggests that each larval stage is not limited by an absolute duration, but is instead terminated when a subset of events that must occur prior to adulthood have been completed. Comparing these results to our more recent studies of developmental timing allows the inference of general principles of temporal organization of development.

371B Plugs and sheaths made to molt

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Temporary plugs and linings protect emergent epithelial organs and body systems from physical damage during specific stages of development. But the interactions among cells and extracellular matrices (ECM) underlying the accretion, clearance, and biomechanical performance of transitory plugs have not been well characterized. Here, we describe provisional matrices that occlude the oral and sensory openings of molting animals, and contain the Zona Pellucida (ZP) domain proteins FBN-1 and NOAH-1. We track the deposition and removal of plug components relative to sequential rearrangements among facial, interfacial, and pharyngeal epithelia using high-resolution confocal fluorescence microscopy. Dual imaging of functional FBN-1::mCherry and GFP-tagged integrins reveals that FBN-1 molecules enmesh integrins embedded in cellular membranes as well as extracorporeal integrins potentially embedded in extracellular vesicles or migrasomes. Further, the endurance of the interim mouth plug stabilizes cell-cell junctions between facial and pharyngeal epithelia while animals molt. Based on additional molecular genetic and biochemical studies, we propose that dynamic interactions among FBN-1 and other matrix molecules, integrins, and cortical actomyosin filaments together route and/or dissipate mechanical forces and thereby preserve the integrity of the feeding apparatus across the molt. Further characterization of the oronasal plugs of *C. elegans* may elucidate the role of human fibrillins in craniofacial development and the etiology of congenital birth defects including orofacial clefts and atresias.

372C *C. elegans* Anterior Morphogenesis: A Tale of Three Tissues

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Morphogenesis encompasses the biological events driving tissue and organ development. The need to build tissues with precision relies on the spatiotemporal coordination of cell shape changes, migration and adhesion. As complex structures are derived from multiple tissue types, studying their organization in vivo is essential for a comprehensive understanding and yet is extremely challenging. In this study we show how cells from three different tissues are coordinated to give rise to the anterior lumen. While most aspects of epidermal morphogenesis and several aspects of pharyngeal morphogenesis have been well-described in *C. elegans*, it is less clear how cells from the pharynx, epidermis and neuroblasts coordinate to define the location of the anterior lumen and supporting structures. Using various microscopy and software approaches, we found that

interactions between pharyngeal, epidermal and neuronal cells are required for anterior morphogenesis. Specifically, to define the position and timing of the anterior lumen and future mouth of the worm. We characterized the movements and patterns of these three cell types and defined key events associated with anterior morphogenesis. We found that the first visible marker of the location of the future anterior lumen is provided by projections from the anterior-most pharyngeal cells (arcade cells) and facilitates patterning of the surrounding neuroblasts. These neuroblasts organize into distinct patterns which subsequently influences the rate of epidermal cell migration towards the anterior. Conversely, the epidermal cells ultimately reinforce the position of the future lumen, as they must join with the pharyngeal cells for their successful epithelialization. We described the crucial involvement of junction (HMP-1, AJM-1), polarity (PAR-6 and PAR-3) and contractility (NMY-2) proteins in this precise patterning. Thus, we have established an innovative model system to explore the in-depth molecular regulation of these different patterns and interactions between multiple cell-types.

373A Establishment of a morphological atlas of the *Caenorhabditis elegans* embryo using deep-learning-based 4D segmentation

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The invariant development and transparent body of the nematode *Caenorhabditis elegans* enables complete delineation of cell lineages throughout development. Despite extensive studies of cell division, cell migration and cell fate differentiation, cell morphology during development has not yet been systematically characterized in any metazoan, including *C. elegans*. This knowledge gap substantially hampers many studies in both developmental and cell biology. Here we report an automatic pipeline, CShaper, which combines automated segmentation of fluorescently labeled membranes with automated cell lineage tracing. We apply this pipeline to quantify morphological parameters of densely packed cells in 17 developing *C. elegans* embryos. Consequently, we generate a time-lapse 3D atlas of cell morphology for the *C. elegans* embryo from the 4- to 350-cell stages, including cell shape, volume, surface area, migration, nucleus position and cell-cell contact with resolved cell identities. We anticipate that CShaper and the morphological atlas will stimulate and enhance further studies in the fields of developmental biology, cell biology and biomechanics (Cao†, Guan†, Ho†, et al. *Nat. Commun.*, 2020, 11: 6254).

374B Computable early *C. elegans* embryo with a data-driven phase field model

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Morphogenesis is a precise and robust dynamic process during metazoan embryogenesis, consisting of both cell proliferation and cell migration. Despite the fact that much is known about specific regulations at the molecular level, how cell proliferation and migration together drive the morphogenesis at the cellular and organismic levels is not well understood. Here, using *Caenorhabditis elegans* as the model animal, we present a data-driven phase field model to compute the early embryonic morphogenesis within a confined eggshell. By using three-dimensional time-lapse cellular morphological information generated by imaging experiments to set the model parameters, we can not only reproduce the precise evolution of cell location, cell shape and cell-cell contact relationship *in vivo*, but also reveal the critical roles of cell division and cell-cell attraction in governing the early development of *C. elegans* embryo. In brief, we provide a generic approach to compute the embryonic morphogenesis and decipher the underlying mechanisms (Kuang†, Guan†, et al. *bioRxiv*, 2020, 422560).

375C Identification of a mitochondrial transfer sequence in a folic acid metabolism gene *mel-32*

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Neural tube defects (NTDs) are common malformities resulting in exposed spinal cord or brain tissue caused by the inability to close the neural tube in embryogenesis. Previous research has shown folate deficiency increases the risk of NTDs. A folic acid metabolism gene, *serine hydroxymethyltransferase* (*SHMT*) is responsible for the synthesis of thymidylates, purines, and

methionine which are important for DNA replication especially during embryogenesis. Typically, eukaryotes have two copies of *SHMT* which are either localized to remain in the cytosol or transferred to the mitochondria. The different localizations are a result of mitochondrial target sequences in the N-terminus. Interestingly, the model system *Caenorhabditis elegans* only have one homolog of *SHMT* called *mel-32* and it was unclear if this gene's product was cytosolic, mitochondrial, or both. To address this question, a bioinformatics approach was taken to identify if *mel-32/SHMT* has a mitochondrial transfer sequence. We identified putative mitochondrial transfer sequences that are present in specific isoforms. Molecular phylogenies of different organisms were then generated to show prominent cytosolic *SHMT* and mitochondrial *SHMT* clustering especially around the phyla Nematoda, Arthropoda, and Tardigrada. By comparing isoforms with different *SHMT* localizations, potential mitochondrial target sequences were identified for organisms that could later be experimentally assessed.

376A *C. elegans prk* mutants exhibit pleiotropic defects.

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The mammalian PIM family of serine/threonine kinases regulate several cellular functions, such as cell survival and motility. In behavioral studies of *C. elegans* nematodes, we have observed that the PIM-related kinases (PRKs) are essential for chemotactic movements of the animals in response to olfactory stimuli sensed by AWB and AWC^{ON} neurons (Kalichamy *et al.,* 2019). When analyzing *prk*-deficient mutants, *prk-1(pk86)* and *prk-2(ok3069),* we noticed that in addition to olfaction, their brood size and growth were also negatively affected. Therefore, we have now analysed the *prk* expression patterns and the pleiotrophic effects of the mutations in more detail. Morphological analyses have revealed that *prk-1* is required for body length, body bends, male tail development and longevity. While a transcriptional fusion reporter of *prk-1* is expressed in head neurons and intestine, *prk-2* is found from posterior parts of the intestine in L2 to L4 larvae and strongly expressed in intestine and seam cells in young adults to adults. Further genetic analyses are underway to fully elucidate the functional roles of PRKs.

377B UPR^{mt} required for anal depressor symmetry and male muscle remodeling

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The mitochondrial unfolded protein response (UPR^{mt}) is normally studied in the context of stress. When mitochondrial function is perturbed, the transcription factor ATFS-1 is trafficked to the nucleus, instead of the mitochondria, to promote gene expression involved in metabolic remodeling and repair of mitochondrial damage. However, the UPR^{mt} is also necessary for normal development under normal conditions. One cell that uses UPR^{mt} is the anal depressor (adp), which is required for defecation; when the adp contracts, waste is expelled from the anus. The H-shaped muscle has left and right sarcomeres that span from the dorsal hypodermis to the rectal epithelium. We used *atfs-1* tissue-specific CRISPR to drive Cas9 expression in the enteric muscles, including the adp, and discovered that the muscle no longer makes the symmetrical H shape, but instead has hyper-expanded asymmetric sarcomeres. This suggests that the adp uses UPR^{mt} to establish symmetry during early development. Furthermore, in L4 males, the adp remodels to change its form and function from a defecation muscle, with a dorsal/ventral sarcomere, to an anterior/posterior-oriented sex muscle. The remodeling process also requires *atfs-1*, since reduction in its function disrupts the disassembly of its dorsal/ventral sarcomere. Additionally, ATFS-1 is expressed in developing male adps, but is below detection in the hermaphrodite muscle, suggesting that ATFS-1 has sexually dimorphic functions during post-embryonic developmental. We asked if artificially inducing UPR^{mt} via ethidium bromide (EtBr), while over-expressing ATFS-1 in the nucleus, could lead to adp defects. While EtBr treatment delays development in both sexes and impairs male tail development, it does not affect adp remodeling, indicating that while too little ATFS-1 prevents muscle remodeling, excess nuclear ATFS-1 is tolerated. Future work will focus on what impact ATFS-1 has on metabolic function in the adp and what role the previously identified WNT/Ca^{2+} pathway has on regulating UPR^{mt}-controlled metabolism.

378C EFF-1 ectopic expression promotes body wall muscle fusion

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In many vertebrates, skeletal muscles normally contain hundreds of nuclei that originate by myoblast fusion. This is also the case in some invertebrates (e.g. in arthropods) but not in nematodes. Adult *C. elegans* has 95 rhomboidal body wall muscles (BWMs) which are bundled in four quadrants, but they are non-syncytial. *Caenorhabditis elegans* protein Epithelial Fusion Failure 1 (EFF-1) and its paralog Anchor cell Fusion Failure 1 (AFF-1) have been described as bona fide fusogens that act to fuse one third of all somatic cells including those of the hypodermis, pharynx, reproductive and excretory systems. Ectopic EFF-1

expression in embryos under the heat shock promoter triggers extensive fusion between cells that normally do not fuse and leads to lethality.

Since there is no evidence that BWMs normally fuse, we hypothesized that ectopic expression of EFF-1 in BWMs may induce their fusion, alter their structure and produce muscle-related phenotypes. We generated transgenic lines expressing EFF-1 under the muscle-specific myosin promoter *myo-3p*. Around 10% of the worms expressing *myo-3p::*EFF-1 showed disappearance of cellular membranes and cytoplasmic merger which likely resulted in uncoordinated, dumpy and larval arrest phenotypes. It appears that EFF-1 has to be present in both adjacent BWMs for fusion to occur as was shown for normal epidermal developmental cell-cell fusions. Our results demonstrate highly conserved roles of EFF-1 mediating cell fusion in BWMs and provide important clues for research on the functions and evolution of muscle fusion in animals.

379A Spontaneous cell internalization of a spatially-confined proliferating blastomere: a mechanical interpretation on worm gastrulation

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Blastomere internalization, namely gastrulation, is a common and significant milestone during development of metazoans from worm to human, which generates multiple germ layers with distinct cell fates and spatial organizations. Although several molecular activities (e.g., cell polarization, intercellular adhesion change, and apical actomyosin cortex contraction) have been revealed to facilitate the process, in this paper, we focus on gastrulation of worm *Caenorhabditis elegans* and demonstrate that even a simple mechanical system, like a group of cells with isotropic interactions, can experience internalization when dividing within a confined space. Beyond a critical cell number, a multilayer structure becomes energetically more favorable than a single-layer structure. Furthermore, in the simulation of *C. elegans* embryogenesis, the critical cell number is near the one measured experimentally. Besides, both mechanical analysis and simulations suggest that a relatively larger cell is more likely to internalize. Interestingly, the first gastrulating cells in *C. elegans* also appear to be the largest due to the regulatory introduction of a gap phase. Last but not least, extra regulation on a limited part of cells to internalize autonomously is found to be able to make the morphogenetic process much more precise and robust. Our work successfully captures the key characteristics in worm gastrulation by simplified modeling and provides a novel and rational interpretation on how this phenomenon emerges and is optimally regulated.

380B Two of the 30 EGF domains in FBN-1/Fibrillin are required for sensory dendrite extension

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Many extracellular matrix (ECM) components are large proteins composed of highly repetitive domains. For example, FBN-1/Fibrillin is a ~2700 aa protein with 30 EGF-like domains. In principle, such repetitive domains could function redundantly, for example to cross-link other matrix components, or they could be distinct modules with specialized functions. In previous studies, disruption of *fbn-1* with a large internal deletion (*tm290)* or RNAi caused almost complete lethality and severe molting defects. In contrast, through a genetic screen for mutants affecting amphid dendrite morphogenesis, we identified two alleles of *fbn-1* (*ns67* and *ns283*) that introduce missense mutations within 32 aa in the first two N-terminal EGF-like domains (EGF1-2). These mutants cause weakly penetrant defects in amphid dendrite extension (8% and 19% defective, respectively), with minimal lethality or molting defects, suggesting that the EGF1-2 domains perform a specialized function related to dendrite extension. To further understand this, we generated a small in-frame deletion encompassing EGF1-2 (*syb239*) and a complete deletion of *fbn-1* (*syb240*) using CRISPR/Cas9. Consistent with our original mutant analysis, *syb239* has weakly penetrant amphid dendrite extension defects (8% defective), while *syb240* exhibits severe embryonic lethality, supporting the idea of a specialized role for EGF1-2. Amphid dendrite extension also requires another ECM protein, DYF-7, which is produced by the amphid neurons and forms «caps» at the dendrite endings that prevent rupture of the developing neuron-glia sensory epithelium. FBN-1 is expressed by hypodermal cells at this stage, and we find that the EGF1-2 domains are required for proper formation of DYF-7 caps. These results show that distinct domains within FBN-1 may be specialized to support different aspects of its function, and suggest that even highly repetitive domains in ECM proteins may reflect modularity rather than redundancy.

381C The role of the kinase MRCK-1 in excretory canal development

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The single-cell excretory canal of *C. elegans* is a powerful model for understanding molecular mechanisms of biological tube development. Coordination of vesicle trafficking, apical-basal polarity, and cytoskeletal support are all required for the development and maintenance of canal integrity. Previously, our lab showed that inactivation of the cerebral cavernous malformation 3 (*CCM3*) gene *ccm-3* caused canal truncations through effects on CDC-42 signaling and recycling endosomes [1].

To gain more insight into the mechanistic basis by which CCM-3 regulates canal development we conducted a genome-wide RNAi screen for genes that caused similar canal truncations as *ccm-3* and identified the myotonic dystrophy-related Cdc42-binding kinase homolog 1 (*mrck-1*). Inactivation of *mrck-1* causes the same canal truncation phenotype as loss of *ccm-3*, as well as reduction of markers for active CDC-42 and recycling endosomes [1]. MRCK-1 is also required for the phosphorylation of MLC-4 during embryogenesis, which regulates actomyosin to promote body elongation [2,3]. Overexpression of a phosphomimetic form of MLC-4 rescues canal truncations in *mrck-1* mutants, but only partially rescues truncations in *ccm-3* mutants. This suggests that *mrck-1* either functions downstream in collaboration with other genes, or independently of *ccm-3* to regulate actomyosin function in the canal.

Using CRISPR genome editing I generated a series of mutations in the domains of MRCK-1 to better elucidate its function in canal development. Mutations in the kinase domain of MRCK-1 completely phenocopy a null mutation in *mrck-1*, whereas deletion of the Cdc42 and Rac1-interactive (CRIB) domain caused very modest canal truncations, indicating that its kinase activity likely drives canal extension. Given the known role of MRCK-1 in promoting the phosphorylation of MLC-4, and the ability of a phosphomimetic form of MLC-4 to rescue canal truncations in *mrck-1* mutants, I am currently investigating the role of MLC-4 and non-muscle myosin in the excretory canal. Through this research I aim to establish how *mrck-1* regulates excretory canal extension, which may provide insights into general mechanisms of biological tube development.

1. Lant et al. (2015) *Nature Comm* 6:6449

2. Gally et al. (2009) *Development* 136:3109-3119

3. Marston et al. (2016) *Current Biol* 26:2079-2089

382A ERM-1 phosphorylation and NRFL-1 redundantly control lumen formation in the *C. elegans* intestine

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Reorganization of the plasma membrane and underlying actin cytoskeleton into specialized cortical domains is essential for the function of most polarized cells in animals. ERM and NHERF proteins are conserved regulators of cortical specialization, that function together as membrane-actin linker and organizers of molecular hubs. ERM protein activity involves a conformational switch from an inactive cytoplasmic form into an active membrane- and actin-bound form, which is thought to be mediated by phosphorylation of a conserved C-terminal threonine residue. NHERF proteins can bind to ERM proteins and act as scaffolding proteins by revealing multiple PDZ domains. However, data on the role of the interaction between ERM and NHERF proteins is scarce. Here, we study the relationship between the ERM and NHERF orthologs ERM-1 and NRFL-1, respectively, and their contribution to intestine formation *in vivo*.

Loss of ERM-1 causes defects in tubulogenesis resulting in an early larval arrest, while ERM-1 phosphorylation defective mutants have milder defects and are viable. Using CRISPR/Cas9-generated *nrfl-1* alleles we demonstrate that NRFL-1 localizes at the intestinal microvilli, and that this localization is depended on an interaction with ERM-1. However, *nrfl-1* loss of function mutants are viable and develop normal intestines. Interestingly, *nrfl-1* mutants combined with *erm-1* phospho-mutants show severe intestinal defects and an early larval arrest, which closely resembles the ERM-1 mutant phenotype. This data indicate that ERM-1 activity involves phosphorylation of the C-terminal threonine residue and recruitment of NRFL-1. NRFL-1 loss did not affect localization, stability or phosphorylation of ERM-1, suggesting that NRFL-1 does not regulate ERM-1 activity. Therefore, the most likely NRFL-1 contribution to intestinal development is by recruiting and anchoring proteins to the apical cortex through its two PDZ domains. Collectively, our data shows that NRFL-1 is important for the morphology of the *C. elegans* intestine in an ERM-1 depended manner.

383B Investigating the Mechanisms of Vesicular Trafficking and Unicellular Tube Growth in the *C. elegans* Excretory Duct Cell

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The *C. elegans* excretory duct cell is a unicellular tube with an apical intracellular lumen greater than the length of the cell itself. The shape of epithelial cells depends on proper allocation of membrane and specific protein to their apical and basal surfaces, but how membrane is asymmetrically distributed to the duct's internal, apical surface is not fully understood. The excretory duct cell is shaped by wrapping towards itself, forming an autocellular junction, and then fusing with itself to erase this junction and become a seamless unicellular tube. Within hours the newly formed duct lumen rapidly elongates, outpacing growth of the exterior basal membrane. Previous work in our lab reported that the fusogen protein AFF-1 was necessary for both duct autofusion and elongation and suggested a novel role for AFF-1 as atypical scission factor in basal endocytosis. Likewise, loss of the apical trafficking regulator RAB-11 inhibited lumen growth. Our group hypothesizes that the duct transcytoses internalized basal plasma membrane to the apical surface to support rapid lumen elongation. To find additional genes involved in duct lumen elongation, we screened candidate mutants in genes associated with vesicular transport and tubulogenesis. We observed a short duct lumen in double mutants for the Patched/Dispatched related proteins DAF-6 and CHE-14, which have been associated with *C. elegans* lumen development and apical transport by studies from the Labouesse and Shaham labs. Patched/Dispatched proteins are generally thought to transport lipids or other hydrophobic small molecules (like Hedgehog) across membranes. We would like to understand how these proteins promote duct tube apical membrane growth.

384C Coordinated tissue growth ensures uniformity of gastro-intestinal size proportions

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Organs need to grow to their appropriate size during development, and even small deviations from the correct size can impair organismal function. For example, discrepancies of only a few percent between the length of our legs severely impair motility. Although, in principle, small differences in the growth rate of different organs could amplify to large differences in their size, deviations from the appropriate organ size proportions are rarely observed in nature. However, the mechanisms involved in maintenance of organ size homeostasis are poorly understood.

We use live imaging of gastrointestinal growth of *C. elegans* to study the mechanism of organ size uniformity among individuals. We followed hundreds of individuals from birth to adulthood and measured pharyngeal and total body growth at a temporal resolution of 10 minutes. Individuals differed substantially in their pharyngeal growth rates. Nevertheless, the heterogeneity in pharyngeal size did not increase during development, suggesting a mechanism that senses and counteracts deviations from the appropriate organ size proportions. Indeed, pharynx-specific inhibition of mTOR slowed down pharyngeal growth but did not alter pharyngeal size proportions, as the slow-down of pharyngeal growth was accompanied by an appropriate growth reduction of other tissues.

In conclusion, we provide strong evidence for the systemic coordination of tissue growth of *C. elegans*. The present experimental system provides an exciting opportunity to identify the molecules involved in this coordination by genetic screens.

385A Differential expression analysis of migrating cells in *C. elegans* embryogenesis

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Cell migration drives morphogenesis, the developmental process in which cells become spatially organized to form tissues. An understanding of the molecular mechanisms and regulatory triggers behind cell migration has clinical relevance because it not only explains how embryos develop but offers insights into immune response and metastatic cancers. However, the mechanisms that regulate the transition between migratory and non-migratory states are not well understood, especially *in vivo*. In *C. elegans*, studies of cell migration have revealed some critical mechanisms, but classical genetic approaches have been limited in part because they miss redundant or pleiotropic genes. To look past these limitations, we conducted a comparative transcriptomic analysis using the scRNA-seq data published by Packer et al (2019), which mapped RNA transcriptomes of embryos onto *C. elegans* developmental lineages. We first identified cells of unambiguous lineage that undergo different types of embryonic cell migration. We then used the differential expression analysis workflow in Monocle 3 (Trapnell, 2019) to compare the transcriptomes of these migrating cells to their non-migrating cellular progenitors and progeny. In our analysis we identify 86 candidate genes from six migratory events, 46 of which come up as differentially

expressed (DE) in more than one lineage. 30 of the candidates have known lethal phenotypes and 42 are associated with cell migration defects in *C. elegans* or in other systems. In comparing migrating lineages, we show that while no single set of mRNAs is associated with all migration events and each individual event has unique characteristics, there are some similarities within migration types. Clustering analysis reveals that cells undergoing intercalation and enclosure, which also share both lineage and fate, have DE gene patterns that are more similar to each other than to other migration types. Cells that ingress form a more diffuse cluster with other ingressing cells, with lineage playing an important role, a result supported by work from other labs (Harrell & Goldstein, 2011). Migrations of cells along the embryo surface also cluster, suggesting that despite differences in lineage and fate these migrating cells share some common mechanisms. Our analysis reveals that changes in gene expression associated with migrating cells are relatively complex but can be explored using DE analysis.

386B Evolution of *fem-1* activity in *Caenorhabditis*

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Self-fertile hermaphrodites evolved independently in three *Caenorhabditis* species: *C. elegans*, *C. briggsae*, and *C. tropicalis*. The convergent evolution depends in large part on the core genes of the sex determination pathway. Among them, the three *fem* genes encode proteins necessary for male sex determination. These genes were discovered in *C. elegans*, where null mutants prevent spermatogenesis in *XX* animals, producing females, and also transform *XO* animals into females. By contrast, the Haag lab showed that *C. briggsae fem-2* and *fem-3* mutants do not exhibit these phenotypes; but instead both *XX* and *XO* animals become hermaphrodites.

While screening for sex-determination mutants in *C. tropicalis,* we isolated *v426*ts*,* and mapped it to the *fem-1* gene. This mutation fails to complement the null allele *ctr-fem-1(v466),* which we produced with CRISPR/Cas9. Moreover, these *C. tropicalis* mutants resemble those of *C. elegans* in all assays. Both *XX* and *XO* animals develop as females, and the null mutations show strong maternal rescue.

For comparison, we produced *C. briggsae fem-1(v508)*, using CRISPR-Cas9. This mutation is a 4 base pair deletion in the second exon that causes a frame shift. As with *C. briggsae fem-2* and *fem-3*, these *fem-1* null mutants make fertile *XX* and *XO* hermaphrodites. To confirm the identity of the *XO* hermaphrodites we used RT-PCR to measure *her-1* mRNA transcript levels in individual animals, and also used *unc-7(v281) fem-1 X* to mark the *X* chromosome in some crosses. Notable features found in the *fem-1 XO* adult progeny of *fem-1* mothers include errors in gonad development, small broods, and sickliness.

To confirm the place of *C. briggsae fem-1* in the sex-determination pathway, we are making double mutants with *tra-2* and *tra-1*. The *tra-1(v181); fem-1(v508)* double mutants become *XX* males, confirming that *tra-1* acts downstream of *fem-1* in *C. briggsae*. The *tra-2* strain is under construction.

Taken together, our results suggest that *C. elegans* and *C. tropicalis* reflect the ancestral state of *fem-1*, and imply that a major shift in its regulation in the germ line occurred during the evolution of *C. briggsae.* Whether this occurred prior to the origin of hermaphroditism in *C. briggsae,* or was an integral part of it, is unknown.

387C Sperm fate is promoted by the *mir-44* microRNA family in the *Caenorhabditis elegans* hermaphrodite germline

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Post-transcriptional regulation of gene expression is typically effected by RNA-binding proteins, microRNAs (miRNAs), and translation initiation factors which are essential for normal germ cell function. Numerous miRNAs have been detected in the germline, however the functions of specific miRNAs remain largely unknown. Functions of miRNAs have been difficult to determine as miRNAs often modestly repress target messenger RNAs and are suggested to sculpt or fine tune gene expression to allow for the robust expression of cell fates. In *Caenorhabditis elegans* hermaphrodites, cell fate decisions are made for germline sex determination during larval development when sperm are generated in a short window before the switch to oocyte production. Here, analysis of *mir-44* family mutants has identified a family of miRNAs that modulate the germline sex determination pathway in *C. elegans.* Mutants with the loss of *mir-44* and *mir-45* produce fewer sperm, showing both a delay in the specification and a delay in the formation of sperm as well as an early termination of sperm specification accompanied by a premature switch to oocyte production. *mir-44* and *mir-45* are necessary for the normal period of *fog-1* expression in larval development. Through genetic analysis, we find that *mir-44* and *mir-45* may act upstream of *fbf-1* and *fem-3* to

promote sperm specification. Our research indicates that the *mir-44* family promotes sperm cell fate specification during larval development and identifies an additional post-transcriptional regulator of the germline sex determination pathway.

388A Dramatic alteration of TRA-2/TRA-1 interactions in the sperm/oocyte decision

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TRA-1 is a Gli transcription factor that regulates sexual identityin worms. Thus, it is subject to complex regulatory interactions that might be invovled in the evolution of hermaphrodites. we are using gene editing to learn how TRA-1 controls the sperm/oocyte decision. To this end, we generated both *cbr-tra-1(v455)* and *cel-tra-1(v472)*, each of which inserts an OLLAS tag near the N-terminus. By comparing them, we showed that *C. briggsae* TRA-1 is cleaved to form a slightly larger product than its *C. elegans* counterpart. The Zarkower and Spence labs showed that cleaved TRA-1 represses male genes.

The *cbr-tra-1(v197v383)* allele alters 30 residues in the TRA-2 binding domain, and disrupt interaction between TRA-2 and TRA-1 in yeast two-hybrid assays. These *XX* mutants make significantly more sperms than the wild type. similarly, we detect extra sperms in our *cbr-tra-2(v403mx)* mutant. Furthermore, both mutations can restore spermatogenesis in *cbr-she-1(v35) XX* animals. Thus, we suspect that *C. briggase* TRA-2 normally binds TRA-1 to block spermatogenesis. This is surprising, since *C.elegans tra-2* mutations that block this interaction cause oogenesis.

The *cbr-tra-1(v197)* allele is a frameshift that truncates the protein upstream of the TRA-2 binding domain. The truncated product makes a functional TRA-1 repressor in a *smg-5* background like *cel-tra-1(e2272)*, allowing *XX* animals to develop into fertile females, and *XO* animals into males that only make oocytes.

By contrast, *cbr-tra-1(v510)* is a stop mutation downstream of the TRA-2 binding domain, near the cleavage site. In a *smg-5* background, it causes both *XX* and *XO* animals to produce only oocytes. however, the *XX* animals do not make a normal soma but instead develop a male gonad and a defective tail. The fact that these animals differ from the shorter truncation cuased by *v197* is also surprising. Since the *v510* mutants retain the TRA-2 binding domain, perhaps TRA-2 is targetting the truncated protein to influence repressor function.

Finally, we have and others in our lab found that several chromatin regulators work with TRA-1 to control the sperm/oocyte decision. Thus, we propose that full-length TRA-1 promotes spermatogenesis by working with chromatin regulatory factors, whereas the cleaved form of TRA-1 represses spermatogenesis. In *C.briggase*, TRA-2 blocks the function of TRA-1 by binding to it.

389B Exploring the role(s) of FOG-2 in the hermaphrodite germ line

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C. elegans hermaphrodites are essentially XX females that evolved the ability to briefly produce sperm in the same germ line that produces oocytes [1]. XX spermatogenesis requires a specific protein/mRNA ternary complex composed of an RNA binding protein, GLD-1, an F-box protein, FOG-2, and the mRNA product of the feminizing gene *tra-2*. GLD-1 and FOG-2 bind the 3' UTR of *tra-2* mRNA and repress its activity during spermatogenesis [2,3,4]. Loss of GLD-1, FOG-2, or the GLD-1-binding sites in the *tra-2* 3' UTR eliminates sperm production. GLD-1 is a highly conserved regulator of meiotic progression and oogenesis, and therefore has many RNA targets [5]. In contrast, FOG-2 is a recently evolved *C. elegans*-specific F-box protein [2, 6] only necessary for hermaphrodite spermatogenesis. It has no known role in males, although they express it [7].

The precise role of FOG-2 in regulating germline sex is unknown, but there are clues. FOG-2 interacts with SKR-1, a component of the E3 ubiquitin ligase complex [6]. This suggests it acts as a canonical F-box protein by targeting another protein for ubiquitin-mediated degradation. However, their similar feminized phenotypes indicated GLD-1 is not that target. If FOG-2 is a regulator of proteolysis, then its F-box should be crucial to its function. We deleted 57 nucleotides encoding 19 conserved amino acids of the FOG-2 F-box via CRISPR. This strain was indeed Fog, but subsequent immunoblotting determined the deleted protein was unstable. Future work will target individual conserved residues with missense point mutations. As another approach to elucidating FOG-2's mechanism of action in sex determination, we are currently seeking its putative ubiquitination target(s) by identifying protein interactors. We are employing both targeted yeast two- hybrid assays and unbiased immunoprecipitation and protein mass spectrometry using a 3XFLAG::TEV::6HIS tagged strain of FOG-2.

Recent work from the Haag Lab suggests that FOG-2 may have roles independent of its association with the *tra-2* 3' UTR, and potentially independent of GLD-1 as well [8]. This led us to wonder what fraction of FOG-2 interacts with GLD-1 and *tra-2* mRNA. We stained the germ line using sm-FISH to *tra-2* mRNA and antibodies to HA-tagged FOG-2 and to GLD-1. Surprisingly, FOG-2, GLD- 1 and *tra-2* do not obviously co-localize. These experiments are consistent with a mode of FOG-2 action other than the aforementioned ternary complex, but much remains to be learned.

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390C Deciphering the functional roles of PIEZO mechanosensors in reproduction

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There are > 6000 rare (or orphan) monogenic diseases identified in humans; many of these diseases are caused by the dysfunction of channel proteins, known as channelopathies. A good example is the recently identified mechanosensitive channels PIEZO1 and PIEZO2, of which ~100 missense mutations are associated with at least 26 dysplasias/diseases in the muscular, lymphatic, connective, and cardiovascular tissues. PIEZO1/2 are excitatory mechanosensitive proteins; they are non-selective ion channels that exhibit a preference for calcium in response to mechanical stimuli. Dysfunction of PIEZOs cause a variety of genetic diseases, including the dysplasia in cardiovascular, respiratory, and connective tissues. However, the cellular and molecular mechanisms of PIEZOs in these diseases are less understood. To further understand the function of these proteins, we investigated the roles of *pezo-1*, the sole *PIEZO* ortholog in *C. elegans*. *pezo-1* is expressed throughout development in *C. elegans*, with strong expression in reproductive tissues, including spermatheca, oocyte, sperm, and somatic sheath cells. A number of deletion alleles as well as a putative gain-of-function mutant caused severe defects in reproduction. A reduced brood size was observed in the strains depleted of PEZO-1. *In vivo* observations show that oocytes undergo a variety of transit defects as they enter and exit the spermatheca during ovulation. Post ovulation oocytes were frequently damaged during spermathecal contraction. Given that PIEZO is an ion channel and may regulate spermathecal contractility through Ca^{2+} signaling pathways, we tested the genetic interactions between *pezo-1* mutants and several cytosolic Ca^{2+} regulators with RNA interference (RNAi). Indeed, the phenotypes of *pezo-1* mutants are enhanced upon depletion of known cytosolic Ca^{2+} regulators. We also observed that loss of PEZO-1 revealed an inability of self-sperm to properly navigate back to the spermatheca after being pushed out of the spermatheca during ovulation. Mating with males rescued these reproductive deficiencies in our *pezo-1* mutants. Reduced brood sizes were also observed in a number of auxin-inducible tissue-specific degradation strains, suggesting PEZO-1 may act in different reproductive tissues to coordinate reproduction. Using CRISPR/Cas9, we generated the patient-specific PIEZO2 allele (p.R2718P) in *C. elegans*, named *pezo-1(R2405P).* Homozygous animals carrying the *pezo-1(R2405P)* mutation displayed reproductive defects similar to the *pezo-1*^{ko} mutants, including reduced ovulation rates, crushed oocytes in the uterus, and reduced brood sizes. Overall, these observations support the idea that *C. elegans* is an appropriate model system to study PIEZO diseases. An undergoing EMS-mediated suppressor screen with this *pezo-1* patient-specific allele should help identify other genetic interactors.

391A Mechanisms in the role of the DBL-1/BMP Pathway in the Innate Immune Response of *Caenorhabditis elegans*

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All organisms have a profound proclivity for an innate immune response to fight infection. The innate immune response is the first line of defense against pathogens, and its impact is not to be underestimated, even in the presence of acquired immunity. In the nematode *Caenorhabditis elegans* various signaling pathways are involved in the secretion of antimicrobial peptides (AMPs), which comprise its primary innate immune response. Among these signaling pathways, our research focuses on the *C. elegans* BMP like pathway, which is regulated by the DBL-1 ligand, and how its signaling pathway regulates the response to bacterial pathogens. BMP's as part of the TGF-β superfamily of signaling proteins play a key role in differentiation and development. Prior work from our lab and others has demonstrated that the DBL-1/BMP pathway is required for normal resistance to a variety of pathogens, and our goal is to elucidate the mechanisms involved. In *C. elegans* the BMP pathway includes SMA-2, SMA-3, and SMA-4 which are members of the Smad family of signaling transducers. SMA-3 is known to be required for normal pathogen resistance and TIG-2, a BMP signaling ligand, has also been shown to be required for the immune response. We are testing how other components of the pathway impact the innate immune response. So far, survival analyses using *Photorhabdus luminescens* have shown that both *sma-2* and *sma-6*, the Type-1 receptor, are necessary for a proper immune response. In another set of experiments using survival on *Serratia marcescens* of strains expressing *sma-3* tissue specifically, we have determined a role for the pharynx in the innate immune response. We hypothesized that a different rate of mechanical digestion in wild type animals might be the reason why they had better survival rates and it might explain the pharynx's role in the immune response. We therefore conducted pharyngeal pumping experiments in *dbl-1* and *sma-3* mutants. The results displayed significant differences between the rates of mutants and controls. In *dbl-1* mutants the rate was significantly higher whereas in *sma-3* mutants the rate was significantly lower. On their own these results don't provide conclusive evidence whether to accept or reject our hypothesis, but suggest that survival is not fully correlated with pumping rate. We plan to analyze their relative survival rates side by side to detect any underlying differences between *dbl-1* and *sma-3* that might provide insight into how to proceed. These experiments will deepen our understanding of the molecular mechanisms and tissue-specific contributions in an organismal response to bacterial pathogens.

392B A Life cycle alteration can correct defects in molting

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Molting is required for *C. elegans* to develop through larval stages and reach adulthood. We have shown that the NIMA-related kinases, NEKL-2 and NEKL-3 (the NEKLs), promote molting through their involvement in the trafficking of epidermal cargos, which include lipid precursors required for steroid hormone signaling. During the course of our studies we observed that, upon experiencing starvation, a significant number of *nekl-2; nekl-3* double mutants grow up without molting defects. Further studies indicated that starvation-induced dauer induction is responsible for preventing molting defects in *nekl-2; nekl-3* mutants. Notably, L1 larvae that are starved or exposed to dauer pheromone enter a lengthened L2 stage known as L2d. L2d comprises a predauer stage from which worms can later molt into dauers (L3d) or proceed with normal development (L3), depending on environmental conditions. Consistent with this, we observed that LOF of DAF-2/IGF-1 and DAF-7/*TGF*-β, which cause constitutive dauer induction, can suppress molting defects in *nekl-2*; *nekl-3* mutants. Molting defects are also suppressed by treatment of *nekl-2; nekl-3* worms with dauer-inducing ascarosides, but this suppression is abolished in backgrounds that are dauer defective. Moreover, we find that uncommitted L2d is sufficient for the observed suppression. Furthermore, suppression by L2d occurs only with weak LOF *nekl* alleles, which typically arrest at the L2/L3 molt, but not with stronger LOF alleles, which typically arrest at L1/L2. Notably, dauer induction has previously been reported to suppress developmental timing defects in heterochronic mutants, but to our knowledge has not been reported to suppress other phenotypes. To investigate the mechanism of L2d suppression, we analyzed the expression of genes that are specifically upregulated during molting cycles. We found that transcriptional upregulation fails to occur with normal timing in *nekl* mutants, but this defect can be partially corrected in *nekl-2; nekl-3* strains following L2d induction. Our current working model is that depletion of NEKLs reduce cholesterol uptake due to trafficking defects. This in turn leads to reduced steroid hormone signaling, which is required for the robust induction of cyclically expressed molting genes. We hypothesize that entry into dauer resets a transcriptional control module, effectively lowering the threshold requirement for steroid hormone signaling necessary to drive molting.

393C Gα/GSA-1 works upstream of PKA/KIN-1 to regulate calcium signaling and contractility in the Caenorhabditis elegans spermatheca

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Correct regulation of cell contractility is critical for the function of many biological systems. The reproductive system of the hermaphroditic nematode C. elegans contains a contractile tube of myoepithelial cells known as the spermatheca, which stores sperm and is the site of oocyte fertilization. Regulated contraction of the spermatheca pushes the embryo into the uterus. Cell contractility in the spermatheca is dependent on actin and myosin and is regulated, in part, by Ca2+ signaling through the phospholipase PLC-1, which mediates Ca2+ release from the endoplasmic reticulum. Here, we describe a novel role for GSA-1/Gαs, and protein kinase A, composed of the catalytic subunit KIN-1/PKA-C and the regulatory subunit KIN-2/PKA-R, in the regulation of Ca2+ release and contractility in the C. elegans spermatheca. Without GSA-1/Gαs or KIN-1/PKA-C, Ca2+ is not released, and oocytes become trapped in the spermatheca. Conversely, when PKA is activated through either a gain of function allele in GSA-1 (GSA-1(GF)) or by depletion of KIN-2/PKA-R, the transit times and total numbers, although not frequencies, of Ca2+ pulses are increased, and Ca2+ propagates across the spermatheca even in the absence of oocyte entry. In the spermathecal-uterine valve, loss of GSA-1/Gαs or KIN-1/PKA-C results in sustained, high levels of Ca2+ and a loss of coordination between the spermathecal bag and sp-ut valve. Additionally, we show that depleting phosphodiesterase PDE-6 levels alters contractility and Ca2+ dynamics in the spermatheca, and that the GPB-1 and GPB-2 Gβ subunits play a central role in regulating spermathecal contractility and Ca2+ signaling. This work identifies a signaling network in which Ca2+ and cAMP pathways work together to coordinate spermathecal contractions for successful ovulations.

394A The role of furrow-associated collagen DPY-7 in regulating stress responses varies during larval development

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Mutation or loss of six collagen genes (*dpy-2, 3, 7, 8, 9,* and *10*) disrupts annular furrows in adult cuticles and causes a short and wide 'Dumpy' body morphology. Loss of these collagens also activates osmotic, detoxification, and antimicrobial defense genes, but not other stress responses. High environmental osmolarity reduces internal turgor pressure, physically distorts the epidermis, and activates the same stress responses, but non-furrow *dpy* mutations and other disruptions of the epidermis (e.g., Lon, Rol, Mlt, and Sqt) do not. These results are consistent with a damage sensor associated with furrows in the adult cuticle that regulates responses to environmental stress. Several cuticle characteristics change between molts, but all stages have annular furrows and express furrow-associated collagen genes raising the possibility that this signaling mechanism functions throughout development. We find high variation in stress gene responses to *dpy-7* mutation with the largest induction in adults and little or no induction in early larvae. Alternatively, stress responses are induced by osmotic stress at all stages demonstrating that environmental response mechanisms are functional in early larvae. These results suggest that furrows can develop despite *dpy-7* mutation or that furrows are not essential for stress response regulation in early larvae. *dpy-7* mutants are not Dpy until the L3 stage suggesting that cuticles of early larvae may be able to compensate. We are currently using fluorescent collagen reporters to visualize annular furrows at all stages in *dpy-7* mutants and investigating deletion of a furrow collagen reported to cause Dpy at all stages. This work was supported by NSF grant IOS-1452948 to KPC.

395B Heparan sulfate proteoglycans, guidance molecules and Rho-family GTPases regulate the number of cellular extensions in developing polarized cells

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During animal development, precise cellular morphologies are established that enable polarized cells to perform specialized functions within the organism. For instance, the *C. elegans* excretory-canal cell has four hollow processes (or canals) that extend from the cell soma along the left and right lateral body regions to collect fluid for osmoregulation. Similarly, neurons project processes to connect with appropriate targets. Heparan sulfate proteoglycans (HSPGs) are conserved glycoproteins that play diverse roles, including to regulate interactions between morphogens and guidance cues and their corresponding receptors to elicit cellular responses and orchestrate morphogenetic events. The heparan sulfate (HS) chains attached to HSPG core proteins are polymerized by glycosyltransferases of the exostosin (EXT) family, whose loss causes morphogenetic defects and embryonic lethality across species. Viable hypomorphic mutants of *rib-1* and *rib-2,* which encode the *C. elegans* HS copolymerase, display the striking phenotype of having supernumerary cellular extensions in a number of polarized cells: several monopolar neurons can develop two full neurites, and the excretory-canal cell can form up to eight projections in these mutants. We define one key HSPG that cell-autonomously controls the number of canals formed by the excretory-canal cell, and find that chemical modifications of its HS chains are important for this function. The supernumerary canals observed in these mutants display the hallmarks of normal canals: they develop at the same time as normal ones, arise from the cell body,

and display normal lumen and cytoskeletal (F-actin and microtubule) organization. Through genetic analysis we demonstrate that HSPGs and specific guidance cues and receptors (of the *unc-6* and *slt-1* pathways) cooperate to regulate the number of formed processes; we also define genetic interactions between HSPGs and downstream Rho-family GTPases known to regulate the cytoskeleton. Our results provide insight into a cellular system operating to guarantee that the proper number of cellular projections is established during polarized cell development, and contribute to understanding the roles of conserved HSPGs in cellular morphogenetic events.

396C Defining the molecular determinants by which EXC-4/CLICs regulate Rho-family GTPase signaling

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The excretory canal (*ExCa*) in *C. elegans*, a unicellular tube that collects excess fluid for homeostasis and osmoregulation, is a powerful model to study the genetic regulation and cell biological processes underlying biological tube formation (tubulogenesis). Several conserved genes involved in *ExCa* tubulogenesis have been implicated in vascular development and disease. One example is *exc-4*, which encodes an ortholog of the chloride intracellular channel (CLIC) family of proteins, and two vertebrate CLICs, *CLIC1* and *CLIC4*, which regulate human umbilical vein endothelial cell (HUVEC) angiogenesis *in vitro* and mouse vascular development *in vivo*. We have recently shown that EXC-4 in the worm, and CLIC1 and CLIC4 in HUVEC, are involved in G protein-coupled receptor (GPCR), heterotrimeric G protein, and Rho-family GTPase signaling (Arena and Shaye, *in preparation.* Mao et al., *in press*). To further understand the role of CLICs in this conserved pathway we are investigating shared and unique functions of EXC-4, CLIC1, and CLIC4. In *C. elegans* EXC-4 constitutively localizes to the *ExCa* apical membrane via an N-terminal putative transmembrane domain (PTMD). In contrast, CLIC1 and CLIC4 are cytoplasmic in HUVEC despite also having N-terminal PTMDs, and they only transiently localize to the plasma membrane, in a PTMD-dependent manner, upon GPCR activation. Importantly, membrane localization is necessary for EXC-4 function in the *ExCa*, and CLIC1 or CLIC4 function in HUVEC. Moreover, previous work showed that *ExCa*-specific expression of an apical membrane-targeted CLIC1 C-terminus could rescue *exc-4* null *(0)*. These results led us to hypothesize that while EXC-4/CLIC membrane localization is achieved via the PTMD, conserved function in Rho-family signaling is achieved via shared features in the C-terminus. However, we have also found that CLIC1 and CLIC4 are not interchangeable in HUVEC: CLIC1 promotes RhoA and Rac1 activity, while CLIC4 only regulates Rac1, and overexpression of one CLIC cannot compensate for loss of the other. Therefore, we further hypothesize that CLIC-specific functions are encoded by differences in their C-termini. We are undertaking structure/function studies of EXC-4, CLIC1, and CLIC4 in the *ExCa* and in HUVEC to test these hypotheses in order to define molecular determinants of EXC-4/CLIC function in Rho-family GTPase signaling.

397A Nfya-1 functions as a substrate of ERK-MAP kinase during *Caenorhabditis elegans* vulval development

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A common bridge between a linear cytoplasmic signal and broad nuclear regulation is the family of MAP kinases which can translocate to the nucleus upon activation by the cytoplasmic signal. One pathway which functions to activate the ERK family of MAP kinases is the Ras signaling pathway which functions at multiple times and locations during the development of *Caenorhabditis elegans* including the development of the excretory cell, germ cells, male tail, and vulva. It has been most extensively characterized during the development of the vulva which is formed from the vulval precursor cells (VPCs), a set of six equivalent, epithelial cells designated P3.p – P8.p. Although LIN-1 appears to be a primary target of ERK MAP kinase during vulval development, it is likely that other developmentally important molecules are also regulated by ERK-mediated phosphorylation. The identification of physiological substrates of MAP kinases has been aided by the identification of docking site domains in substrate proteins that contribute to high-affinity interactions with kinases.

Our laboratory has identified the *C. elegans* protein, T08D10.1/Nfya-1, as a potential ERK MAP kinase substrate in this manner, and we have initiated a characterization of its role during Ras-mediated development. T08D10.1 possesses significant homology to the CCAAT-box DNA-binding domain of the vertebrate nuclear transcription factor-Y, alpha (NF-YA) family of proteins. NF-Y proteins act as part of a complex to regulate the transcription of a large number of genes, in particular, genes that function in the G1/S cell cycle transition. T08D10.1/Nfya-1 is predicted to code for a protein containing multiple potential phosphorylation sites for ERK MAP kinase and a D-domain docking site. We demonstrate through biochemical analysis of purified Nfya-1 protein that it can act *in vitro* as a high affinity substrate for activated ERK MAP kinase. We demonstrate through mutant analysis that *nfya-1* acts to inhibit vulval development and functions downstream or in parallel to *let-60/*

ras. Mutants also exhibited a low penetrance protruding vulva (Pvul) phenotype. Analysis of gene expression using transgenic animals expressing a GFP reporter indicates potential expression of *nfya-1* in the L3 stage VPCs.

398B Probing the molecular mechanism of receptor tyrosine kinase activation through the analysis of heterodimers of the *C. elegans* FGF receptor, EGL-15

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Receptor tyrosine kinases (RTKs) are a class of cell-surface receptors that activate Ras and Raf-MEK-ERK signaling pathways to mediate many important cell-cell communication processes. In the absence of signal, RTKs exist predominantly as individual monomers, and their intracellular kinase domains are in an inactive conformation. Upon binding signal, RTKs dimerize, leading to the activation of their kinase domains and triggering downstream events. Protein structure models suggest that the activated dimers are asymmetric, in which one monomer acts as an "activator," and the other as a substrate "receiver." Using *C. elegans*, we have discovered a system in which simple, but powerful genetic tools can be used to probe this mechanism of kinase activation in RTKs.

Genetic analysis of EGL-15, the *C. elegans* fibroblast growth factor receptor (FGFR), has provided many insights into the molecular mechanisms of RTK signaling. In the hypodermis, EGL-15 dysregulation causes defects that include the hypermorphic "clear" (Clr) phenotype and the hypomorphic "suppressor of clear" (Soc) phenotype. We have used *egl-15* heterozygous genotypes which express two different forms of EGL-15 on the surface of their cells to understand how the structures of EGL-15 heterodimers affect kinase activation.

egl-15(n1457) is a nonsense mutation that truncates the EGL-15 carboxy-terminal domain (CTD). Based on the *egl-15(n1457 ΔCTD)* homozygote phenotype, this truncated protein forms EGL-15 homodimers with compromised activity, but that are sufficiently active to be at the Clr/Soc phenotypic threshold. Interestingly, we discovered that heterozygotes between *egl-15(n1457 ΔCTD)* and several *egl-15* substitution mutations have less activity than *egl-15(n1457 ΔCTD)/nDf19*, a deletion that eliminates *egl-15*. These results suggest that certain EGL-15 mutant monomers can inhibit the activity of EGL-15(ΔCTD) monomers when the two dimerize. We have utilized a quantitative fluorescent biosensor of ERK activation, ERK-nKTR, to visualize the impact of *egl-15* dysregulation on signal transduction. We find that ERK is strongly activated in the hypodermis of genotypes that display the hypermorphic Clr phenotype. By combining this quantitative assay with our analysis of mutant alleles that inhibit *egl-15(n1457 ΔCTD)*, we hope to gain insight into the molecular mechanism of kinase activation.

399C A genome-wide RNAi screen for factors of tissue growth coordination

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Reaching appropriate organ size proportions is crucial for organismal function. However, size proportions are intrinsically challenged by stochastic fluctuations in the growth rate of organs. Although, in principle, small heterogeneities in the growth rate can amplify to large differences in their size during development, organ size proportions are remarkably uniform among isogenic individuals. We therefore hypothesised that organs coordinate their growth and size with each other.

To test this hypothesis and understand the mechanism involved, we used the AID/TIR1 to systematically inhibit mTOR signalling in individual tissues. The mTOR pathway is a conserved and central regulator of cellular growth that is thought to act cell autonomously. However, we find that tissue-specific inhibition of mTOR also has large systemic effects on other tissues. For example, pharynx-specific depletion of mTOR is associated with a global reduction of body growth. Depletion of mTOR in the pharynx led to a 2-fold reduction in the adult body size, but did not cause a deviation from the appropriate pharynx-to-body size proportions. To unravel the inter-cellular signalling network that coordinates organ growth, we are currently conducting a genome-wide high content RNAi screen for supressors and enhancers of this phenotype.

400A Detection of clinically relevant ERK/MAPK signaling inhibitors using *C. elegans*

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The ERK/MAPK pathway is evolutionary well conserved and commonly mutated in human cancer. The BRAF^{V600E} substitution is present in many cancer forms and can be specifically targeted by BRAF-inhibitors, often given in combination with MEK inhibitors. However, acquired and inherent resistance to these drugs hampers treatment response and new drugs are needed to improve long-term survival in several patient groups. Here, we find that *Caenorhabditis elegans* animals expressing the BRAF^{V600E} homolog LIN-45^{V627E} form readily scored vulval development phenotypes that are suppressed by experimental- and clinically approved RAS-, BRAF-, MEK- and ERK inhibitors. Using control mutants, we were able to counter screen compounds *in vivo* and determine pathway-specific effects: BRAF^{V600E} inhibitors suppressed signaling from LIN-45^{V627E}, but had no effect on wild-type LIN-45 and were masked by a downstream activating mutation. MEK inhibitors on the other hand effectively suppressed pathway signaling irrespective of LIN-45/BRAF status and were also masked by a downstream activating mutation. The model also identified pathway-specific effects from a panel of experimental ERK/MAPK pathway inhibitors. Assay Z-factors for MEK inhibitors ranged from 0.53 to 0.87, demonstrating that our model is likely capable of identifying novel drugs in a compound screen. We conclude that our model is likely capable of detecting novel and clinically relevant ERK/MAPK pathway inhibitors *in vivo*.

401B Uncovering a novel endocannabinoid (2-AG) pathway required to modulate cholesterol metabolism in *Caenorhabditis elegans*

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Cholesterol is an essential constituent of eukaryotes membranes and its derivate metabolites also serve as a signaling molecule that is crucial for growth, development and differentiation. Dysregulation of cholesterol metabolism is strongly associated with the development of cardiovascular disorders and neurodegeneration. *Caenorhabditis elegans* is especially suited to genetically study organismal orchestration of cholesterol metabolism. This worm requires exogenous cholesterol to survive and perturbations in cholesterol trafficking result in an early development larval arrest. Thus, tight regulation of cholesterol storage and distribution within the organism is critical. We have recently demonstrated that the endocannabinoid 2-arachidonoylglycerol (2-AG) plays a key role in *C. elegans* modulating sterol mobilization (Galles et al, Sci Rep. 2018; 6398). However, the mechanism by which 2-AG controls cholesterol trafficking in *C. elegans* is not known. Recent reports have shown that *C. elegans* has two cannabinoid-like receptors named NPR-19 and NPR 32, which are involved in nociception and regenerative axon navigation, respectively (Oakes et al, J. Neurosci. 2017; 2859, Pastuhov et al, Genes Cells. 2016; 696). Here we show that neither NPR-19 or NPR-32 are involved in 2-AG-mediated cholesterol trafficking. Furthermore, we found that the insulin-IGF1(IIS) signalling pathway is essential for the 2-AG suppression of larval arrest induced by cholesterol depletion. Studying the linkage between IIS and 2-AG we found two *C. elegans* single mutants, *ocr-2* and *osm-9*, were insensitive to the 2-AG-mediated mobilization cholesterol in sterol-depleted worms. OCRs and OSM-9 proteins belong to *C. elegans* Transient Receptor Potential Vanilloid (TRPV) subfamily channels (Colbert et al, J. Neurosci. 1997; 8259). TRPV channels were previously reported as a sort of cannabinoid receptors in mammals (Ahluwalia et al, Eur J. Neurosci. 2003; 2611). Thus, we propose that OCR-2 and OSM-9 are endocannabinoid receptors in *C. elegans*, defining a novel signalling pathway mediated by 2-AG that modulates sterol metabolism in *C. elegans*.

402C The Alimentary Cuticle of *C. elegans* Plays Multiple Roles in Mediating Xenobiotic Sensitivity

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Forward genetic screens with small molecules (SMs) in C. *elegans* carried out in our and other laboratories have repeatedly shown that some SMs aggregate in the anterior pharynx of worms as dark objects. Our group first identified the accumulation as at least partly, if not wholly composed of SM itself manifest as birefringent crystals and/or globular spherical objects. The crystallizing molecules are all lethal to worms. Our data indicates that the process of SM crystallization begins in the pharyngeal cuticle of the anterior pharynx, and over time, extends to the plasma membrane, ultimately causing extensive

cellular damage. The crystallizing molecules can be grouped into 24 distinct structurally similar families (called scaffolds), and tend to be generally more hydrophobic and polar than molecules that do not aggregate. To understand the molecular basis of crystal accumulation, we screened 1.3 million randomly mutagenized wild-type F_2 genomes and isolated 46 mutants that resist the lethal effects of a crystallizing SM, wact-190. This uncovered loss or reduction-of-function mutations in genes encoding components of a putative sphingomyelin synthesis pathway, SMS-5, TTM-5 and SPTL-2. Additionally, loss or reduction-of-function mutations in the ABC transporter PGP-14 also confer robust resistance to all crystallizing SMs. *sms-5* and *pgp-14* are moderately and highly expressed, respectively, in pharyngeal cells that line the pharynx cuticle. Both *sms-5* and *pgp-14* mutants show reduced accumulation of the hydrophobic, crystallizing SMs. Interestingly, both mutants show higher accumulation and sensitivity to a different set of lethal SMs with different physical properties relative to the wildtype. Together, these results indicate that (1) the anterior pharyngeal cuticle may be an important route of SM entry and a "sink" for many hydrophobic molecules, and (2) loss of SMS-5 or PGP-14 activity leads to altered barrier properties. Remarkably, *sms-5* mutants are hypersensitive to cholesterol-deprived conditions, suggesting *sms-5* is specifically required for accumulation of hydrophobic nutrients and other SMs in the pharynx cuticle and in turn into pharyngeal cells. PGP-14 maybe required for transporting a hydrophobic molecule related to SMS-5-catalyzed polar lipid synthesis into the developing cuticle. Taken together, our data suggest that SMS-5 and PGP-14 contribute to a lipid barrier in the anterior pharynx cuticle to limit access of small molecules to the animal.

403A Two RapGaps in *C. elegans* differently regulate development and behavior.

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RAP GTPase is a Ras-like small GTPase family known to regulate cell adhesion and cell to cell junction. RAP GTPase is activated when bound to GTP and deactivated when GDP is attached to the protein. RAP Guanine nucleotide Exchange Factor (RAPGEF) can detach the bound GDP to let free GTP bind to the RAP and be activated. RAP GTPase Activating Protein (RAPGAP) promotes hydrolysis of bound GTP to inactivate the RAP signal. In human cells, RAP signaling activated by RAPGAP can promote cancer invasion and metastasis. Knockout of RAPGEF arrests the cell junction at zipper state and stops it from cell junction maturation. In *C. elegans*, RAP GTPase and its upstream regulator PXF-1, *C. elegans* ortholog of human RAPGEF2, regulate VPC primary cell fate reinforcement, cuticle formation, hypodermal cell organization, and male ray formation. This study aims to find the role and the target protein of two *C. elegans* RAPGAPs, *rgap-1* and *rgap-2*. *rgap-1* deletion mutant exhibits higher autophagy level and shorter lifespan than N2. Also, *rgap-1* deletion mutant is more sensitive to serotonin and levamisole mediated egg-laying and has a larger brood size. On the other hand, *rgap-2* seems to be in a different pathway in organismal development and longevity. With further study, we will investigate the expression pattern and the functional role of *rgap-2*, and investigate genetic interaction between those two RAPGAPs.

404B SEL-5 kinase interacts with retromer complex to regulate QL.d migration and excretory cell canals outgrowth

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During *Caenorhabditis elegans* development multiple cells migrate long distances or extend processes to reach their final position and/or to attain proper shape. These migrations/outgrowths are regulated by several signaling pathways. QL neuroblast descendants (QL.d) movements are an example of EGL-20/Wnt signaling-regulated migration. When Wnt signaling is disrupted QL.d migrate improperly and reach ectopic position in the worm's anterior. To identify new regulators of Wnt pathway we looked for enhancers of a weak retromer mutant *vps-29* QL.d migration defect. We identified serine/threonine kinase SEL-5/AAK1 as a strong enhancer of *vps-29* QL.d migration phenotype. AAK1 was previously shown to regulate Wnt signaling in Wnt receiving cells. However, our data assign SEL-5 requirement to the Wnt producing cells during QL.d migration. By GFP tagging of the endogenous *sel-5* locus we confirmed that *sel-5* is expressed in Wnt producing cells. Its role there is not yet clear but its function is not required for regulation of MIG-14/Wls level. Compound *sel-5 vps-29* mutants also display shortening of the posterior excretory canals. Interestingly this SEL-5 role seems to be distinct from the role in QL.d migration. Firstly, SEL-5 function is cell-autonomous in case of the excretory canal length, and secondly, mutations in Wnt pathway genes *lin-44/wnt*, *lin-17/Frizzled* and *mig-14/Wls* lead to an excretory canal overgrowth instead of its shortening. We thus identified SEL-5 as both Wnt dependent and Wnt independent regulator of cell migration and cell growth in *C.elegans*.

405C Integrative role of the DBL-1/BMP signaling pathway with BLMP-1/BLIMP1 in *Caenorhabditis elegans* development

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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? 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 in a stage-specific manner. Additionally, ChIP-seq, RNA-seq, and co-immunoprecipitation analyses suggest that the DBL-1 pathway and BLMP-1 act together to control expression of some common target genes, further linking these conserved molecular mechanisms during development.

406A PPK-1, the *Caenorhabditis elegans* homolog PIP5K regulates *let-7* miRNA expression through interaction with the nuclear export protein XPO-1

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C. elegans seam cells are lateral epidermal cells that divide with a stem cell-like lineage during larval development. It is known that the development timing of seam cells is strictly regulated by the heterochronic pathway. LIN-28, an RNA-binding protein and the *let-7* microRNA (miRNA) play pivotal roles in controlling the timing of seam cell development. However, the mechanisms of action and the downstream affecters of *lin-28*/*let-7* are poorly understood. Thus, the purpose of this project is to identify novel target genes in the *lin-28*/*let-7* pathway in *C. elegans*.

In our lab, we reported the use of CLIP-Seq to identify 2000 mRNAs interacting directly with the LIN-28 RNA binding protein. To identify functional LIN-28 targets, we first looked for the overlap among our set of LIN-28 CLIP hits, the set of 201 known *let-7* suppressors and 213 *let-7* enhancers, and our unpublished RNA-seq data from staged L2 *lin-28* mutant animals. We found 16 candidate genes at the intersection of these groups. We validated these 16 candidate genes via qRT-PCR experiments in WT, *lin-28* mutant and *lin-28(gf)* animals. From these experiments, we identified 8 candidate genes whose mRNA levels are dependent on *lin-28* levels.

Of these genes, we found PPK-1, the *Caenorhabditis elegans* homolog phosphatidylinositol-4-phosphate 5' kinase (PIP5K), functions in the *lin-28*/*let-7* pathway. As we expected *lin-28* mutants showed decreased *ppk-1* mRNA levels. *ppk-1(RNAi)* worms showed weak precocious phenotypes and increased mature *let-7* expression. *In vitro*, we found that PPK-1 has a physical interaction with nuclear export protein XPO-1, which is already known as a heterochronic gene that regulates mature *let-7* expression. *In vivo*, we also found that PPK-1 and XPO-1 co-localize in seam cells. Taken together, this indicates that PPK-1 interacts with XPO-1 to regulate mature *let-7* expression. As *lin-28*/*let-7* also acts in human cancer cells, we are exploring the functions of PPK-1/XPO-1 homologues in human cancer.

407B TOM-1/Tomosyn is an inhibitor of growth cone protrusion and works with the UNC-6/Netrin receptor UNC-5

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Previous work from the Lundquist lab showed that the UNC-6/Netrin receptors UNC-40 and UNC-5 regulate growth cone protrusion. UNC-40 stimulates protrusion whereas UNC-5 inhibits protrusion, and asymmetric distribution of protrusive activity across the growth cone results in directed growth cone migration away from UNC-6/Netrin (the Polarity/Protrusion model). *unc-5* mutant VD growth cones display unpolarized and excessive protrusion. UNC-5 inhibits protrusion using the FMO flavin monoxygenases, likely via actin inhibition, and by restricting growth cone microtubule entry via UNC-33/CRMP, which might in turn regulate vesicle entry which drives protrusion. To explore the role of vesicle fusion in growth cone protrusion, we analyzed *tom-1/tomosyn* mutants. Tomosyn normally occludes formation of the SNARE complex by interacting with and inhibiting syntaxin-1. VD growth cones of *tom-1 null* mutants were similar to wild-type. However, *tom-1* null mutants suppressed the effects of constitutively-activated MYR::UNC-5, which alone causes small growth cones with little protrusion. This suggests that TOM-1 is normally required for the inhibitory effects of MYR::UNC-5 on growth cone protrusion. Mutations specifically affecting *tom-1* long isoforms showed small and non-protrusive growth cones, and did not suppress MYR::UNC-5. This suggests that TOM-1 short and long isoforms might have opposing roles, with TOM-1 short normally inhibiting protrusion, and TOM-1 long stimulating protrusion. Short isoform specific mutations suppressed MYR::UNC-5, and long isoform specific mutations suppressed excess protrusion in *unc-5* loss-of-function mutants, consistent with this idea. Finally, transgenic expression of full-length *tom-1(+)* resulted in small and non-protrusive growth cones in both wild-type and *unc-5* mutant backgrounds, consistent with a role of TOM-1 in inhibiting protrusion downstream of UNC-5. In the polarity/protrusion model

of growth cone outgrowth, UNC-6/Netrin inhibits growth cone protrusion via the UNC-5 receptor. Previous studies showed that UNC-5 inhibits protrusion via the FMOs and possible actin destabilization, and by preventing MT entry via UNC-33/CRMP. These results suggest that UNC-5 inhibits protrusion via a third pathway, employing TOM-1/tomosyn to prevent vesicle fusion and growth cone protrusion.

408C Parallel Rap1>RalGEF>Ral and Ras signals sculpt the *C. elegans* nervous system.

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The Ras small GTPase is the most mutated oncoprotein, driving both tumorigenesis and metastasis. Oncogenic Ras effectors Raf and PI3 Kinase cascades are well studied and pharmacologically targeted. Ras directly binds a third oncogenic effector, RalGEF, which in turn activates a "Ras-Like" cousin, Ral. Yet the downstream consequences of RalGEF-Ral activation are poorly understood. Consequently, we are studying the functions of RalGEF-Ral in *C. elegans* development.

We found that mutations of RalGEF and Ral enhance migration phenotypes of mutants in genes with established roles in cell migrations. We tested these findings by using the canal associated neurons (CANs) as our model, while validating with HSN cell migration, neurite guidance, and animal locomotion. We found that Ral functions cell autonomously as a permissive developmental signal. Ras, the canonical activator of RalGEF>Ral in cancer, functions as an instructive signal. Unexpectedly, we identified a function for Rap1, a close relative of Ras, in the same process RalGEF>Ral, suggesting that Rap1 and not Ras activates RalGEF>Ral. These findings demonstrate the role of canonical RalGEF>Ral signaling in development of the nervous system, and implicate an unexpected upstream activator, Rap1.

409A Regulation of aging and recovery in arrested L1 larvae

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C. elegans arrest post-embryonic development as a strategy to survive unfavorable conditions. After hatching in the absence of food, larvae arrest at the first larval stage (L1). Blast cell divisions occurring during L1 cease and cells enter quiescence. Arrested L1s can survive several weeks without food and show increased resistance to stress. L1 larvae arrested for long periods undergo a process of aging during which animals accumulate different signs of aging. When fed, arrested L1s resume development after a process of recovery where aging is reversed. There is a direct correlation between the higher level of ageing during prolonged arrest and the time needed to recover. We have observed that prolonged L1 arrest delays specifically the reactivation of blast cell divisions. Once postembryonic development is resumed, the subsequent events occur normally. Insulin signaling modulates the rate of L1 ageing affecting proliferative potential after quiescence, what directly impacts recovery time.

Different endogenous and exogenous factors can directly influence recovery after arrest. We show that variable yolk provisioning to the embryos as a consequence of maternal age confers inter-individual variability in recovery after quiescence of genetically identical animals. Larvae coming from older mothers recover faster. Furthermore, we have revealed that density also affects recovery from L1 quiescence by modulating insulin signaling. L1 larvae arrested at high density show sustained DAF-16 activation during arrest, and reduced expression of the insulin-like peptide DAF-28. The effect of density is mediated by small soluble compounds of unknown nature. Finally, we have identified the disaccharide trehalose as one of these molecules. Trehalose mediates the communication between arrested L1 larvae and modulates the insulin signaling in the animals that sense the signal.

Overall, we have contributed to the consolidation of L1 arrest as an in vivo model to study the mechanisms involved in cell quiescence, and the role of insulin signaling in the process.

410B Elucidating the role of SUP-17/ADAM10 in the BMP signaling pathway in *C. elegans*

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Bone Morphogenetic Protein (BMP) signaling is a highly conserved cascade that is involved in many essential biological events across metazoans, like cell migration, proliferation, and differentiation. Numerous hereditary disorders ranging from cancer to

cardiovascular disease can arise in humans when this pathway malfunctions. Consequently, gaining a better understanding of how this pathway is tightly regulated could help us to address and, ultimately, treat these medical conditions.

In *C. elegans*, the BMP pathway is known to regulate body size, postembryonic mesoderm development, and several other developmental processes. Multiple factors have been identified to be involved in modulating BMP signaling. One such factor is the ADAM (a disintegrin and metalloprotease) protease SUP-17, whose mammalian ortholog is ADAM10. SUP-17/ADAM10 is a single-pass transmembrane protein that plays a fundamental role in ectodomain shedding, a post-translational modification where either a transmembrane or membrane-associated protein is cleaved at the extracellular domain through juxta-membrane processing. Our research aims to elucidate the role of SUP-17/ADAM10 by identifying its substrate(s) in BMP signaling. Towards this goal, we have used the CRISPR/Cas9 technique to tag multiple known transmembrane and membrane-associated proteins involved in BMP signaling with GFP and FLAG. We are conducting western blot analyses on wild-type and *sup-17* mutant worms carrying each of these tagged proteins. We also plan to use an unbiased proteomic approach to identify SUP-17/ADAM10 substrate(s) in BMP signaling. Results from these studies will shed light on how SUP-17/ADAM10 functions to regulate BMP signaling.

411C Physical constraints on cuticle stretch guide *C. elegans* developmental trajectories

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Organismal growth is regulated on a genetic level, as changes in gene expression patterns and signaling dictate much of development. However, environmental conditions (e.g. nutrients and temperature) also have strong impacts on growth. For a comprehensive understanding of organismal growth, the links among genetics, environment, and metabolic regulation must be considered. Studies of single cells have revealed that growth regulation can be achieved using time or size sensing control methods. In multicellular organisms, regulatory mechanisms must not only control single cell growth but also integrate it across organs and tissues during development. The nematode *Caenorhabditis elegans* enables the investigation of growth control in metazoans because it has conserved metazoan pathways and processes and can be cultured by the thousands in controlled laboratory conditions. We developed a high-throughput phenotyping platform that facilitates a quantitative assessment of *C. elegans* growth at high precision. Using this platform, we collected growth measurements of thousands of individuals throughout the 72 hours of larval development, measured feeding behavior to pinpoint developmental transitions associated with decreased feeding, and quantified highly accurate changes in animal size and shape during development. We observed simultaneous increases in animal length, decreases in width, and maintenance of volume at each larval transition, suggesting that body shape in addition to size plays a role in the control of *C. elegans* growth. We propose a model of growth control whereby *C. elegans* senses body size through physical constraints on cuticle stretch and undergoes larval-stage transitions when the cuticle reaches its maximum capacity for stretch. This work lays the foundation for a mechanistic dissection of how both genetics and environmental cues control organismal growth.

412A Differential regulation of developmental stages supports a linear model for *C. elegans* postembryonic development.

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During *C. elegans* postembryonic development, each of the four larval stages entails a process of feeding, a period of quiescence, and ecdysis. The repetitive nature of this process resembles oscillatory process as circadian rhythms. Nevertheless, each larval stage has a defined duration and entails specific events. A central question remains as to whether the overall speed of postembryonic development is controlled by a timer that affects all larval stages equally, as opposed to a linear process, where progression of development is achieved by the completion of stage specific events. The perturbation of a regulator of developmental timing would yield slower or faster animals, with larval stages that scale proportionally to the duration of total development. Contrarily, if development proceeds in a linear manner, based on completing stage-specific events, perturbations that affect overall duration of development might have different impact on different stages, depending on the specific events that take place during each stage. The answer to this question calls for precise quantification of postembryonic development in response to varied perturbations that alter overall developmental timing.

We have measured the duration of each stage of larval development for over 2,500 larvae, upon perturbations in temperature, in food quantity and quality, and amount of insulin signaling. Importantly, the assay we used monitors development continuously, with a time resolution of five minutes, and resolves the transitions between molts and intermolts. We observed that interventions that alter developmental timing have a differential effect on the discrete stages of larval development.

Furthermore, our high-resolution measurement of the effect of temperature has unveiled characteristic features of temperature dependence in *C. elegans* postembryonic development. Altogether, our results support a mechanism based on a linear progression of postembryonic development, where the events that take place in each stage are differentially impacted by environmental perturbations.

413B Beta-catenin centrosomal localization regulates Wnt signaling in C. elegans development and human cells

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The Wnt/beta-catenin signaling pathway is central to metazoan development as well as adult tissue homeostasis and Wnt signaling is routinely dysregulated in cancer and congenital disorders. Caenorhabditis elegans embryos serially employ a modified Wnt/beta-catenin pathway embryos to drive asymmetric cell division (ACD) and rapidly diversify cell fate. Wnt-dependent ACDs rely on nuclear asymmetry of the transcriptional coactivator SYS-1/beta-catenin between daughter cells to differentially activate Wnt-responsive target genes. Interestingly, SYS-1 dynamically localizes to mitotic centrosomes in a symmetrical fashion during many embryonic ACDs. This centrosomal localization pattern enables proteasomal processing and limits SYS-1 levels in daughter cells; uncoupling SYS-1 from the centrosome leads to increased nuclear SYS-1 in both daughter cells and loss of robust control of Wnt-dependent cell fate. Photobleaching experiments reveal that centrosome-bound SYS-1 turns over rapidly, a process that requires the proteasome. These data demonstrate that centrosomal processing of SYS-1 represents a novel form of beta-catenin negative regulation specifically at mitosis that reinforces SYS-1 regulation by Wnt signaling. Intriguingly, beta-catenin centrosomal localization is well-conserved between nematodes and mammals so we examined the functional contribution of centrosomes to Wnt signaling, beta-catenin regulation, and posttranslational modifications using HEK293 cells. After pharmacological depletion of centrosomes, the cellular response to Wnt signaling is attenuated despite normal beta-catenin synthesis and degradation rates. The attenuated Wnt response is due to accumulation of a novel high-molecular-weight form of phosphorylated beta-catenin. Wnt signaling normally operates by inhibiting a destruction complex that phosphorylates and degrades beta-catenin, but the high-molecular-weight form of phosphorylated beta-catenin is unexpectedly increased by Wnt signaling. Furthermore, gel-shift analyses and mass spectrometry suggest the high-molecular weight form of phospho-beta-catenin results from mono-ubiquitination. These studies have thus identified a pool of beta-catenin effectively shielded from regulation by Wnt. We present a model whereby centrosomes across species regulate beta-catenin stability: centrosomes actively degrade beta-catenin during ACD and prevent inappropriate beta-catenin modifications that antagonize normal stabilization by Wnt signals.

414C Coordinating neuronal signaling pathways with anterior epidermal cell migration

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We study the cell patterning that regulates anterior morphogenesis in *C. elegans* embryos. We recently described how the epidermal, pharyngeal and neuroblast cells must be properly coordinated for development of the anterior lumen. However, the signaling events that coordinate these cell types are still not well understood. We found that soon after the ventral epidermal cells meet at the ventral midline, PAR-6 (polarity protein) foci associated with different subsets of cells form distinct patterns in the anterior of the embryo. Specifically, we observed two pentagons, a larger focal point and a more ventrally positioned semi-circle of foci. While the larger focal point corresponds to the arcade cells (anterior most cells of the pharynx), the pentagons appear to correspond to neuronal precursor and/or glial cells (UNC-119, MIR-228), while the semi-circle of foci may correspond to neuronal precursor cells (UNC-119, HLH-16), although some of these could come from the epidermis. The dorsal foci within each pentagon move in concert with the dorsal epidermal cells, while the semi-circle of foci move with the ventral epidermal cells. Ventral epidermal cells migrate using F-actin-rich projections, and we observed that these projections come close to, but do not cross the semi-circle of foci suggesting they are guided by nearby cues. We found that blocking neuroblast cell division and disrupting the PAR-6 patterns caused delays in epidermal cell migration by decreasing the number of F-actin projections. Based on these findings, we propose that signals associated with the neuronal and/or glial cells control anterior epidermal cell migration. To identify these signals, we are performing tissue-specific RNAi to guidance cues and their receptors including ephrins (*enf-1/2/3*), netrin (*unc-6*), slit (*slt-1*) and sax/robo (*sax-3*), as well as Wnt (*lin-44 , mom-2, mig-5*, etc), and determining how they disrupt either 1) the pentagon or semi-circle of foci and/or, 2) migration of the anterior epidermal cells. This work will continue to provide new insights on the mechanisms underlying the multi-tissue cooperation required for successful anterior morphogenesis of *C. elegans* embryos.

415A Understanding the regulation and function of the CRISP protein LON-1 in C. elegans

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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. The C. elegans BMP pathway regulates body size, mesoderm patterning, and a number of other processes. Previous studies have shown that the Cysteine Rich Secretory Protein (CRISP) LON-1 negatively regulates body size and that lon-1 transcription is negatively regulated by BMP signaling. Using a sma-9 suppressor screen, we have found that LON-1 has a role in regulating postembryonic mesoderm development, like all other BMP pathway components. 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 found that a 4.3 kb lon-1 promoter region, which is sufficient for mediating BMP regulation of lon-1, contains several highly conserved putative Smad-binding sites. We are currently testing the hypothesis that lon-1 transcription is directly regulated by BMP signaling through the binding of Smad transcription factors in the lon-1 promoter.

To dissect the mechanisms by which LON-1 modulates body size and regulates BMP signaling, we have used CRISPR/Cas9 and generated an endogenously V5 tagged LON-1. This V5-tagged LON-1 is fully functional and is detectable on western blots. We plan to use this strain to dissect how LON-1 interacts with other BMP pathway components as well as to identify novel factors that interact with LON-1.

416B Calumenin functions in cuticle collagen modification

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Calumenin is an acidic, low-affinity Ca^{2+} binding protein that belongs to the CREC family (Cab4, reticulocalbin, ERC-55 and calumenin). It has been involved in various malignant diseases and muscle contraction-relaxation processes in vertebrates. *C. elegans* has the single gene of calumenin, *calu-1*. It has been proposed to have pleiotropic roles in body size, muscle function, and cuticle formation. As *calu-1(tm1783)* mutant displayed severe cuticle defects such as irregular annulus and fused alae, we investigated the mechanistic roles of calumenin in cuticle development. The worm cuticle is an extremely flexible exoskeleton, which is indispensable for normal development and survival. It is firstly synthesized in the embryo and four times more in each larval stage prior to molting. Collagen, the major component of cuticle, is synthesized as procollagen and further modified in the endoplasmic reticulum (ER). Since calumenin is a Ca^{2+} binding protein resident in ER, we firstly examined the function of calumenin in collagen modification in ER. The first step of modification of procollagen is prolyl 4-hydroxylation, which is conducted by prolyl 4-hydroxylase (P4H; DPY-18/PDI-2). The next step is the trimerization by peptidyl-prolyl *cis-trans* isomerase (PPIase). Genetic interactions of calumenin and P4H or PPIases were examined by establishing multiple mutants and cuticle-related phenotypes of mutants were identified.

417C Nutritional status and fecundity are synchronised by muscular exopheresis

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Organismal functionality and reproduction depend on metabolic rewiring and balanced energy resources. However, the crosstalk between organismal homeostasis and fecundity, and the associated paracrine signaling mechanisms are still poorly understood. Using the *Caenorhabditis elegans* we discovered that large extracellular vesicles termed exophers, attributed in neurons and cardiomyocytes to the removal of damaged subcellular components, are released by body wall muscles to support embryonic growth. We found that exopher formation (exopheresis) is a non-cell autonomous process regulated by developing embryos in the uterus. Our data suggest that exophers serve as transporters for muscle-generated yolk proteins used for nourishing and improving the growth rate of the next generation. We propose that the primary role of muscular exopheresis is to stimulate the reproductive capacity, thereby influencing the adaptation of worm populations to the current environmental conditions.

418A Wide-Spread Non-Canonical CED-3 Caspase Activities Regulate Gene Expression Dynamics Including Antagonizing PMK-1 p38 MAPK Stress-Priming Function to Support Development

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Recent studies across diverse animal phyla have revealed non-canonical activities of apoptotic caspases involving specific modulation of gene expression, such as our finding of LIN-28 inactivation in worms and others findings of Nanog and Pax7 inactivation in mice to limit pluripotency of stem-like cell types to promote differentiation. We still do not know how broadly these non-canonical caspase functions span across various biological processes or their underlying molecular mechanisms. Using a forward genetic screen, we identified the CED-3 caspase working in diverse biological processes in *C. elegans*. Specifically, we recently showed that CED-3 caspase negatively regulates an epidermal p38 stress-responsive MAPK pathway to promote development. We also showed that PMK-1 (p38 MAPK) functions to prime animals for adverse environmental conditions prior to experiencing the stress. However, this priming function comes with the cost of retarding post-embryonic development. CED-3 caspase counters this stress-priming function by inactivating PMK-1 through proteolytic cleavage thereby promoting development. Strikingly, using RNA-seq and ribosomal profiling, we find that CED-3 and PMK-1 inversely regulate a key sub-set of more than 300 genes. A key enrichment amongst these genes were stress-responsive and pathogen resistance factors. Now, our ongoing studies are working to identify the molecular and physiological mechanisms of CED-3 and PMK-1 antagonism in regulating cell signaling and gene expression dynamics during development and stress-response states.

419B Insulin signaling and osmotic stress response regulate arousal and developmental progression at hatching

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The progression of animal development depends on the coordination of organism-wide responses with environmental conditions. Two transcription factors that function in interneuron differentiation, *fax-1* and *unc*-42, are required for arousal and progression from embryogenesis to larval life by potentiating insulin signaling. The combination of mutations in either transcription factor and a mutation in *daf-2* insulin receptor results in a novel peri-hatching arrest phenotype: embryos are fully developed but inactive and often remain trapped within the eggshell. This pathway is opposed by an osmotic sensory response pathway that promotes developmental arrest and a sleep state at the end of embryogenesis in response to elevated salt concentration. The quiescent state induced by loss of insulin signaling or by osmotic stress can be reversed by mutations in genes that are required for sleep, including *ceh-17, aptf-1,* and *flp-11.* Therefore, countervailing signals regulate late embryonic arousal and developmental progression to larval life, mechanistically linking the two responses. Tissue-specific expression of *daf-2* indicates that integration of the response depends on both the nervous system and the intestine or pharynx. Our findings demonstrate a role for insulin signaling in an arousal circuit, consistent with evidence that insulin-related regulation may function in control of sleep states in many animals. The quiescent arrest response may serve as an adaptive response to the osmotic threat from high salinity environments.

420C Relatives of Ras regulate function of the *C. elegans* exocyst complex in development

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Among three Ras effectors of roughly equivalent oncogenicity, Raf and PI3K are well studied but the downstream mechanisms of Ras-RalGEF-Ral signaling remain poorly understood. Ral (Ras-like) is a small GTPase related to Ras. Ral uses a heterooctameric protein complex named exocyst as a signaling intermediary (as exocyst components-Exo84 and Sec5 are Ral effectors) and also performs essential activities to regulate exocytosis functions of the exocyst, which precludes conventional biochemical bootstrapping to identify signal transduction components downstream of the exocyst. Rap1 (Ras proximal) is another small GTPase that sometimes signals in parallel with Ras. Delineating the mysterious functions of Ral in signaling and Ral and Rap1 in functions of the exocyst are important for therapeutic targeting of oncogenic Ras and understanding the cell biological functions of the exocyst complex.

We are using genetic, biochemical and cell biological analyses in *C. elegans* to identify roles of Ral and Rap1 in control of exocyst functions during development. The three aims of this project are: 1. Determine the contribution of Ral to functions of the exocyst complex. 2: Investigate whether the Ral signaling function works to regulate exocytic functions of the exocyst separately from the signaling-independent functions. 3: Delineate Rap1 contributions to exocyst functions.

We found that Ral is needed for the exocyst function, as deletion of Ral aggravates developmental defects conferred by maternally rescued (M+Z-) deletion of Sec5. Yet signaling-defective Ral does not alter the phenotype of M+Z- Sec5 mutant animals. Furthermore, constitutively activated Ral reduces the severity of M+Z- Sec5 mutant defects, implicating signaling-dependent and -independent contributions of Ral to exocyst-dependent development. Rap1 similarly interacts with the M+Z- Sec5 mutant and the M+Z- Ral mutant, suggesting a model similar to that of Ral. We will continue to test the contributions of Ral and Rap1 to exocyst-dependent development using the chemogenetic tool Auxin-Inducible degron (AID). We will also use the development of elaborate arborization of the axons of PVD neurons as a more precise readout of exocyst function, and also assay the impact of Sec5, Ral and Rap1 depletion on the transport of marked exocytic cargoes. Besides, we will use biochemical tools and will do confocal imaging to measure the physical interaction and colocalization of HA and fluorescent tagged Ral and exocyst components.

In conclusion, this study will further investigate the role of two Ras relatives, Ral and Rap1, in the exocyst. This will help delineate the downstream effectors of the poorly understood Ras>RalGEF>Ral pathway, and help better understand the relationship of Rap1 with the exocyst, as well as the crosstalk of Rap1 and Ras effectors.

421A A cilia-independent function of BBSome mediated by DLK MAPK signaling in *C.Elegans.*

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Bardet-Biedl Syndrome (BBS) is a genetic disorder affecting primary cilia. BBSome, a protein complex composed of eight BBS proteins, regulates the structure and function of cilia in diverse organisms, and its malfunction causes BBS in humans. Here, we report a new function of BBSome. In a genetic screen conducted in C. elegans to identify genes regulating the photoreceptor LITE-1, a non-ciliary protein expressed in ciliated sensory neurons, we isolated bbs mutants. Functional analysis revealed that BBSome regulates LITE-1 protein stability in a cilia-independent manner. Through another round of genetic screen, we found that this new function of BBSome is mediated by DLK MAPK signaling. We further showed that BBSome regulates the expression of DLK. Interestingly, we found that BBSome also regulates DLK expression in mammalian cells, suggesting a conserved mechanism. These studies identify an unexpected cilia-independent function of BBSome and uncover DLK MAPK signaling as a novel BBSome effector.

422B The effects of venlafaxine on behavior and central nervous system of *Caenorhabditis elegans*

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The growing consumption of psychoactive drugs, including antidepressants and antipsychotics, along with the low levels of total metabolization of these drugs, are increasing their residues in the environment. After being used, the drugs are metabolized and excreted, sometimes still in their active form, and may or may not suffer degradation when in contact with the environment. Thus, these psychoactive drugs can reach the aquatic environment and affect non-target organisms, such as zebrafish (*Danio rerio*) and *Caenorhabditis elegans*, in their natural environments. Our main hypothesis is that venlafaxine (VEN), a drug that works with the selective inhibition of the reuptake of noradrenaline and serotonin, can exert effects on the endocrine and behavioral system of zebrafish and toxicological effects on the behavior and nervous system of *C. elegans*. The integrity of the natural behavioral repertoire is vital for the maintenance of the species, reinforcing the importance of studies that show the potential of drugs in altering these models. Here we evaluate acute exposure to VEN, in three concentrations followed by a behavioral assessment. The behavior of *C. elegans* was analyzed by the body bands, pharyngeal beat, and defecation cycle; while the biochemical analyzes of the enzyme Acetylcholinesterase to assess the toxicological effects of VEN on the nervous system. We have shown that the nematode *C. elegans* resists changes in behavior and the nervous system. Thus, this model response was not a clear indicator of water contamination and possible zebrafish effects, probably because of the cuticle and natural behavior of the nematode. Results with zebrafish exposure to VEN have shown changes in the novel tank test, mainly the robust anxiolytic-like pattern, suggesting that VEN may be more dangerous to species conservation, since fish presenting an anxiolytic-like behavior becomes more susceptible to predation. We are working on biomarkers to study differences in the responses to these models when exposed at VEN.

423C Neural and Molecular Mechanisms of Microbe-sensing in the Control of Animal Behavior

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Animals use the chemical senses of taste and smell to represent the environment. How complex and distributed sensory representations of the world are integrated to form sensory percepts and instruct behavior remains poorly understood. The nematode *C. elegans*, with its well-defined genetics and compact, accessible nervous system, permits cell-specific manipulation and monitoring of neural activity to determine how sensory information is integrated and how neural activity generates behavior.

Using a genetically encoded integrator of neural activity - CaMPARI - I found that exposure to *E. coli* and *E. faecalis* elicit different responses in interneurons of freely behaving *C. elegans*. These microbes have different effects on *C. elegans* biology and elicit different behavioral responses: *C. elegans* avoids *E. faecalis*, which is highly pathogenic, but is attracted to *E. coli*, which is nutritive. I discovered that AIB and AIZ interneurons, which receive convergent inputs from many chemosensory neurons, are strongly inhibited by exposure to *E. coli* but remain active in the presence of *E. faecalis*. I also observed this effect using microbe-conditioned media, indicating that differential activation of chemosensory neurons generates the observed difference in interneuron activity.

Using the calcium indicator GCaMP, I mapped sensory responses to *E. coli* and *E. faecalis* and found that distinct populations of chemosensory neurons respond to chemical cues derived from these microbes. These data indicate that chemosensory representations of these microbes are complex and distributed, and they may be integrated in AIB and AIZ interneurons. Our next goal is to manipulate activity in chemosensory neurons and monitor how interneuron activity changes to determine the computation used to integrate complex sensory information.

424A Does programmed organismal death promote fitness at the *C. elegans* colony level?

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Is aging a form of programmed death which, like programmed cell death, promotes fitness? According to evolutionary theory, the answer is no, at least for species that reproduce as outbred dispersed populations. However, it is theoretically possible for adaptive death to evolve in species with clonal, colonial modes of existence, including bacteria, budding yeast and, potentially, *C. elegans* (1-3). Adaptive benefits of programmed organismal death in post-reproductive adults include increasing food availability for kin (consumer sacrifice) and consumption of dead adults by kin (biomass sacrifice)(1-3). Occurrence of adaptive death in *C. elegans* could partially explain the presence of so many genes where loss of function increases lifespan (1).

We recently created an *in silico* model of a *C. elegans* colony on a virtual food patch to probe the likelihood that earlier adult death could increase fitness at the colony level. For the latter, dauer yield per colony was used as a metric. Behavior of the model supported the hypothesis that traits that reduce individual worm fitness can increase colony fitness (4). Increased colony fitness was sometimes observed not only when adult lifespan was shorter but also when brood size was reduced. Notably, reduction of brood size could increase the efficiency with which food was converted into dauer yield.

Our findings supported the hypothesis that colony fitness is a function of population structure (4). To test this we have been altering *C. elegans* colony structure by varying founder animal number, and measuring colony fitness using a dauer yield assay. Results will be presented at the meeting. This work draws attention to the possibility that many characteristics of individual nematodes may promote fitness at the colony level rather than the individual nematode level.

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425B Microbiome perturbations moderately modulate *Caenorhabditis elegans* health and life history traits

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Bacteria are the sole food source of *C. elegans* and many must be advantageous to their health, but some are detrimental. *Stenotrophomonas* bacteria are members of the natural *C. elegans* core microbiome described by Zhang *et al*. (2017). Surprisingly, we have shown that many *Stenotrophomonas* isolates are detrimental to *C. elegans* health, although they are

an abundant member of the core microbiome. 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) consisting of representative bacteria (Dirksen *et al*., 2020) to determine the effects of perturbing the microbiome with various *Stenotrophomonas* strains. We will analyze the effects on microbiome community composition and host health and fitness. We have found that individual CeMbio members have varying effects on host survival compared to the whole community, indicating that the effect of the community on survival is likely additive. We used the effects of individual *Stenotrophomonas* strains on *C. elegans* health in monoculture and their isolation source (worm or substrate) 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 is resilient to perturbations at least in terms of the effect on host survival. We also found that development time, used as a measure of host fitness, is mostly unaffected as compared to the intact community. Future work will determine if other measures of fitness, such as brood size, are affected by these microbiome perturbations, and how community composition of the gut microbiome might be altered.

426C Skin-penetrating nematodes exhibit life-stage-specific interactions with host-associated and environmental bacteria

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Skin-penetrating nematodes of the genus *Strongyloides* infect over 600 million people, posing a major global health burden. Their life cycle includes both a parasitic and free-living generation. During the parasitic generation, infective third-stage larvae (iL3s) actively engage in host seeking. During the free-living generation, the nematodes develop and reproduce on host feces. At different points of their life cycle, *Strongyloides* species encounter bacteria from various ecological niches. However, the microbial interactions between *Strongyloides* and bacteria remain uncharacterized. We first investigated the microbiome of the human parasite *Strongyloides stercoralis* using 16S-based amplicon sequencing. We found that *S. stercoralis* free-living adults have a distinct microbiome, suggesting that they selectively associate with specific fecal bacteria. We then investigated the behavioral responses of *S. stercoralis* and the closely related rat parasite *Strongyloides ratti* to an ecologically diverse panel of bacteria. We found that *S. stercoralis* and *S. ratti* showed similar responses to bacteria. The responses of both nematodes to bacteria varied dramatically across life stages: free-living adults were strongly attracted to most of the bacteria tested, while iL3s were attracted specifically to soil bacteria. The behavioral responses to bacteria were dynamic, consisting of distinct short- and long-term behaviors. Finally, a comparison of the growth and reproduction of *S. stercoralis* free-living adults on different bacteria revealed that the bacterium *Proteus mirabilis* inhibits *S. stercoralis* egg hatching, greatly decreasing parasite viability. Our results identify bacteria that serve as key sensory cues for directing movement, as well as bacteria that decrease the parasite's reproductive fitness.

428B Gut-brain-axis signaling in regulation of the *Caenorhabditis elegans* microbiome

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The animal gut is critical for both acquisition and interpretation of nutrients and other molecules in the environment, in addition to cultivating a robust community of microbes. Communication of nutritional and microbial information between the gut and the brain, referred to as the gut – brain axis, occurs via signaling molecules like host-derived neuropeptides and neurotransmitters, in addition to microbially produced metabolites. Thus, altering the gut – brain axis and the communication within can have influence host physiology and metabolism, but also impact the regulation of the host's gut microbiome. Studies conducted within the last decade have highlighted the importance of balanced relationships within the microbiome, but the molecular mechanisms of microbiome regulation of the gut – brain axis remain largely uncharacterized due to the complexities in other systems and difficulties with high-throughput testing. We use *C. elegans* to address this because it contains a comparable gut whose composition is well defined, is amenable to various high – throughput techniques, and is transparent, which allows us to view differences in composition using fluorescent bacteria.

Insulin signaling members, specifically the insulin receptor and downstream pathway factors, have previously been shown to alter microbiome composition.

To determine which neuropeptides, and their respective receptors, are associated with gut microbiome regulation, we have conducted RNAi experiments coupled with 16s rRNA sequencing to assess gut composition. The resulting data has shown that roughly 20 of 104 RNAi clones assayed alter composition of the microbiome, broadly or in taxa-specific manner, in N2 animals when knocked – down via RNAi in a tissue – specific manner. Specifically, *flp –14, ins –1, ins –11, ins –18, ins –21,* and *ins –24*, promote colonization of *Ochrobactrum* when knocked down in the gut alone using an intestinal – specific RNAi strain. These knockdown findings will be further validated using fluorescent bacteria colonization phenotypes as a proxy for sequencing. Insulin signaling members, specifically the insulin receptor and downstream pathway factors, have previously been shown to alter microbiome composition. The data obtained from the RNAi screening suggests that the normal function of these 20 or so neuropeptides is to relay information about the microbiome or otherwise that could influence microbe's ability to colonize the gut. Through these and related studies, we hope to better characterize the gut – brain axis signaling pathways and identify novel molecular factors that regulate the microbiome in *C. elegans*.

429C Behavioral analysis of *P. pacificus* mutants encoding for a novel repeat-containing protein

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Most parasitic nematodes exhibit species-specific host-seeking behavior, oftentimes triggered by chemo attraction to host-secreted odors, such as pheromones. The entomophilic nematode, *Pristionchus pacificus*, has a nuanced relationship with its host beetle. The host beetle produces a pheromone, ZTDO, which is not only a strong attractant to *P. pacificus* but also induces developmental arrest in embryos and paralysis in early larval stages but surprisingly, not in J4 or adults. This phenomenon leads us to speculate that there is a co-evolutionary battle occurring between the host's ability to produce a potent nematocide and the nematode's ability to resist paralysis in order to maintain a non-pathogenic relationship. In *C. elegans,* mechanisms of paralysis due to various anthelmintics have been found to act on the betaine-gated channel ACR-23, a nicotinic acetylcholine receptor. Because various paralytics act on the same receptor we hope to find overlap between ZTDO induced paralysis and the betaine pathway. To better understand the genetic component of ZTDO induced paralysis, we investigated a ZTDO-hypersensitive mutant, *Ppa-obi-1(tu404),* a gene which encodes a lipid-binding domain that is hypothesized to facilitate odorant reception.

To identify potential genes involved in the pathway for ZTDO resistance, we performed a suppressor screen on *Ppa-obi-1* and isolated three mutant suppressor lines. Two of these candidates (*csu63,csu64*) show partial resistance to ZTDO in the J4 stage when compared to their *Ppa-obi-1* parent, suggesting that we have disrupted components of the pathway mediating *obi-1* J4 larvae hypersensitivity. One of the leading gene candidates is PPA09604, which has multiple independent mutations found in both *csu63* and *csu64* alleles in a reading frame encoding for a novel protein. Interestingly, both alleles also suppressed the *Ppa-obi-1* hypersensitivity to betaine. The predicted structure of PPA9604 leads us to believe that this gene may encode a transcriptional repressor which do not have homologs outside of the *Pristionchus* genus. PPA9604 expression is found in the pharyngeal gland cells, intestine, and the vulva. To characterize this novel protein, we used a co-CRISPR strategy to phenocopy the suppressor mutations. We generated three loss-of-function alleles and found that all three alleles modestly reduced hypersensitivity in J4 larvae, in the presence of ZTDO. We are currently determining if these alleles also suppress the *Ppa-obi-1* hypersensitivity to betaine.

431B Interaction of genetic variation and diet on stress resistance in *Caenorhabditis tropicalis* isolates

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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.

We 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. Genetic analysis suggests that cold

stress resistance is a dominant trait, and initial mapping reveled a potential QTL on chromosome III. Currently, we are working on refining and validating the QTL associated with cold stress resistance, 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.

432C The involvement of host genes in shaping the *C. elegans* gut microbiome

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The recent advent of microbiome research in *C. elegans* provides new trajectories for exploring host-microbe interactions. *C. elegans* harbors a distinct gut microbiome that is shaped by environmental conditions, age, and host genotype. Taking advantage of *C. elegans*' genetic tractability, we only started to discover the roles of host genes being involved in regulating the microbiome. Previous work in the lab demonstrated a novel role of TGFβ/SMA signaling in shaping gut microbiome composition. Disruption of TGFβ/SMA signaling changed gut microbiome composition, specifically resulting in a bloom of *Enterobacter*, a common natural commensal of *C. elegans*. Which downstream targets of TGFβ/SMA signaling contributed to this change is yet unknown.

To address this question, we carried out RNAseq analyses exposing the *dbl-1(nk3)* and *sma-3(e491)* knock-out mutants, as well as the *dbl-1* over-expressor to CeMbio, a recently established synthetic community representing the natural *C. elegans* microbiome. We identified genes up-regulated in worms raised on the synthetic community, compared to those raised on the non-colonizing *E. coli*, in agreement with previous results. We further identified a subset of these genes that was dependent on *dbl-1* or *sma-3,* potentially involved in TGFβ/SMA-mediated host-microbiome interaction. Current work is investigating the roles of identified candidate genes in shaping microbiome composition using knock-out mutants, over-expression strains, and reporter strains.

433A Evidence for the inclusion of *Caenorhabditis elegans* in Environmental Risk Assessment routines

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Caenorhabditis elegans has long been used as a choice for a laboratory model organism, and it has been linked to several discoveries that have led to Nobel Prizes. Although *C. elegans'* journey as an invaluable model in developmental biology and neurobiology is long, dating to the 1960s, its application in other research fields such as environmental toxicology is more recent. As a well-known experimental organism bearing high sensitivity to different environmental contaminants, and representing important functional levels in soil and aquatic ecosystems, *C. elegans* has high potential to be extensively integrated within Environmental Risk Assessment (ERA) routines. The major advantages supporting the inclusion of *C. elegans* in lower tiers of ERA, where a first screening of potential hazardous scenarios to the biota of both environmental compartments is performed, as well as the large array of endpoints that can be tested in this context, are herein presented. In addition, its sensitivity to contaminants such as metals and pesticides, as well as specific strengths and limitations, are compared to other laboratorial model organisms commonly used in ERA, such as *Daphnia magna* (crustacean) and *Eisenia fetida* (earthworm). The inclusion of *C. elegans* in ERA routines is encouraged, since it may provide ecologically relevant insights on the effects of contaminants, thus improving the establishment of appropriate environmental protection benchmarks.

434B Assortative mating and the potential for sperm-mediated reproductive interference in co-occurring nematodes *C. macrosperma* and *C. nouraguensis*

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Co-existence of species is shaped by many abiotic and biotic factors. One interesting factor in determining how species can co-exist is their order of arrival in a new habitat. This "priority effect" phenomenon is most-studied in plant communities, but displacement of first colonizers can occur in animal species that may interfere with one another's reproductive success in ways that may allow one species to sexually exclude the other. In this study, we characterize reproductive life-history traits and test for reproductive interference through adverse effects of inter-species mating between *C. macrosperma* and *C. nouraguensis,*

two co-occurring species of *Caenorhabditis* from the Nouragues Reserve (French Guiana). *C. macrosperma* displays sperm gigantism, also making this an interesting system for studying divergence in reproductive traits.

We provide rarely quantified measures of lifetime fecundity for males and females, showing higher intrinsic population growth for *C. nouraguensis. Caenorhabditis* often are assumed to mate non-assortatively, leading to costs to female fitness through ectopic sperm cell migration. However, here we demonstrate mate discrimination between these species. We also quantify the asymmetry of sperm-mediated costs to female lifespan and fitness, and track migration of sperm inside the female to ectopic somatic locations in the cross direction with greatest adverse effects. Lastly, using this life-history information we modelled and empirically tested whether fitness costs imposed by *C. macrosperma* in inter-species matings could offset the intrinsic growth advantage of *C. nouraguensis* with ecological implications for species co-existence.

With this research, we provide rare insight into different life-history and reproductive traits of two *Caenorhabditis* species and demonstrate the importance of ecological context for understanding the evolution of gametic and behavioural reproductive traits for species co-existence.

435C A population state shift supports aging as a cause of adult death

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Birth and death drive population dynamics and determine whether a population survives or is doomed for extinction. Individual traits that influence development, diapause, reproduction, aging and lifespan must impact the age structure and survival of a population. However, the relationships between individual traits and population dynamics are still a major challenge in the emerging field of ecology-development (eco-devo), because wild populations exist in complex ecosystems that are challenging to investigate and undesirable to manipulate.

Here, we introduce a laboratory ecosystem based on the model organism *C. elegans* that can be used to measure and manipulate worm populations over hundreds of generations. To complement this experimental system, we developed a computational simulation that realistically models the ecosystem and makes it possible to monitor the life history of every single worm in the population. With this integrated systems approach, we investigated the role of aging in population dynamics.

The first question we addressed was why some populations support old animals whereas, in other populations, all animals die young. We discovered that old age as a cause of death is influenced by three conditions: maximum lifespan, rate of adult culling, and progeny number/food stability. More specifically, the populations displayed an unexpected tipping point for aging as the primary cause of adult death. In populations with high progeny survival and regular starvation almost all adults died young. In contrast, a reduction of progeny survival over the tipping point caused a dramatic shift in the population with the consequence that nearly all adults died of old age. By defining these conditions, we establish a conceptual framework that explains why animals as different as mayflies and elephants die of old age in the wild. In conclusion, we created a powerful experimental platform to investigate the relationships between individual developmental processes, developmental plasticity, and population dynamics including extinction.

436A Carboxyesterases and intestinal granules in the biosynthesis of novel families of nematode small molecule signals

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Signaling molecules derived from attachment of diverse primary metabolic building blocks to ascarosides play a central role in the life history of *C. elegans* and other nematodes; however, the extent to which various inter- and intra-organismal signaling pathways are controlled by small molecules is unclear. Using comparative metabolomics, we show that a pathway mediating formation of intestinal lysosome-related organelles (LROs) is required for biosynthesis of a large library of small molecules, including most modular ascarosides as well as previously undescribed modular glucosides (1). Similar to modular ascarosides, the modular glucosides are derived from highly selective assembly of moieties from nucleoside, amino acid, neurotransmitter, and lipid metabolism. We further show that a family of carboxylesterases (CEST›s) that localize to intestinal organelles are required for the assembly of both modular ascarosides and glucosides, suggesting that modular glucosides,

like the ascarosides, serve signaling functions. For example, biosynthesis of some modular glucosides is starkly upregulated in long-lived *daf-2* mutants, suggesting that insulin signaling is modulated by small molecules produced in a lysosome-dependent manner. Parallel studies in *C. briggsae* and other nematode species indicate that assembly of modular metabolites via CEST enzymes is widely conserved in nematodes. Further exploration of LRO function and *cest* homologs in *C. elegans* and other animals may reveal additional new compound families and signaling paradigms.

(1) H. H. Le, C. J. J. Wrobel, S. M. Cohen, J. Yu, H. Park, M. J. Helf, B. J. Curtis, P. R. Rodrigues, P. W. Sternberg, F. C. Schroeder. Modular metabolite assembly in C. elegans depends on carboxylesterases and formation of lysosome-related organelles. eLife, 9, e61886, **2020**

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437B Dissecting the genetic architecture underlying mouth dimorphism in *Pristionchus pacificus* identifies *cis* -regulatory variation in a supergene locus.

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Developmental (phenotypic) plasticity describes the property of a genotype to respond to environmental variation by producing distinct phenotypes. In *Pristionchus pacificus,* the mouth form is developmentally plastic, resulting in two alternative mouth forms: the *eurystomatous* (Eu) predatory form has two strong teeth, whereas the alternative *stenostomatous* (St) form has a single tooth and is bacteriovorus. The switch between the two forms is environmentally sensitive, and a previous forward genetic approach showed a key switch function for the sulfatase-coding gene *eud-1*, mutations in which result in all-St worms. In this study we used *P. pacificus* natural isolates with different Eu/St ratios to generate Recombinant Inbred Lines (RILs), and performed Quantitative Trait Locus (QTL) analysis to dissect the genetic architecture underlying mouth dimorphism in *P. pacificus*. Our result showed the involvement of one major locus on the X chromosome, spanning a recently described multi-gene locus containing *eud-1,* its paralog, and two more genes encoding α-N-acetylglucosaminidases (*nag*), all of which were shown to be involved in mouth form regulation. RNA-seq analysis of parental strains revealed 40% higher expression of *eud-1* in the high Eu parental strain, and CRISPR-Cas9 mutants of the two sulftases paralogous in the high Eu parental strain showed a complete switch to the St form. With the absence of non-synonymous substitutions in *eud-1* between the parental lines, we used CRISPR-Cas9 technology to perform variant swapping in the *eud-1* regulatory region to define potential causative SNPs behind the mouth-form dimorphism. Our experimental analysis identified variations in different *cis*-regulatory components of *eud-1*. Copy number differences in a potential Forkhead transcription factor binding site within the promoter/enhancer region, besides a SNP in the *eud-1* first intron between the parental lines caused differences in mouth-form ratios phenotype. Mutant lines showed an additive effect of these *cis*-regulatory elements, with a systematic change in the mouth-form phenotype and downregulation of *eud-1* expression. Currently, we are using CRISPR-Cas9 technology to examine the potential involvement of various Forkhead genes in controlling *eud-1* expression, while also expanding our analysis to test variations in the causative region within 30 more *P*. *pacificus* natural isolates.

438C Translation of *fem-3* is regulated somatically to prevent abnormal TRA-1 activation

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Because the maternal germline produces oocytes, translational regulation plays a critical role in the control of messages that are stored for later use in embryogenesis. In *C. elegans,* the sex-determination gene *fem*-3 has been a key model for this process, since mutations in the 3'-UTR increase FEM-3 activity in the germ line, causing all cells to differentiate as sperm. These mutations were thought to define a germline-specific site for regulation by the two FBF proteins, which are members of the PUF family of translational regulators.

Recently, we identified a role for this translational regulation in the soma of *XX* animals. One of the strongest 3'-UTR mutations is *fem-3(q96*gf*)*. We studied its interactions with *tra-2(mx)* alleles, which affect the germ line by disrupting the physical interaction of TRA-2 with TRA-1. When we made double mutants between *C. elegans fem-3(q96)* and any of three different *tra-2(mx)* mutations, we observed significant masculinization of tail-tip retraction, spicule formation and ray formation.

Although *C. briggsae* lacks FBF proteins, the regulatory site in the *fem*-3 3'-UTR is conserved, and might be targeted by other members of the PUF family. Thus, we made orthologous mutations in *C. briggsae fem-3,* which had only a small effect on germ cells, by slightly increasing spermatogenesis in hermaphrodites. However, they caused masculinization of the tail when were

combined with our *C. briggsae tra-2(mx)* allele *v403.* Indeed, this double mutant combination has such a severe effect on *C. briggsae* that homozygous strains are difficult or impossible to maintain. We conclude that the regulation of *fem-3* translation is not limited to the germ line. Furthermore, this regulation has been conserved during *Caenorhabditis* evolution.

What somatic factors are controlled by FEM-3 and TRA-2? The simplest possibility is their known target TRA-1. Null mutations in *tra-1* cause the soma to become male. They probably act by eliminating TRA-1 repressor, which shuts off male genes. By contrast, we have some mutations, like *cbr-tra-1(v48),* which alter the C-terminus of TRA-1, a region that is cleaved off when the repressor is formed. Homozygous *tra-1(v48)* mutants make oocytes instead of sperm, but the *XX*animals make normal female bodies, and the *XO* animals make normal male bodies. Furthermore, gene dosage tests show that *v48* prevents full-length TRA-1 from activating target genes.

Surprisingly, TRA-1 activator mutations like *v48* suppress the *tra-2(mx); fem-3(gf)* masculinization in both *C. elegans* and *C. briggsae.* By contrast, they enhance the masculinization of *tra-2(null)* mutant tails. Thus, we suspect that important aspects of the complex regulation of TRA-1 by TRA-2 and FEM-3 are designed to prevent aberrant sex-determination from causing developmental disorders.

439A Sex-determination in the male/female species *C. nigoni*

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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. The sex-determination pathway controls spermatogenesis and oogenesis, so it must have been modified to create hermaphrodites. 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, along with recessive visible mutations to use as balancers. Genomic edits were made by injecting gravid C. nigoni females with Cas9 RNPs. C. nigoni tra-1(v481) masculinizes the somatic tissues of putative XX animals. Moreover, it appears to arrest gonad development and these Cni-tra-1(v481) XX animals are unable to sire progeny. TRA-1 is known to control gonad development in C. elegans, but phenotypes like ours have not been described. More recently, I generated the alleles Cni-tra-2(v498) and Cni-fem-3(v496), and balanced them with Cni-unc-104(v494) and Cni-unc-129(v495), respectively. More detailed analyses are ongoing; however, diminished brood sizes and reduced mating efficiency seen within isolate JU1422 have made the characterization of these mutants slow-going.

The sister species, C. briggsae and C. nigoni, can form fertile female hybrids. To determine what C. nigoni genes are responsible for the dominant feminization that has been observed in these hybrids, I will use my new sex-determination mutants to make heterozygous hybrids for each gene. Identifying which genes are responsible for repressing XX spermatogenesis in these animals could indicate which genes have key differences between these sister species, differences that might be critical for the origin of self-fertility. These comparative studies should lead to interesting hypotheses about which genes were critical for the evolution of self-fertility in C. briggsae. Using gene editing, we can swap any genes of interest between the two sister species. A definitive test would be to swap one of these genes into C. nigoni and observe its effect, it may be possible to drive self-fertility in a male/female species this way.

440B Significant differences in the sex determination pathways between *C. inopinata* and *C. elegans*

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In the nematode genus *Caenorhabditis*, most species are gonochoristic (male/female), and several hermaphrodite species (male/hermaphrodite), including *C. elegans*, are thought to have evolved independently from gonochoristic species. In this study, we compared the sex determination pathways between *C. elegans* (hermaphrodite species) and its closest species, *C. inopinata* (gonochoristic species), to elucidate the genomic changes responsible for the differences in their reproductive modes.

Based on the comparison of genome sequences, we found three significant differences in the sex determination pathway genes in *C. inopinata*. 1) *xol-1*, which is on Chr. X and responsible for the male sex determination in *C. elegans*, is duplicated and localize on Chr. I and Chr. X, but the latter is disrupted by transposon insertion. 2) A transposon insertion disrupts the coding region of *her-1*, which is essential for the male development in *C. elegans*. 3) *fog-2*, which is essential for temporal masculinization in *C. elegans* hermaphrodite germline, is absent in *C. inopinata*. Next, to investigate functional conservation of the sex determination genes, each of their expression in *C. inopinata* was inhibited by RNAi, and sex determination phenotypes were analyzed. Similar to *C. elegans*, RNAi-knockdown of *tra-2* and *tra-1* caused masculinization of *C. inopinata* female germline. However, knockdown of more upstream genes involved in both sex determination and dosage compensation (*sex-1, xol-1, sdc-2*) and genes involved in male sex determination (*her-1, fem-1*) did not show any detectable phenotypes, suggesting that these genes do not play significant roles for sex determination in *C. inopinata*.

These results suggest that the sex determination pathways in *C. elegans* and *C. inopinata* are surprisingly different from each other, and only the downstream part, including *tra-2* and *tra-1*, seems to be conserved; in *C. inopinata*, an alternative set of genes is likely to regulate the early male/female decision and dosage compensation.

441C Chromosome dynamics in sex determination of the parthenogenetic nematode *Strongyloides ratti*

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The genus *Strongyloides* is unique among nematodes in having alternate free-living and parasitic generations. The parasitic female (PF) inhabits the small intestine of its mammal host and produces clonal offspring by mitotic parthenogenesis. Eggs produced by PF are excreted with the feaces and develop into either infective larvae (iL3) directly or free-living males and females. The free-living adults mate and produce eggs which develop into iL3. It was reported that *Strongyloides ratti*, a rat parasite, has three chromosomes and one of them is a sex chromosome, which determines their sex by XX/XO system. However, it remains unclear how mitotic parthenogenetic PF produces XX and XO eggs though it is presumed that the male karyotype (XO) is generated by missegregation of the sex chromosome like the *C. elegans* male generation.

In this study we investigated details of the chromosome structure of each developmental stages of *S. ratti* using the single-worm sequencing technique and the multiplex colored FISH observation. In the single-worm analysis, we expected a half depth of mapping coverage in sex chromosome compared to autosomes in free-living males (XO). However, the sex-chromosome of the male exhibited ~1/3 depth of the autosomes. Furthermore PF and free-living female (XX) showed lower coverage depth of the sex-chromosome and the auto:sex ration varied from 1:0.8 to 1:0.5. Additionally, in germline depleted PF, sex-chromosome showed almost equal coverages as autosomes. These results indicate that chromosome manipulations occurs in the PF germline to produce free-living males and the *S. ratti* sex determination system is not a simple XX/XO. Multiplex colored FISH analyses, recently developed for *C. elegans* in-detail chromosome observation, provided visual clues to understanding the dynamics of *S. ratti* chromosomal structure and the puzzling sex-determination system of *Strongyloides*.

442A Comparative analysis of cellular dynamics of *C. inopinata* and *C. elegans* zygotes

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C. inopinata is the closest species to *C. elegans*, but these two species have various morphological and ecological differences. Notably, *C. inopinata* is nearly twice as long as *C. elegans*, while its embryonic size is ~20% longer. In this study, we compared the cellular dynamics of *C. inopinata* and *C. elegans* zygotes. To visualize chromosomes and cell membrane, *C. inopinata* strains that express GFP::histone and GFP::PH (Pleckstrin-Homology) domain were constructed by the microparticle bombardment and cellular behaviors in zygotes of the two strains were analyzed by DIC and fluorescent live imaging. We found differences between the two species in 1) the position of the polar bodies and pronuclei formation, 2) the position of pronuclear meeting and metaphase plate formation, and 3) the mitotic spindle oscillation. While polar bodies in *C. elegans* almost always form in the anterior end of the embryo, those in *C. inopinata* appeared at random positions. In *C. elegans* zygotes, the female and male pronuclei formed in the anterior and posterior end, respectively, migrate to the center to meet; in *C. inopinata* zygotes, the pronuclei appeared at random positions, and the position of pronuclear meeting and metaphase plate formation was more posterior than in *C. elegans*. While the mitotic spindle of most *Caenorhabditis* nematodes, including *C. elegans*, oscillates during anaphase, that in *C. inopinata* did not. The mitotic spindle in *C. inopinata* was formed at a skewed angle, then gradually

rotated to a horizontal position. Despite the differences in the location of the pronuclear meeting and metaphase plates, the relative position of the cleavage furrow was equivalent to each other. Thus, some microtubule-dependent aspects of cellular dynamics of *C. inopinata* zygotes were different from those in *C. elegans*.

443B NHR-1 and NHR-40 in *C. elegans* – an outgroup approach to the origin of a novel trait

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A longstanding prediction of evolutionary theory is that phenotypic plasticity, the ability of one genotype to produce different phenotypes based on environmental input during development, plays a significant role in the evolution of morphological novelty. Nematodes of the Diplogastridae family are characterized by the presence of such a novelty: they possess cuticular teeth at the base of their mouths. Additionally, these structures are also polyphenic; different environmental cues can induce development of either a predatory form with two large teeth or a bacterivorous morph with only one dorsal tooth. Previous work demonstrated that two nuclear receptors, *Ppa*-NHR-1 and *Ppa*-NHR-40, regulate the morphogenesis of these teeth in *Pristionchus pacificus*. Interestingly, nematodes outside of the Diplogastridae, like *Caenorhabditis elegans*, lack this morphological novelty and the associated phenotypic plasticity entirely. Instead, these worms are monomorphic with simple, triangular flaps at the base of their mouths. However, *Ppa-nhr-1* and *Ppa-nhr-40* are the only genes of the *P. pacificus* mouth-form regulatory network that retain 1:1 orthologs in *C. elegans*. Therefore, we investigate whether these transcription factors also have a conserved function in regulating the development of the *C. elegans* mouth.

Using the CRISPR/Cas9 system, we generated small indels in the coding sequences of *Cel-nhr-1* and *Cel-nhr-40* and isolated frameshift mutants to ensure a loss-of-function. Additionally, we aim to investigate the localization of both nuclear receptors by tagging them with fluorescent protein markers (e.g., *mNeonGreen*). In order to identify potential mutant phenotypes, we quantify differences in mouth shape between wildtype and CRISPR-edited strains by applying landmark-based geometric morphometrics in combination with *k*-medoid and model-based clustering.This data will be complemented with future RNA-seq experiments, which will identify the regulatory targets of Cel-NHR-1 and Cel-NHR-40. Taken together, this data will allow a comparison of the cellular localization, the regulatory targets, and the functional relevance of NHR-1 and NHR-40 for stoma development between *C. elegans* and *P. pacificus*. Ultimately, this study will reveal whether these nuclear receptors share a conserved function in stoma development that predates the evolution of the morphological novelty, or if a neo-functionalization accompanied its origin.

444C Exploring the mechanisms of MSS-mediated sperm competition in *C. briggsae*

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By impacting the relative reproductive success of otherwise fertile conspecifics, sperm competition plays a profound role in the evolutionary trajectory of entire species. Sperm competition has been observed in numerous organismal clades, yet our understanding of the molecular mechanisms that mediate it *in vivo* remains extremely limited. MSS (male secreted short) proteins are small sperm surface glycoproteins essential for successful competition in obligately outcrossing *Caenorhabditis*[2]. In all three known self-fertile species, however, these *mss* genes were lost. This parallel loss may be because male fertilization success both increases subsequent male frequency and depresses population growth relative to pure selfing[3]. While MSS proteins provide a competitive advantage to sperm that bear them, the molecular basis for this is currently unknown. MSS-decorated sperm may reach fertile oocytes faster and/or spatially exclude sperm lacking these proteins. Alternatively, MSS-containing sperm may possess an enhanced fertilization ability compared to wild-type sperm even without spatial stratification among sperm types. To test these hypotheses in real time, we mated feminized (*she-1*) XX *C. briggsae* with vital dye-stained males that either possessed or lacked a *mss(+)* transgene derived from *C. nigoni*[2]. After mating, the distributions of sperm in various sections of the hermaphroditic gonad (spermatheca, uterus, vulva) were quantified. While there were no significant differences between the number of *mss(+/-)* sperm in each broad gonad area, *mss(+)* sperm were more commonly observed proximal to the spermathecal valve regardless of mating order. More intriguingly, ectopic *mss(+)* sperm were frequently observed beyond the spermathecal valve (similar to[1]). This suggests that *mss(+)* sperm reach receptive oocytes more efficiently than *mss(-)* counterparts. Time-lapse imaging revealed that invasivity occurs during ovulation, when the valve is briefly open. Ectopic sperm cannot fertilize pre-ovulatory oocytes, but may be in position to be the first to do so upon ovulation. To clarify the structural determinants of MSS action, we used microparticle bombardment to overexpress two endogenous MSS-related proteins (MSRPs) in *C. briggsae, msrp-3* and *msrp-8*. Initial phenotyping indicates that they do confer competitive advantages to the sperm bearing them. Important control experiments are in progress, but these initial results suggest that

the *mss* genes have properties (regulatory or coding) that distinguish them from related paralogs, and that are responsible for competitiveness.

[1]Ting *et al.* (2014) *PLoS Biology* 12: e1001915

[2]Yin *et al.* (2018). *Science* 359: 55-61

[3]Yin and Haag (2019). *PNAS* 116: 12919-12924

445A Widespread changes in gene expression accompany body size evolution in nematodes

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Body size is a fundamental trait that drives multiple evolutionary and ecological patterns. *Caenorhabditis inopinata* is a fig-associated nematode that is exceptionally large relative to other members of the genus, including *C. elegans*. We previously showed that *C. inopinata* is large primarily due to postembryonic cell size expansion that occurs during the larval-to-adult transition. Here, we describe gene expression patterns in *C. elegans* and *C. inopinata* throughout this developmental period to understand the transcriptional basis of body size change. We performed RNAseq in both species across the L3, L4, and adult stages. Most genes are differentially expressed across all developmental stages, consistent with *C. inopinata*'s divergent ecology and morphology. We also used a model comparison approach to identify orthologs with divergent dynamics across this developmental period between the two species. Notably, among such genes were two transcription factors previously shown in *C. elegans* to be important for body size that are regulated by the TGF-β signaling pathway. Multiple hypodermal collagens were also observed to harbor divergent developmental dynamics across this period. *C. elegans*-specific ontology enrichment reveals genes with divergent developmental dynamics tend to be expressed in neurons and regulate behavior; they also include genes important for molting and body morphology. A comparison of such genes with previous *C. elegans* experiments reveals overlap with stress response, developmental timing, and small RNA/chromatin regulation. These results have identified candidate genes that will be further investigated to test their roles in cell size divergence and broaden our understanding of the genetic bases of body size evolution.

446B Regulatory differences in wild *C. elegans* strains from investigation of allele-specific expression

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Across individuals and species, genetic variation leads to variation in gene expression, resulting in further phenotypic variation. One way to understand how genotype regulates gene expression variation is to look within hybrid or heterozygous individuals: any differences in expression from the two alleles within an individual must be caused by *cis* acting variants located on the same chromosome as the gene of interest, because the global (or *trans*) environment is shared across the alleles.

We have applied such allele-specific expression (ASE) analyses to uncover *cis* regulated gene expression changes between wild strains of *C. elegans* and the reference strain N2. Specifically, we performed RNA sequencing on three biological replicates from each of the strains N2, CB4856, EG4348, JU1088, and QX1211, and from F1 crosses between N2 and the other four strains. We performed RNA alignment and quantification using multiple methods to identify optimal ASE analytical frameworks for this species. For example, we mapped RNA reads to the genome and transcriptome; aligned as well as pseudo-aligned reads; and used both the reference genome alone and pseudo-diploid genomes that incorporated the variants from other strains. We further tried multiple methods for gene-level quantification and ASE analysis. Tools and methods investigated included STAR, WASP, Salmon, bowtie2, and EMASE.

A preferred analysis identified 2535 genes with ASE – and thus *cis* regulatory differences – across the strains, with strains with higher nucleotide divergence from N2 having more genes affected by *cis* regulatory changes, as expected. For example, less-diverged JU1088 showed evidence for differential *cis* regulation at 255 genes, while more-diverged CB4856 showed evidence at 702 genes. The vast majority of genes with differential *cis* regulation from N2 showed this differential regulation in only one strain (n = 2263), while 52 genes showed differential regulation vs. N2 in three or all strains.

Comparison of ASE differences to expression differences between the parental strains enables characterization of regulatory divergence as in *cis* vs. in *trans* across *C. elegans.* The evolutionary forces underpinning the observed regulatory divergence may be elucidated via associations of regulatory patterns with genome and population genetic parameters. Such characterizations and analyses will be useful in comparing *C. elegans* regulatory evolution with that of other species and of regulatory evolution across *Caenorhabditis* species.

447C Genetic architecture of alcohol sensitivity in *C. elegans*

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Developing medical treatments for alcohol use disorders (AUD) may benefit from identifying molecules that underlie behavioral responses to alcohol. Forward genetics and candidate gene screens using *C. elegans* have identified molecules required for behavioral responses to alcohol, including the BK potassium channel, which is a direct target of alcohol, as well as the CRF and SWI/SNF pathways, which modify alcohol responses. These and other molecules identified in *C. elegans* appear to work in conserved ways in mammalian models of AUD and humans.

Despite their success, genetic screens likely miss other genes of large effect if, for example, those genes are essential for development, functionally redundant, or are rare. By leveraging natural phenotypic and genetic variation in 200 wild isolates from across the globe, rather than mutagenesis using the lab strain N2, we uncovered new conserved genes that contribute to alcohol sensitivity in *C. elegans*. Our genome-wide association study revealed four significant quantitative trait loci (QTLs), three of which have never been implicated in alcohol response in nematodes or mammals, as well as the BK channel gene, *slo-1*, which validated our approach. Candidate QTL genes included an FBOXA gene located within an expanded cluster of other FBOX genes, an essential nuclear transporter gene, and a rare isoform-specific allele of protein kinase D (*dkf-2*). Additionally, we found that deletion of *dkf-2* in an N2 background conferred high resistance to intoxication. One extremely resistant wild isolate, JU830, which harbors all resistant genotypes at the QTLs, was six standard deviations above mean resistance. In contrast to the small indel and SNP variation associated with the four QTL in other strains, closer inspection of the BK channel *slo-1* locus in JU830 identified large indels in a number of its introns as well as its 5'UTR. Our results suggest that *C. elegans* underwent selection for alcohol sensitivity and resistance in nature. The three novel genes identified here, especially the protein kinase D, might represent druggable targets for novel treatments for alcohol addiction and abuse.

448A Utilizing Quantitative Molecular Techniques to Capture Expression Level Differences in *C. elegans* Wild Isolates

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In the *C. elegans* embryo, a network of interacting proteins are responsible for establishing polarity along the anterior-posterior axis and coordinating the first few cell divisions. This process is very well studied and is used as a model for cell polarity in metazoans. Morphologically, polarity establishment is highly stereotyped and near-invariant across *Caenorhabditis* and within *C. elegans*. However, the underlying molecular and genetic pathways show variability, even at the intraspecific level. In this study, we have developed a method to capture expression level differences of proteins involved in polarity establishment across wild isolates of *C. elegans*. Using microfluidics and single molecule fluorescent in situ hybridization (smFISH), we are able to achieve excellent spatial and temporal resolution for quantification of expression levels in different wild isolates. This ultimately allows us to compare subtle differences in the molecular pathways involved in polarity establishment and maintenance across different strains of *C. elegans*.

449B Natural variations in reproductive aging phenotypes reveal the importance of early reproductive period in *Caenorhabditis elegans*

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Although reproductive capacity is a major factor for individual fitness, aging of the reproductive system precedes somatic aging, which may reduce total brood size. Genetic bases and evolutionary theory of this phenomenon have been addressed in *Caenorhabditis elegans*, but these studies lack current knowledge about the natural history of *C. elegans*. To enhance our understanding of reproductive aging in *C. elegans*, we measured the number of progeny and the X-chromosome nondisjunction rate of 96 wild strains during early, late, and total reproductive periods. We found that these traits exhibit phenotypic natural variations and few outliers, and that brood size and X-chromosome nondisjunction rate were not genetically correlated. In addition, although a previous theory predicted that reproductive aging would contribute to generating an optimal total number of offspring, total brood size might not be converged to an optimal value, and early brood size was more constant among wild strains than total brood size. Thus, we speculated that reproductive aging seemed to be a byproduct produced through achieving a rapid increase of the initial population size, which might be related to the boom-and-

bust lifestyle of *C. elegans*. We also identified significantly associated loci and candidate genetic variants for X-chromosome nondisjunction rate in late and total reproductive periods. Our results may provide an insight into reproductive aging in *C. elegans* wild strains.

450C The evolution of developmental genetic biases explains the evolution of evolutionary trends

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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 trends 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 vulval precursor cells (VPC) in two clades of nematodes that have divergent evolutionary trajectories of cell fate variation. In *Caenorhabditis* species, among the six VPCs termed P3.p to P8.p, only P3.p cell fate shows significant evolutionary variation within and among species. In contrast, in *Oscheius* species (same family as *Caenorhabditis*) evolutionary variation of cell fates is highest in different precursor cells: P4.p and P8.p.

We have 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. We compared this mutational variance with natural genetic variation within and across species of both genera. Our phenotypic analysis of vulva cell fates on over 85,000 nematodes shows, within each species and genus, a strong alignment of the axes of variation upon random mutation with those of wild variation. 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.

This demonstrates a differential overall sensitivity of VPC fates in *Caenorhabditis* versus *Oscheius*, which can explain the evolution of evolutionary rates.

451A Biochemical and structural characterization of a tRNA-synthetase-based selfish element in *C. tropicalis*

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Selfish genetic elements have evolved manifold ways to persist in genomes and populations without offering any apparent benefit to the host. In a particularly radical example, toxin-antidote elements (TAs) secure their position in host populations by selectively removing progeny that do not inherit a copy of the respective underlying gene pair. Numerous TAs have been identified and mechanistically dissected in bacteria. However, only few examples have been reported in eukaryotes. Importantly, the underlying molecular mechanisms of eukaryotic TAs remain completely unresolved to date, hindering our understanding of this ongoing arms-race between the host genome and parasitic elements.

Initially discovered in *C. elegans*, we recently greatly expanded the repertoire of known animal TAs with the discovery of multiple TAs in a cross between two isolates of the nematode *Caenorhabditis tropicalis* (Ben-David, Pliota et al., 2021). Here, we focus on a single TA from this cross, aiming to uncover its mechanism of action (for genetic and in vivo characterization see poster by Tikanova et al.). We identified and validated the novel toxin and antidote genes, which we named *klmt-1* (**K**iller of **L**1 and embryos **M**aternal **T**oxin) and *kss-1* (**K**lmt-rescue by zygotically-expre**SS**

ed antidote), respectively. We found that KLMT-1 shares 52% sequence identity with the beta subunit of the phenylalanyl-tRNA-synthetase (PheRS) and both N and C-terminal ends are predicted to be intrinsically disordered. We hypothesized that

KLMT-1 kills worms by competing with the beta subunit in the heterotetrameric PheRS complex, thereby interfering with amino acid charging. In line with this hypothesis, we found that KLMT-1 is capable of binding the alpha subunit (FARS-1) of the PheRS in pulldown experiments and that FARS-1/KLMT-1 form an oligomeric complex *in vitro*. Furthermore, using a metabolic labelling approach, we quantified the activity of the *C. tropicalis* PheRS in bacteria and found that the presence of KLMT-1 strongly reduces the ability of the enzyme complex to charge its tRNA. Intriguingly, removal of the disordered termini of KLMT-1 further increased its inhibitory effect, indicating potential ways of spatial or temporal regulation of TA activity. Our findings serve as the basis to formulate a model for KLMT-1 toxicity, thereby for the first time providing mechanistic insight into a TA from animals and will be further substantiated by structural investigations of the FARS-1/KLMT-1 complex.

452B *C. elegans* has lost a regulatory motif that represses *fog-3* transcription in *C. briggase*

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In *C. elegans*, FOG-1 and FOG-3 directly promote spermatogenesis, and the activity of each gene is regulated by the TRA-1 transcription factor. as we reported earlier, the *C.briggase fog-3* promoter also has several TRA-1 binding sites and can work in *C. elegans*. To begin studying its native functuion, we made *cbr-fog-3(v501)*, a frameshift mutation that creates a recessive null allele. It feminizes the germline in *XX* and *XO* animals but does not affect the soma. These phenotypes are just like those seen in *C.elegans*.

Our phylogenetic analysis revealed that *C. briggase fog-3* promoter contains a second regulatory element, that is the unique 26 bp sequence is located near the first TRA-1 binding site, and is conserved in most *Caenorhabditis* species, but is not found in *C.elegans*. to study this site, we made the *cbr-fog-3(v506)* mutation, which scrambles the 26 bp conserved element. Surprisingly, *fog-3(v506)* causes dominant feminization of germ cells in both *XX* and *XO* animals. This phenotype is not suppressed by mutations in *tra-2* or *tra-1*, and we can only maitian the strain by mating with wildtype males.

To learn how this 26 bp element works, we used single worm RT-PCR to detect *fog-3* transcript levels in young *XX* animals. Our prelimary results suggested that *v506* mutants produce higher levels of *fog-3* transcripts than the wild type. this surprising result indicated that too much FOG-3 might disrupt function of the FOG-3/FOG-1 complex recently described by the Kimble lab.

To test this model, we greated a cbr-fog-3(v501v506) double mutant. The addition of the *v501* frameshift mutation makes this double mutant recessive. we infer that *v506* normally causes a dominant Fog phenotype through the production of too much FOG-3 protein. Thus, all our data indicate the 26 bp element mediates repression of *fog-3*, in addition to nearby TRA-1 binding sites. Furthermore, the loss of repression results in too much FOG-3, which might disrupt FOG-3/FOG-1 complex formation and function. The *C. elegans fog-3* promoter might compensate for the lack of this site by the presence of extra TRA-1 binding sites.

453C Genetic background-dependent expression of *clec-62* in *Caenorhabditis elegans*

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The pursuit of genes underlying quantitative traits is often accompanied by creating introgression lines (ILs). ILs consist of a single genetic locus introgressed in an otherwise homogeneous background. We previously generated a genome-covering IL panel by introgression of segments of the strain N2 in a CB4856 genetic background. This population complements a previously created IL panel with CB4856 segments in an N2 genetic background. Combining both panels allow the study of the effect of the genetic background at a higher resolution than previously possible with chromosome substitution strains.

We exposed 150 strains of a N2xCB4856 recombinant inbred line population and the two IL panels to a normal growth regime (20°C on standard NGM plates). After 48 hours of growth, we collected the animals, flash froze them and proceeded to isolate RNA. Via qPCR, we measured the expression of *clec-62*, finding that only in the N2-background population QTL were detected. In other words, no QTL were detected in the RIL and reciprocal IL population. Thereby we show the strong role of the genetic background on trait variation.

Here we present a case of either closely-linked loci that were only segregated in one population, or an epistatic interaction depending on the genetic background. We are currently in the process of building a double introgression panel to experimentally test the effect of two combined backgrounds.

454A Evolution of selfishness from a core tRNA synthetase in *C. tropicalis*

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Selfish genetic elements maximize their own transmission despite being neutral or even harmful to their host. One of the most extreme examples of this behavior are toxin-antidote elements (TAs). TAs comprise two linked genes: one encodes a toxin and the other its cognate antidote. The toxin is expressed in the parental germline, and the antidote is expressed zygotically. In crosses between individuals that carry the TA and ones that do not, the toxin is delivered to all of the progeny by the egg or sperm, but only embryos that inherit at least one copy of the TA are able to express the antidote and therefore survive.

We recently identified numerous TAs in *C. elegans*, *C. tropicalis*, and *C. briggsae*. However, the molecular underpinnings of all known eukaryotic TAs are still largely unknown. A critical barrier remains dissecting the molecular mechanisms of TA elements, which is very challenging given their lack of homology to well-studied proteins. Here we report the fine mapping and *in vivo* characterization of a *C. tropicalis* TA that evolved from a core essential gene: *klmt-1/kss-1*. The toxin, *klmt-1* (Killer of L1 and embryos Maternal Toxin), evolved via gene duplication from *fars-3*, the beta subunit of phenylalanyl-tRNA synthetase (PheRS), which is responsible for charging $tRNA^{Phe}$. We hypothesize that KLMT-1 can bind FARS-1 and thus interfere with $tRNA^{Phe}$ charging and translation. We found that KLMT-1 can bind FARS-1, the alpha subunit of the PheRS complex *in vitro* (see poster by Ross et al.). To test it *in vivo*, we are performing Co-IP experiments in endogenously tagged strains. Interestingly, KLMT-1 levels decrease rapidly during development and are hardly detectable by the time of hatching, suggesting that the toxin is actively being degraded. We hypothesize the degradation of the toxin is driven by its cognate antidote. The antidote KSS-1 (Klmt-rescue by zygotically-expreSSed antidote) has a predicted F-box domain on its N-terminus. F-box proteins act as adaptors between target proteins and the SCF complex, which mediates proteasome-mediated degradation. In support of this model, we found that KSS-1 can bind several SKP-1 orthologs of *C. tropicalis*. Furthermore, *in vivo* studies revealed that loss of *kss-1* drastically increases the half-life of the toxin. Unlike other eukaryotic TAs, *klmt-1/kss-1* originated from a core essential protein. Understanding the molecular mechanism of this TA will provide key insights into the inception of selfish genes.

455B Why do some wild-type strains fail at germline RNAi?

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The discovery that experimental delivery of dsRNA can induce gene silencing at target genes revolutionized genetics research, both in uncovering essential biological processes and in creating new tools for developmental geneticists. Our ability to silence *C. elegans* genes by RNA interference (RNAi) arises from the fact that worms induce a native cascade response upon exposure to exogenous dsRNA, a response that we now know is associated with antiviral immunity but also coincides with a suite of processes affecting things like gene regulation and genome defense against transposons. However, previous work has shown that wild-type strains of *C. elegans* vary dramatically in their response to exogenous RNAi. Here, we investigate why some strains fail to mount a robust RNAi response to germline targets. We observe diversity in mechanism: in some strains, the response is stochastic, absent in most individuals but rapidly turned on in a few; in other strains, the response is consistent but much delayed. Increased activity of the argonaute PPW-1, which is required for germline RNAi in N2, partially rescues the response in some strains, but dampens it further in others. Across strains, we also observe variability in gene expression of known RNAi factors, and strain-specific instances of pseudogenization, gene loss, and allelic divergence. Our results support the conclusions that secondary argonautes share overlapping functions, that a “just right” level of overall activity promotes a robust response, and that the weak germline RNAi we observe in some strains is explained by diverse genetic variants at shared RNAi genes.

456C Compensatory evolution in mitochondrial tRNAs

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Deleterious mutations can accumulate in natural populations, and reversal mutations are likely to be rare. However, new mutations at other sites can potentially compensate for prior deleterious genetic changes, reversing fitness. In the same vein, mutations that are neutral at the time of substitution may effectively “pre-compensate” future mutations that would otherwise be deleterious, allowing mutations to accumulate that are deleterious under some conditions. Identifying such

compensatory or conditional mutations is difficult, however, without an *a priori* expectation of sequence function. Here we use the canonical structure-function relationship of the cloverleaf-like tRNA secondary structure, as well as the high mutation rate of tRNAs, to study compensatory evolution. Specifically, we are exploring mutations in mitochondrial tRNAs (mt-tRNAs) in *Caenorhabditis* nematodes, including interactions between mt-tRNAs and associated factors encoded in the nuclear genome.

457A Dose-response relationships reveal complex patterns of natural variation in susceptibility to diverse toxicants

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Genetic differences among individuals can modify the influences of environmental exposures that induce both acute and chronic disease. Toxic exposure is a known risk factor in the onset of many human diseases, but the contributions and abundance of specific alleles to xenobiotic-induced disease risk at the population-level are virtually unknown. Because of the limited power and scale of toxicological assessments across genetically diverse human subjects, a tractable model system is required to quantify gene-by-environment interactions in a controlled manner. Toxicological assessments using *C. elegans* have revealed previously unknown and translational features of xenobiotic metabolism, but investigations of natural variation in these responses are extremely limited. To understand population-wide differences in xenobiotic responses, we measured the susceptibility of genetically diverse *C. elegans* wild strains to an array of toxicants, including several heavy metals, mitochondrial poisons, organophosphate insecticides, fungicides, herbicides, and one flame retardant. We measured phenotypic responses to each compound by adapting a high-throughput fitness assay using the Molecular Devices ImageXpress Nano automated imaging microscope and developed open-source software to extract and analyze animal morphology measurements from images. Using this platform, we exposed eight *C. elegans* wild strains to increasingly concentrated doses of 24 diverse toxicants and estimated dose-response curves for each of these toxicant-strain combinations as a function of animal length. Wild strains varied significantly in susceptibility to most compounds, with some strains exhibiting generalized resistance or susceptibility to many compounds. This result suggests that certain genetic backgrounds might retain pleiotropic xenobiotic response alleles that attenuate the metabolism of diverse xenobiotics. Between 20-80% of the variance in susceptibility to at least one dose of each toxicant could be explained by genetic differences among strains, confirming that natural genetic variation among wild strains plays a significant role in population-wide toxicant susceptibility. To resolve the number of xenobiotic response alleles and their effects at a population-level, we plan to extend this phenotyping platform to hundreds of wild strains and perform genome-wide association mappings.

458B Evaluating the power and limitations of *C. elegans* genome-wide association mappings

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A central goal of evolutionary genetics is to understand the genetic basis of traits that contribute to adaptation and fitness. Genome-wide association (GWA) mappings scan the genome searching for individual genetic variants with significant correlations with phenotypic variation in a population, or quantitative trait loci (QTL). GWA mappings are a popular choice for quantitative genetic analyses because the QTL that are discovered segregate in natural populations and, therefore, contribute to complex trait variation in the wild. Our research group established and currently curates the *C. elegans* Natural Diversity Resource (CeNDR), which captures genetic diversity worldwide. We have leveraged this genetically diverse strain collection to dissect the genetic underpinnings of a broad suite of traits. Experimental choices, such as the size and composition of the mapping population, as well as the abundance, effect size, and population frequency of causal alleles determine how accurately GWA mappings reflect the underlying genetic basis of complex traits. To date, we lack an empirical evaluation of power and precision to robustly identify QTL using GWA mappings. We developed an open-source GWA mapping pipeline (NemaScan) and used a simulation-based approach to provide benchmarks of mapping performance among wild *C. elegans* strains. We selected markers to be QTL ranging in abundance, effect size, and genomic location, simulated phenotype distributions, and then conducted GWA mappings. Only a fraction of trait heritability was explained by detected QTL on average, confirming that GWA is most effective for identifying large-effect alleles. Detection power was highly sensitive to both the number and genetic composition of strains in the mapping population, clarifying the importance of strain selection prior to undertaking GWA analysis. Artefacts of QTL detection occurred within small populations when the phenotype perfectly segregated among strains with highly differentiated haplotypes. The unique evolutionary history of *C. elegans* has generated complex patterns of genetic variation among many strains. Our simulation-based evaluation of GWA performance provides the community with guidance for experimental choices that can maximize the potential for QTL discovery.

459C Natural variation in the aldehyde oxidase, *gad-3,* confers oxidative stress resistance between *C.elegans* strains

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In this study we sought to identify allelic variants responsible for mediating paraquat (PQ) resistance between *C.elegans* strains N2 and MY16. We observed that MY16 is two to three-fold more resistant then N2 to oxidative stress from PQ exposure. To identify causative alleles, we developed an experimental evolution approach which relies on multiple rounds of strong selection on standing genetic variation. Genetic variation is created by making strains obligate outcrossing (by introducing a *fog-2* mutation) and forcing random mating for twelve generations to create starting pools (n=3). A similar approach was taken by Burga et al. 2019. In our method, which we call Evolutionary Segregant analysis (ESA), we start with a pool of 30k worms from the F12 generation. We performed a strong selection by recovering only ~ 0.3% of the starting population resistant to PQ. This protocol was performed for five rounds with appropriate controls. All samples were sequenced using RAD-seq. The mapping data pointed to a ~150kb region on LGV. To identify causative alleles, we focused on candidate genes with a role in oxidative stress. From this group, *gad-3* seemed like a solid candidate. *Gad-3* (B0222.9) is an evolutionarily conserved aldehyde oxidase downstream of the sirtuin (Sir-2.1) pathway and has a role in oxidative stress response and longevity (Park et al., 2009). GAD-3 dependent production of hydrogen peroxide is responsible for longevity increases from sirtuin (*sir-2.1*) pathway over-expression (Schmeisser et al., 2013). GAD-3 protein differs by three mutations between strains. We focused our efforts on testing a mutation in a widely conserved Iron-sulfur cluster domain of GAD-3. Using CRISPR, we performed congenic allele swaps for both MY16 and N2 strains. In both cases, the MY16 allele conferred increased resistance to PQ exposure. In addition, we found that hydrogen peroxide production is reduced with the *gad-3* N2 allele in an MY16 background. Ongoing experiments are testing the reverse allele swap. Our results suggest that GAD-3 dependent production of hydrogen peroxide is mainly responsible for oxidative stress resistance between these natural isolates.

460A Natural variation in differential *C. elegans* responses to the broad-range anthelmintic emodepside

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Emodepside is an anthelmintic drug effective against parasitic nematodes, including gastrointestinal and filarial nematodes. Resistance to emodepside has not yet been reported, and nematodes that are resistant to other anthelmintic drug classes are susceptible to emodepside. The study of emodepside mode of action and potential mechanisms of resistance in parasitic nematodes is challenging because of their high levels of genetic variation, the inability to culture animals in the laboratory, and a limited genetic toolbox. *Caenorhabditis elegans* is an ideal model organism to study phenotypic responses to emodepside because of its tractable life cycle, small and well characterized genome, and the availability of genome-editing tools. The *C. elegans* laboratory strain N2 is sensitive to emodepside. In the presence of the drug, N2 animals have reduced movement, pharyngeal pumping, and egg-laying, and are developmentally delayed. Mutagenesis studies have implicated a calcium-activated potassium channel, SLO-1, in emodepside mode of action.

Responses to emodepside differ across wild isolates of *C. elegans*. If these differences are heritable, we can use *C. elegans* to discover natural emodepside resistance mechanisms that could be shared with parasitic nematodes. Here, we determined if natural genetic variation across the natural population can explain differences in emodepside responses. In a genome-wide association study, we measured the phenotypic response to emodepside for 154 wild isolates. We found that several genomic regions on chromosome V are associated with differences in emodepside responses. Candidate genes in these regions include UGT enzymes and *slo-1*. Variation in the genomic region that includes *slo-1* is associated with increased susceptibility to emodepside. Currently, we are testing the roles of the candidate genes in emodepside responses. Our results show that other genes in addition to *slo-1* affect the response to emodepside and that wild strains vary in the function of *slo-1*.

461B Genome-wide association study for nictation behavior of the nematode *C. elegans*

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Dispersal is crucial for many organisms. There are various strategies for efficient dispersal, and phoresy is one example. Animals can disperse efficiently by attaching to another organism, like hitchhiking. *Caenorhabditis elegans* dauers show a stage-specific standing behavior, called nictation, that can facilitate phoretic interactions of dauers. Standing and waving their

body, dauers can interact with other organisms such as isopods. It helps dauers to escape from harsh conditions and move into a better environment for re-growing and reproduction. As *C. elegans* are found most frequently as dauer larvae in the wild, nictation is thought to play an important role in their life cycle. However, the genetic basis and the underlying regulatory mechanism of nictation are not well understood. Here, we try to figure out the genetic factors that regulate nictation and make nictation diversity between *C. elegans* wild isolates. Wild isolates from the worldwide region showed diverse nictation fractions in the same experimental conditions. A genome-wide association mapping of the nictation of 137 wild isolates identified a quantitative trait locus (QTL) for nictation. Using near-isogenic lines, we identified a QTL of 90 kb interval, and also found that this QTL affects other dauer-related phenotypes. Now we are testing and confirming candidate genes using CRISPR mutants and RNAi experiments. Elucidating nictation regulatory mechanisms will provide new insights into the genetic basis of phoretic behavior.

462C The genetic architectures of gene expression variation in wild *C. elegans*

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Natural variation in gene expression is a major source of phenotypic diversity among individuals. Genetic variants underlying gene expression differences are known as expression quantitative trait loci (eQTL). In *Caenorhabditis elegans,* both local eQTL (located close to the genes they influence) and distant eQTL (located farther away from the genes they influence) have been identified using linkage analysis from a panel of recombinant inbred advanced intercross lines (RIAILs) between the laboratory reference strain, N2, and a wild strain, CB4856. However, the eQTL detected using RIAILs were limited to genetic variants between N2 and CB4856. Here, we investigated the natural variation in gene expression of 205 genetically distinct *C. elegans* wild strains by performing RNA-sequencing on synchronized young adult hermaphrodites. We obtained reliable expression of 25,896 protein-coding transcripts (16,106 genes). We used genome-wide association (GWA) analysis to identify 3,342 local eQTL for 3,342 transcripts (2,777 genes), and 2,835 distant eQTL for 2,206 transcripts (2,082 genes). We found that most of the narrow-sense heritability for transcript expression variation is explained by detected eQTL. Of the 2,835 distant eQTL, 1,670 eQTL significantly clustered in 54 hotspots across the *C. elegans* genome. We will further explore causal genes underlying these distant eQTL hotspots and their functions. Additionally, we applied mediation analysis to the eQTL data and other organism-level quantitative traits to elucidate the genetic effects on phenotypic variation mediated by gene expression. For instance, instead of relying on time-consuming investigations of rare genetic variants missed in GWA studies and prior knowledge of the trait as published in our study of *C. elegans* responses to benzimidazoles (Hahnel et al. 2018), the significant mediating effect of the expression of ben-1, the causal gene, was quickly and successfully identified by mediation analysis. Our results suggest that mediation analysis using expression data facilitates identification of causal genes in GWA studies in *C. elegans*.

463A Natural variation in fertility is correlated with species-wide levels of divergence in *Caenorhabditis elegans*

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Life history traits underlie the fitness of organisms and are under strong natural selection. A new mutation that positively impacts a life history trait will likely increase in frequency and become fixed in a population (e.g. selective sweep). The identification of beneficial alleles that underlie selective sweeps provides insights into the process of natural selection that occurred during the evolution of a species. In the global population of *Caenorhabditis elegans*, we previously identified selective sweeps that have drastically reduced chromosomal-scale genetic diversity in the species. Here, we measured the lifetime fertility of 121 wild strains, including many recently isolated divergent strains from the Hawaiian islands, in standard laboratory conditions. We found that strains with larger swept genomic regions on multiple chromosomes have significantly higher fertility than strains that do not have evidence of the recent selective sweeps. We used genome-wide association (GWA) mapping to identify three quantitative trait loci (QTL) underlying fertility variation. The haplotype structure in each QTL region revealed correlations with recent selective sweeps in the *C. elegans* population. Moreover, North American and European strains showed significantly higher fertility than most strains from Hawaii, a hypothesized origin of the *C. elegans* species. These results suggest that beneficial alleles that cause increased fertility are associated with selective sweeps during the worldwide expansion of *C. elegans*. Additionally, we mapped previously collected fertility data (fertility of the first four days in the adult stage) of wild *C. elegans* strains and *C. elegans* recombinant inbred advanced intercross lines that were grown in various conditions and detected eight QTL across the genome using GWA and linkage mappings. Altogether, these 11 QTL show the genetic complexity of fertility across this species.

464B Isolation, Characterization, and Antibiotic Resistance Profile of Staphylococci from the Indoor Air of the Students' Halls of Residence at the Obafemi Awolowo University, Ile Ife, Nigeria

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It has become a public health concern to evaluate the safety of the only readily consumable and unfiltered part of the biosphere, the air, most especially from the densely populated region like the students' hostel. This study was conducted to investigate the colony-forming unit per unit volume of staphylococci in the indoor air of students' halls of residence, the biochemical characteristics, and antibiotic resistance profile of the isolates. Using the open-plate method to collect samples from the indoor air as described by Omeliansky, identification of the isolates was based on standard methods such as colonial morphology, Gram stain reaction, catalase, and deoxyribonuclease tests. The antibiotic susceptibility profile was on the standard disk diffusion method using commercially available antibiotic discs (Abtek, Habdiscs) including ciprofloxacin 5 µg/mL, gentamicin 10 µg/mL, and tetracycline 30 µg/mL. A total of 26 staphylococci isolates were obtained from which none (0%), 4 (15%), and 10 (37%) of the isolates were resistant to ciprofloxacin, gentamicin, and tetracycline, respectively with no intermediate. Using a mathematical model, it is deduced that the year of release of the antibiotics has a non-linear correlation with the recorded rate of resistance of the staphylococci to the antibiotics, which predicts an efficacy duration window of 104 years for each of the antibiotics. These findings predict a future increasing pattern of antimicrobial resistance, which may lead to serious public health threats if antimicrobial stewardship measures are not put in place.

465C Neglected strongylid nematodes: Metabarcoding reveals hidden transmission patterns between great apes and humans

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Strongylid nematodes such as hookworms and nodule worms are important pathogens of terrestrial vertebrates, including humans and non-human primates (NHPs). Despite being more closely related to *C. elegans* than *Pristionchus*, strongylids have evolved to live for months or years inside vertebrate hosts, sometimes forming complex communities within those hosts. More than 400 million people are infected with hookworms worldwide. Severe strongylid infections can cause heavy enteritis, intestinal lesions, iron-deficiency anemia, weight loss, stunting in childhood, and adverse birth outcomes. Because the human population and human settlements have rapidly grown in recent decades, people often live close to wild, free-living animals, which enables mutual pathogen transmission. These cross-species infections can have devastating effects on both sides, as was previously recorded for many diseases of either viral or microbial origin; such infections are especially risky in the case of phylogenetically closely related humans and non-human primates (NHPs). We used metabarcoding DNA amplification and short-read Illumina sequencing to assess the strongylid diversity and infections within humans and great apes (western lowland gorillas and chimpanzees) living in close proximity on the northern border of DJA Faunal Reserve in Cameroon. We analyzed 46 human, 60 western lowland gorilla and 31 central chimpanzee strongylid-positive samples. Great apes exhibited greater strongylid diversity than humans, with *Oesophagostomum* (nodule worms) and *Necator* (hookworm) being the most prevalent genera. We also detected rare strongylid taxa and observed several zoonotic strongylid species that were shared between humans and great apes. Our work shows that the human-animal contact can cause mutual exchange of strongylid pathogens, and that complex strongylid nematode communities can be detected time- and cost-efficiently through metabarcoding. Effective conservation efforts in the regions of increased human-animal contact are warranted.

466A Deconstructing Male Fertility: Functional and Evolutionary Characterization the NSPF Gene Family

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In organisms with internal fertilization, the male ejaculate must interact with the female reproductive tract and ova as well as potentially competing with sperm of other males to determine that male's reproductive success. Nematodes represent an excellent group for studying and manipulating post-insemination molecular interactions because of the high opportunity for sperm competition, wealth of genetic tools, and unique sperm morphology. In particular, nematode sperm contain subcellular

vesicles known as membranous organelles (MOs) that fuse with the cell membrane upon sperm activation to release their soluble contents into the extracellular space. Mutant screens show that correct MO fusion is necessary for male fertility, yet the function of these fusion event remains unknown. We hypothesize that one function is to act as a source of seminal fluid proteins which are involved in post-insemination reproductive tract dynamics.

Our previous novel proteomic approach to characterize the functional protein complement of MOs and identified the newly-designated Nematode-Specific Peptide family, group F (NSPF) as the second most abundant MO-localized proteins[1]. Comparative genomic analysis of 10 *Caenorhabditis* species indicated that the amino acid sequence and gene synteny are highly conserved. Together, the proteomic data along with the molecular evolution analyses suggest that these genes play a critical role in reproduction. To directly asses function, we constructed a CRISPR-based knockout of the NSPF genes. Single-generation fertility assays of young and old males under non-competitive and competitive conditions suggested a small but non-significant effect size, with advantage to wildtype over knockout males. To overcome this effect size limitation, we exploited experimental evolution to compound the effects over the course of 20 generations, using 10 replicate populations to compete the knockout allele against the wildtype allele in an otherwise homogeneous genome of a *fog-2* feminized *C. elegans* background. We calculated a selective disadvantage of 0.7% for the knockout allele. These results confirm that NSPF genes are an important component of male reproductive success and potentially act as part of a larger male-female signaling network. Ongoing transgenic work aims to test this functional hypothesis.

[1]Kasimatis *et al.* (2018) BMC Genomics 19:593.

468C A recombination modifier greatly affect C.elegans linkage map without inducing a direct fitness cost

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Evolution of recombination has been the subject of numerous theoretical studies. These works focus on how a gene that modifies recombination rate could be indirectly selected for by affecting recombination between fitness loci. However, direct selection may act on recombination modifiers. Indeed, these modifier genes are implicated in processes such as meiosis and may directly affect fitness. To address this question empirically, we use *Caenorhabditis elegans*. These nematodes have a marked recombination landscape with low recombination rate in the center of the chromosomes and high recombination rate in the arm domains. Loss of function of the Rec-1 gene equalizes recombination rate between markers in the center and the arms of the chromosomes. Although limited side effects have been reported, loss of Rec-1 function could have an impact on fitness. To measure this, the frequency of the Rec-1 mutant allele was followed during competition with the wild type. To detect epistasis, these competitions were performed in three different genetic backgrounds. To obtain a high-resolution recombination landscape and to determine if the effect of Rec-1 depends on the genetic background, mutant recombinant lines were generated from 3 different genetic backgrounds. These Rec-1 recombinant lines were sequenced to construct a linkage map.

469A Climate Change and Extinction – Lessons from *C. elegans* Population Dynamics

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Biodiversity loss is one of the major challenges for the 21st century. Ecosystems suffer from our modern civilization through pollution, agriculture, overexploitation, industrialization, coastal disturbance, landscaping, and ultimately climate change. Humans have a track record of driving species extinct ranging from passenger pigeons over baiji dolphins to western black rhinoceros. In order to preserve biodiversity and minimize extinction, a deep understanding of population dynamics is necessary.

To address this challenge, we are using our previously developed experimental platform comprised of a laboratory ecosystem with *C. elegans* and a complementary computational simulation. With this platform, we can model how overexploitation, habitat loss, and climate change destabilizes populations to extinction. We found that gradual changes in predation rates, habitat sizes, temperature, and food availability have little effect on population dynamics. In contrast, the populations collapsed if the environmental conditions reached a certain threshold (tipping point) and/or several environmental stressors accumulated. For example, *C. elegans* populations can only resist starvation for extended periods if predations rates are low and the temperature is optimal. We have defined the conditions for population collapse in wild type *C. elegans* in order to screen wild isolates, mutants, and *in silico* mutants for stabilizing traits. One future application is to modify the computational

simulation to model different nematode species and to predict for example which plant-parasitic nematode populations are stabilized by increasing temperature due to climate change.

470B Genomic mechanisms of asexual reproduction

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The eukaryotic innovation of genetic diversification through recombination confers survival advantages by providing the ability to rapidly purge deleterious mutations and fix beneficial mutations in a population. In animals, asexual lineages have evolved independently many times from sexual ancestors, but they are usually short-lived "evolutionary dead ends". Surprisingly, then, some rare asexual lineages are exceptionally long-lived and thus appear to enjoy unusual evolutionary success. Very little is known about the genetic mechanisms that drive transitions from sexual to asexual reproduction, but two common features are modified meiotic programs and altered genome organization.

We have previously published the genome and transcriptome of *Diploscapter pachys*, a parthenogenetic nematode from a long-lived (est. ~18M years) asexual lineage with abridged meiosis and a karyotype of 2*n* = 2. Our analyses revealed that the *D. pachys* genome is highly heterozygous and resulted from end-to-end fusions of ancestral chromosomes. *D. pachys* appears to lack clusters of ancestral telomeric repeats (TTAGGC) and canonical telomere maintenance proteins found in yeast, mammals and *C. elegans* – suggesting that its chromosomes may have atypical ends. *D. pachys* also appears to skip meiotic recombination and the reductional meiotic division, and several genes for homologous pairing and recombination are not detected in the current assembly.

To better understand the evolutionary trajectory of *D. pachys* from sexual reproduction to parthenogenesis, we are undertaking a comparative analysis of genome evolution across the clade of parthenogenetic *Diploscapter/Protorhabditis* species, as well as a related sexual outgroup. For each species, we are in the process of generating phased diploid chromosome-level assemblies using long-read DNA and RNA sequencing complemented with chromatin conformation capture. This will allow us to establish the patterns of chromosomal fusions and heterozygosity, which will inform hypotheses on their evolutionary history and potential crossover suppression through genomic rearrangements. With our new assemblies and transcriptomes, we will also define the nature of the chromosome ends as well as the complete repertoire of telomeric and meiotic genes in this clade. Finally, we plan to determine how the expression patterns of heterozygous alleles have evolved to adapt to non-recombining diploid genomes of the parthenogens in the *Diploscapter/Protorhabditis* clade.

471C Investigating the Diversity and Distribution of *Caenorhabditis elegans* in Georgia

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The *Caenorhabditis elegans* Natural Diversity Resource (CeNDR) maintains a library of strains of *C. elegans* collected from every continent except Antarctica (*Caenorhabditis elegans* Natural Diversity Resource [CeNDR], 2020). However, currently, there is only one *C. elegans* strain from the state of Georgia cataloged in CeNDR (CeNDR, 2020). In an attempt to expand upon the diversity of these collected strains and in an effort to study the distribution of *C. elegans* in Georgia, we set forth to collect a number of wild nematode isolates. Samples of rotting and decaying vegetation were collected from a variety of locations across Georgia. Data such as temperature, location, and sample type were recorded along with images of each collection site using the Nematode Field Sampling app within the data collection app Fulcrum. Nematodes were isolated from these samples and screened by visual inspection for morphological similarity to *C. elegans*. Potential *C. elegans* strains were then cultivated. To confirm that an isolate is indeed *C. elegans*, we will perform PCR and gel electrophoresis. For worms with an appropriately sized PCR band, we intend to submit samples of the PCR product for Sanger sequencing. We will then use NCBI BLAST to compare the sequencing results of the wild isolates with known species. Finally, we will submit frozen isolates of *C. elegans* to CeNDR for cataloging and whole-genome sequencing.

Caenorhabditis elegans Natural Diversity Resource. (2020, August 30). Global Strain Map [Interactive Map]. Retrieved March 24, 2021 from https://www.elegansvariation.org/strain/global-strain-map

472A Direct estimate of the distribution of fitness effects (DFE) of spontaneous mutations in *C. elegans*

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The distribution of fitness effects (DFE) of new mutations is a fundamental parameter in population genetics, and has practical application in the context of modeling the genetic basis of complex heritable disease in humans. However, the DFE is very difficult to estimate empirically. At present, nearly all estimates of the DFE rely on either statistical inference from the standing site-frequency spectrum or from laboratory estimates of fitness in mutation accumulation (MA) lines, with no reference to the underlying specific mutations. We report results from a new method to estimate the DFE from competitive fitness data from a set of recombinant inbred lines (RILs) derived from a cross between two *C. elegans* MA lines, combined with whole-genome sequence data from the set of >500 RILs and the parental lines, as well as from ~40 fully-sequenced MA lines. Of five distributions fit to the data, all produce similar results, but the best fit is to a normal distribution nearly centered at 0, with almost no weight beyond +/- 2%. Averaged over two fitness assays, nearly ten years apart, the average fitness effect of a new mutation is about -0.2%. These results are in strong contrast with estimates of the average mutational effect inferred from MA line fitness data not informed by sequence data, which are much larger. We suspect that a few mutations of large effect may have been lost during the inbreeding phase of the construction of the RILs.

474C High-throughput phenotyping of *C elegans* wild isolates reveals that microsporidia genotype-specific interactions are common in *C. elegans*

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Animals are under constant selective pressure from a myriad of diverse pathogens. Microsporidia are ubiquitous animal parasites, but the influence they exert on shaping animal genomes is mostly unknown. Several microsporidian species have been isolated in wild strains of *Caenorhabditis elegans* and are shown to be a natural parasite of the worm, making them a powerful tool for the study of host-pathogen interactions *in vivo*. Using the multiplexed competition assay, PhenoMIP, we measured the impact of 4 different species of microsporidia on 23 wild isolates of *C. elegans*. This screen resulted in the identification of 15 strains with altered resistance or sensitivity to infection. Of these identified strains, JU1400, is sensitive to the epidermal-infecting species *Nematocida ferruginous* by lacking tolerance to infection. Conversely, it is resistant to the intestinal-infecting species *Nematocida ironsii*, but this infection delays the development of JU1400 animals. Furthermore, coinfection experiments with other microsporidia species demonstrates that JU1400 can specifically recognize and eliminate the invaded *N. ironsii* parasite. Genetic mapping of JU1400 revealed that several loci contribute to the resistance of *N. ironsii* and that sensitivity to *N. ferruginous* is cause by a single locus on the left most part of chromosome I. Construction of near-isogenic lines confirmed that these two opposing phenotypes to different microsporidia species are caused by separate alleles. Overall, our results demonstrate that *C. elegans* can rapidly evolve to recognize and respond to specific microsporidia infections.

475A Genomic architecture of 5S rDNA cluster and its variations within and between species

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Ribosomal genes (rDNAs) are arranged in purely tandem repeats, preventing them from being reliably assembled onto chromosome. The uncertainty of rDNA genomic structure presents a significant barrier for studying their function and evolution. Here, we generate ultra-long Nanopore and short NGS reads to delineate the architecture and variation of the 5S rDNA cluster in the different strains of *C. elegans* and *C. briggsae*. We classify the individual rDNA units into 25 types based on the unique sequence variations in each unit of *C. elegans* (N2). We next perform manual assembly of the cluster using the long reads that carry these units, which led to an assembly of rDNA cluster consisting of up to 167 5S rDNA units. The ordering and copy number of various rDNA units are indicative of separation time between strains. Surprisingly, we observed a drastically lower level of variation in the 5S rDNA cluster in the *C. elegans* CB4856 and *C. briggsae* AF16 strains than *C. elegans* N2 strain, suggesting a unique mechanism in maintaining the rDNA cluster stability in the N2. Single-copy transgenes landed into the rDNA cluster shows the expected expression in the soma, supporting that rDNA genomic environment is transcriptionally compatible with RNA polymerase II. Delineating the structure and variation of rDNA cluster paves the way for its functional and evolutionary studies.

476B Dissecting the Molecular Mechanism of the *peel-1/zeel-1 S*elfish Genetic Element

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Selfish genetic elements persist in species despite their negative impacts on host fitness. Toxin-antidote selfish elements kill progeny that do not inherit the genetic element thus increasing their representation in the next generation. The *peel-1/zeel-1* gene pair is a toxin-antidote system responsible for a hybrid incompatibility between *C. elegans* Bristol N2 and Hawaiian CB4856 strains. PEEL-1 is a sperm-delivered toxin that arrests embryonic development. However, embryos inheriting the *zeel-1* antidote gene can escape PEEL-1 toxicity. We are investigating the molecular mechanism of PEEL-1 toxicity and ZEEL-1 anti-toxicity. We used a forward genetic screen to discover that the small, conserved protein, F47B7.1 is necessary for PEEL-1 toxicity. Expression of PEEL-1 and F47B7.1 together induces cell swelling and cell death in HEK293T mammalian cells. ZEEL-1 expression suppresses this toxicity. Therefore, I have reconstituted the toxin and antidote activity in a heterologous system, suggesting that we have identified the minimal set of proteins involved in toxicity and anti-toxicity. My continued work uses *C. elegans*, HEK293T cells, and *in vitro* biochemistry to dissect the molecular mechanism of toxicity and anti-toxicity.

477C Studying inter-species genome size variation using C. nigoni and C. briggsae hybrids

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The genome size variation is an “old” question in evolutionary biology. However, its causes and consequences are still a debate. There are various intraspecies models to study genome size variations, which usually hold very limited size change between pairs. As a result, sophisticated and pricy detection methods are required to monitor the change of genome size. And the impact of large size variation can not be tested.

The inter-species models, however, could have significant genome size variation. One close sister pair among *Caenorhabditis* clade, the *C. briggsae* and *C. nigoni*, has around 30% genome size difference. They can cross with each other and produce fertile females, and can be used to study genome size variation and hybrid incompatibility. One major disadvantage of inter-species model is that, the F1 male is either dead for sterile, which hinder the monitoring of further offspring by F1 crossing.

We generated a lot of introgression strains with *C. nigoni* genome background with a small fragment from *C. briggsae* genome. 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 both fertile F1 females and males. By breeding the F1 worms with 10 x 10 crossing, we can monitor the competition of the two haplotypes, which has 30% size difference, by checking the ratio of two haplotypes using NGS sequencing.

We have sequenced some F7 and F20 lines. And the long haplotype, the *C. nigoni* haplotype, has become dominant (78%) in F7 populations, especially in the X chromosome (92%). With this speed, we expected to see a 100% recovery of ZZY10253 genotype after F15. But to our surprise, the F20 lines still maintained at least 8% of *C. briggsae* haplotype, and all these lines (n=8) have two autosomes remained. These remained autosomes may reflect an interaction between the X introgression fragment and autosomes, which grant these individuals some advantage in growing.

478A Reproductive incompatibility among populations of *Caenorhabditis inopinata*

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Caenorhabditis inopinata, the sibling species of *C. elegans,* recently discovered in Ryukyu islands Japan, has a distinct lifestyle from other known *Caenorhabditis* species. The gonochoristic species inhabits in syconia of a fig tree (*Ficus septica)* and uses the pollinator wasp (*Ceratosolen* sp.) as a vector to move from old to new syconia.

We isolated *C. inopinata* from fig syconia collected in seven Ryukyu islands and Taiwan (distance in 730 km), and established over 20 single female lines. Phylogenetic analyses of those lines revealed that *C. inopinata* can be separated largely into two geographical groups (Okinawa and Ishigaki groups). Although we couldn’t identify clear morphological differences, the genetic distance of the two groups calculated by genome comparisons was relatively large as an intra-species diversity. We therefore sought to clarify mating and reproductive compatibilities between the two geographical groups.

Using three *C. inopinata* lines each from Okinawa and Ishigaki groups, respectively, we observed mating behaviours, embryo developments and reproductions for all line combinations and directions (male - female). Mating behaviours were observed in all combinations though sexual attraction levels seem lower in inter-group than intra-group combinations. However, offspring (F1) numbers were smaller in inter-group combination, especially incomplete embryo developments and almost no offspring were observed at Okinawa male and Ishigaki female combination. These results suggest that speciation in *C. inopinata* has possibly been accelerated by the characteristic lifestyle and the island effect in this small area.

479B Using First-year Students To Do Research While Learning Biology

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The First-year Innovation and Research Experience (FIRE) program at the University of Maryland College Park follows the Course-based Undergraduate Research Experience (CURE) model to provide an opportunity for ~600 students to get hand-on experience in 15 research streams in the natural, social, and applied sciences. Unlike the traditional apprenticeship model where only a small fraction of undergraduates gets the opportunity to gain research experience towards the end of their undergraduate career, the FIRE program places students into the research setting during their first year at university. One of the FIRE research streams, the Transgenerational Brain Initiative (TBI), uses *C. elegans* as a model system to study how double-stranded RNA (dsRNA) expressed in neurons leads to silencing of a germline gene for multiple generations. The previous studies on how dsRNA-derived signals move through the animal to enter the germline to trigger and maintain germline gene silencing used multicopy arrays that overexpressed and possibly mis-expressed dsRNA. To better study this phenomenon under more controlled and physiological conditions requires new genome-edited animals with single-copy transgenes. Each year, ~40 students join the stream where in the first semester they learn basic methods in molecular biology such as pipetting, PCR and plasmid miniprep. In the second semester students learn molecular cloning with restriction enzymes to clone their transgene into homology repair plasmids ready for CRISPR/Cas9 genome editing. About 10 students will return for a second year to be peer research mentors who help train the new class while pursuing their own research project. Presented here is the progress made by the students of FIRE-TBI in their efforts to create and characterize a collection of strains expressing dsRNA and reporter genes from single-copy transgenes in different subsets of neurons.

480C Using student annotations of published data in the *C. elegans* database, WormBase, to foster collaboration during an online laboratory course

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Course-based Undergraduate Research Experiences (CUREs) provide the same benefits as individual, mentored faculty research while expanding the availability of research opportunities. One important aspect of CUREs that students engageme in collaboration through their research. We developed a partnership with the *Caenorhabditis elegans* (*C. elegans)* database, WormBase, in which students submitted annotations of published manuscripts to the website. This activity provided students with a collaborative research activity that benefits the *C. elegans* community and enhanced students' understanding of molecular genetics during the COVID-19 pandemic.

WormBase relies on community annotators to read published articles and input phenotypic data. Students were introduced to C. elegans nomenclature and the structure of WormBase at the beginning of the course, and submitted a total of nine unique annotations directly to WormBase. Student annotations were curated by WormBase to ensure correctness and to reduce overlap from redundant annotations. Due to the stress on students during this time of crisis, qualitative data were collected in lieu of quantitative pre-post analyses. Students described their learning experiences in terms of interactions with the scientific community and the "real world", content knowledge and competencies, and changes in perspectives and use of resources. Students also reported that this activity was helpful in their understanding of critical molecular genetics concepts. The shift to online learning during the COVID-19 pandemic created an immediate need for meaningful, collaborative experiences in CUREs. By partnering with WormBase, students gained insight into the scientific community and contributed as community members.

481A Wormfinding: a semester-long CURE for introductory biology

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Engaging students in inquiry-based laboratory experiences is widely considered superior to traditional "canned" labs in improving attitudes towards science and retention of foundational principles. We developed and implemented a laboratory course grounded in worm discovery, involving worm isolation, DNA barcoding for phylogenic placement, phenotype analysis, and chemotaxis experiments. Throughout the course, students gained familiarity in molecular biology, animal behavior, and bioinformatics methods, as well as experience in experimental design, analysis, and presentation skills.

Our introductory biology course is a mixed-majors course that fulfills a general education laboratory requirement. Wormfinding was used during two semesters (three total sections) in place of the traditional laboratory. This lab is robust and can be completed by novices in a once-per week, three-hour sessions format over the course of 15 weeks. Prep and cost was similar or less than the labs it replaced.

In the most recent offering of the course, 22 out of 30 students were able to isolate a strain from the environment that was able to be maintained throughout the semester.

Quality DNA barcoding sequence reads were found for all but one strain. Students used NCBI's BLAST to find genus-level matches to worms from around the world, and imaged their worms to compare morphology between strains. Students designed and executed their own chemotaxis experiments, and maintained laboratory notebooks via entries into a printed template. At the course's end, students drew conclusions about whether their genetic and phenotypic data fit together, and shared their discoveries through poster presentations and data uploads to our lab database (nematode.umwblogs.org).

We designed our course in alignment with the principles of Creating Significant Learning Experiences (LD Fink). The lab guide's protocols are contextualized in a way that encourages students to care about their work and think critically and creatively, all while reinforcing the foundational knowledge requisite for an introductory course. Following IRB guidelines, students participated in the Grinnell CURE pre/post surveys and evaluations of their learning. At semester's end, most students indicated that they enjoyed the course, gaining confidence in their participation in the process of research, a more mature perspective on the nature of science, and a positive experience overall.

482B Using worms in a Molecular Biology course to teach cloning.

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Cloning genes is an important molecular biology skill. I developed a semester-long upper-level undergraduate laboratory where students clone a gene from *Auanema rhodensis* or *C. elegans*. Because *A. rhodensis* is a species with three sexes, the course focuses on candidate genes involved in sex determination. Students learn about sex determination from the primary literature, propose a gene to clone, obtain the sequence to target, and design primers. Next, they attempt the various steps of cloning into a TA cloning vector. In an undergraduate setting, with a twice per week meeting, this process can take the whole semester. Most students have generated a new vector with a portion of their gene of interest by the end. The laboratory manual I developed could easily be adapted to cloning genes involved in any biological process, and from most nematode species.

483C Creating choice in molecular genetics lab through the use of toxicology

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Some pedagogy techniques focus on giving students choice – what assignments to do, what topic path to study, or what topics within the course an individual wants to learn. The basis behind this is that by giving students choice, they have ownership in their learning and will be more invested in their coursework. For many science courses, however, the choice of what topic to study is not a viable option; students cannot choose to learn about translation and not transcription or leave out entire units of key concepts in a discipline. Regardless, creating choice and ownership is still important in student learning and can be done in a more structured way. In my 300-level molecular genetics lab, students choose a toxicant to investigate for two lab experiments using *C. elegans*. One lab experiment (Lab 1) focuses on utilizing reverse transcription and PCR to analyze gene expression. The second lab experiment (Lab 2) is a toxicology measure where students pick an outcome (movement, growth, reproduction, or death) to observe after exposure to their toxicant of choice. Students work in pairs to pick a toxicant to study. They perform a literature search to determine two concentrations to test for the experiments and an outcome to test in Lab 2 based on their readings. Lab 1 allows students to learn RNA isolation, PCR, and gel electrophoresis while testing a hypothesis related to gene expression in response to their toxicant. Lab 2 provides more opportunities for students to have choice in which they explore an effect of the toxicant that interests them. Students present their research through the writing of an

introduction, results, and discussion sections in the form a journal style paper. Student choice with these two labs provides them with the opportunity to have ownership of the experiments through structured lessons. Students also practice skills of developing research statements and hypothesize through literature research and investigation. These labs are reported as being a favorite within the course and students become invested in conducting the experiments because they got to choose what was being tested (toxicant) and what they wanted to test (outcome).

484A Screening bacterial isolates for novel therapeutics: a CURE approach

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Natural products, also called secondary metabolites, are historically the most effective source for therapeutics. The microbial branch of the tree of life remains the largest untapped resource of secondary metabolites, and *Caenorhabditis elegans* is an ideal platform to screen through bacterial strains for useful drugs. Variation of *C. elegans* bacterial food sources have uncovered fitness benefits and costs beyond what can be explained by just changes in nutritional content. We hypothesize that altering bacterial food sources will uncover strains that harbor unique secondary metabolites that suppress conserved cell signaling and regulation pathways. To test our hypothesis, the spring semester cohort of the genetics teaching lab at Michigan Tech University piloted a Course-Based Undergraduate Research Experience (CURE) aimed towards isolating and screening wild bacterial species for enhancement or suppression of the *C. elegans* Sythetic Multivulval (SynMuv) phenotype. The SynMuv phenotype occurs when a SynMuv class A gene mutation is paired with a a SynMuv class B gene mutation. Each gene class redundantly antagonize RTK/Ras/MAPK signaling that directs vulval cell differentiation during development. In this 3-month-long course, we harvested environmental samples from soil, snow, ice, and compost. After isolating bacterial colonies, we performed gram-staining and 16S sequencing to identify 1 isolate per student. We next tested bacterial toxicity and food preference compared to OP50 *E. coli* control. We then assessed whether feeding wild bacterial isolates increased penetrance of the multivulva phenotype in the SynMuv A strain *lin-8(n2731)* or the SynMuv B strain *lin-52(bn151).* In parallel, we assessed whether feeding wild bacterial isolates decreased penetrance of the multivulva phenotype in the double mutant *lin-8(n731); lin-52(b151).* Enhancement or suppression of the single or double mutant strains would suggest that the bacterial isolate harbors a secondary metabolite that suppress the conserved RTK/Ras/MAPK signaling pathway or its negative regulators. Altogether, our CURE component of the genetics teaching lab provides students with the experience needed to establish their path towards their own individual undergraduate research journey.

485B Utilizing CRISPR to Reinforce Genetics

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An essential yet privileged place of learning for the scientist is the laboratory, however at the undergraduate level access to research experience is often limited to a chosen few students. Therefore, to allow a more diverse cohort of students a research experience, I have designed and implemented three iterations of a Course-based Undergraduate Research Experience (CURE) that aligns with my overall research goal to better understand the genetic networks promoting gonad development in the *C. elegans*. In the first two iterations of this class, students used CRISPR to delete DNA regulatory regions and determine whether these DNA regions were necessary for the proper protein expression of the *fkh-6* gene, which is normally expressed in the gonad and required for proper gonadal development in *C. elegans*. In the third iteration students worked to delete the open reading frame of genes of interest, but this work was cut short when classes moved online due to the SARS-CoV-2 pandemic. During the semester-long CURE, students designed the constructs needed to edit the genome and performed the needed cloning experiments. Following the injection of the construct, students then screened the progeny of the injected animals for potential CRISPR edits by genotyping and verified that the intended edit was present within the genome of the animal. Finally, students analyzed the edited strains for the resulting expression pattern of the FKH-6 protein and the overall gonadal morphology of the newly generated strain. In these first two classes, seven of the eight laboratory groups were successful at making genomic edits using CRISPR, six of which were the intended edit, indicating that CRISPR can be accomplished in a classroom setting with inexperienced researchers, many of whom had never handled a micropipette prior to this course. Even more exciting, students were able to identify DNA regulatory regions that when deleted led to the feminization of male animals. To facilitate a stronger research experience, the students were also required to present a published paper related to their project as well as discuss their research in lab meetings with their peers in the course. At the completion of the semester, the students presented their project in a poster format open to the university as a whole. Work is now currently underway to develop this CURE as a required course for all sophomore biology majors in connection with the Genetics course.

486C Making online exams more secure in an introductory cell/molecular biology course by using banks of questions made with Python

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The move to remote instruction at the onset of the pandemic changed the way that instructors give and proctor exams. The availability of resources to cheat during exams has greatly compromised the ability to assess individual learning outcomes. Restricting exams to short time intervals, randomizing question/answer order with one-at-a-time presentation of questions (and no backtracking) are considered best practices to make cheating more difficult. Another accepted best practice, but more time-consuming to implement, is to make alternate versions of exam questions. When teaching became remote for a majors-level introductory cell/molecular biology course (250+ students) in spring of 2020, I began generating question pools semiautomatically by scripting. The ease of programming in Python makes this language ideal for such an application. Questions involving simple genetics and molecular biology, factual recall, and diagram labeling, are particularly amenable to generating variant questions. Exam score distributions resembled those for in-person exams, suggesting that online exams without proctoring can generate results that are comparable to in-person exams, although there are caveats. I will outline a general approach for constructing question banks through programming and give specific examples.

487A Virtual Active Science Engagement (VASE): unveiling the hidden curriculum of academic science through peer networking, career discussions, and skill building

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The Covid-19 pandemic caused profound impacts on many, if not all, aspects of STEM research and learning for undergraduate students. One of particular interest is the loss of an already difficult thing to maintain in a classroom setting: engagement. To promote engagement with peers, mentors, and science in the summer of 2020, I designed Virtual Active Science Engagement (VASE) as a semi-structured mentoring and peer-networking program for a few undergraduates from across the USA for 8 weeks. VASE is now in its third term and over 25 meeting sessions. Growing from just six students to over forty, VASE students are globally distributed across seven different time zones: many students are first-generation in STEM or from other communities historically underrepresented in STEM. VASE students range in age from 16 to 24 (late high school to post baccalaureate) with career goals ranging from nanoengineering and parasitology to many fields of medicine and biomedical research. My objective was to provide a space for students to engage in small peer-network group discussions to explore science as a career, identify opportunities for research experiences, and gain confidence to visualize themselves as scientists. VASE meetings highlight the diversity of careers and paths into STEM with invited guest speakers while guided discussions unveil the hidden curriculum of academic science. Discussion topics have included how to read a scientific research article, find funded research projects, and give a scientific talk or journal club presentation. VASE students are also encouraged to participate in Virtual Itty Projects (VIPs). VIPs are brief, hands-on projects with the purpose of providing students with real research experiences and deeper understanding of how research is conducted. VIPs are performed alongside volunteer scientists (like you!), who donate a few hours of their time to train undergraduates in a useful scientific skill. Here I present one VIP, in which a dozen undergraduate students learned how to use ImageJ to analyze *C. elegans* cuticle integrity. We hypothesize that even a short period (<4 hours) of engagement with guided research projects will allow students to build confidence, expand their professional network, and hone their scientific interests. VASE is a virtual space for students to actively engage with motivated peers, compassionate guest speakers, an invested mentor, and the pursuit of science.

488B Collection of Wild Isolates as a Remote Hands-on Research Experience

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In response to the COVID-19 pandemic, teaching was moved to remote learning design including experiential lab courses. With classes offered remotely, many students opted to stay home and continue their classes online. However, as lab courses provide students some of the only hands-on laboratory experience for many students prior to graduation, it was our aim to create a hands-on lab experience that was transportable and therefore accessible to all enrollees. The major challenge in

creating a remote *C. elegans* lab experience was that our university would not permit the shipment of *E. coli*, in any form, to students. This created a challenge for shipping worms to students, as well as for providing a food source once worms arrived. To circumvent this obstacle, we designed the class around the idea of students each collecting wild-type worms locally. As well, a vegan broth was utilized as a substitute food source. The lesson plan consisted of multiple assignments beginning with a literature review to develop an appreciation for the contributions and potential power of using wild isolates in research. Following this, students independently developed their own collection protocol. Materials that would allow for the extraction of worms from soil using the Baermann funnel method were then shipped or provided for pickup. Finally, following collection students determined if the worms were *Caenorhabditis* by screening animals for self-replication and by physical observation of the pharyngeal structure. As part of the final assignment, students completed the wild isolate submission form from the *Caenorhabditis elegans* Natural Diversity Resource (www.elegansvariation.org). Students were encouraged to be innovative in their method design and implementation and to utilize notebooks to record unique steps and deviations from initial method proposals. Upon completion, students were required to return a sample of their worm collection to the university for further identification. By the conclusion of the term, half of the students had successfully collected worms. In the future, this lesson plan can be expanded to include additional identification procedures such as crossing wild-isolates with the N2 strain and/or DNA sequencing.

489C Development of a GFP RNAi Experimental Module in *C. elegans*

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The Molecular Genetics course at Wabash College is a primary literature-driven course designed to explore the molecular and cellular experimental evidence which underpins our current understanding of gene expression. The laboratory component is a semester-long investigative Course-based Undergraduate Research Experience (CURE). In this laboratory, each student designs a unique RNAi construct to disrupt gene expression in *C. elegans* and assesses the phenotypic defects via behavioral assays and microscopy. In the lab's current design, we accomplish two main course goals, including 1) student engagement in scientific practices (asking questions, developing hypotheses, designing experiments, and gathering / analyzing data), and 2) novel scientific discovery (addressing scientific questions where the outcome of an investigation is unknown to both the students and the instructor). Although the students enjoy the laboratory and the opportunity to engage in an authentic research project, they do not obtain experience using various molecular and cellular readouts of gene expression beyond basic microscopy. To expose students to essential techniques that they frequently encounter in the primary scientific literature, we developed a collaborative 1–2-week laboratory module in the course. In this module, students complete a GFP reverse genetics experiment and assay GFP gene expression using: 1) protein visualization/localization by microscopy, 2) protein abundance via Western Blotting and 3) mRNA levels via quantitative PCR (qPCR). The addition of this module to the course allows for additional course goals, including: 1) exposure to fundamental cell biology techniques; 2) empirical observation of the transfer of genetic information; 3) a practical demonstration of qualitative vs. quantitative data; and 4) empirically demonstrates the difference between a gene knock-out (deletion) and RNAi (reduced but not eliminated expression). We will report specific details about the experimental timeline and reagents for this module.

490A Characterization of RNAi phenotypes in *C. elegans* from understudied genes in a Cell and Molecular Biology course

Jessica Sullivan-Brown[1], Jessica Sowa[1] 1) West Chester University.

The characterization of relatively understudied genes in *C. elegans* using RNA interference is an excellent approach for designing undergraduate research-based labs. Here we describe a successful lab course in which Cell and Molecular Biology students at West Chester University of Pennsylvania (WCU) make novel discoveries associated with knock down of *dcaf-13,* a likely oncogene in humans. Each class represents the flow of a typical study of gene function, beginning with a journal club reading the primary literature. Then students use bioinformatics to identify the human protein sequence of DCAF13, perform a BLAST search to find *C. elegans* homologs, and use WormBase to identify the *C. elegans* gene sequence. An RNAi knock down experiment is then performed with *dcaf-13.* Throughout the lab, students characterize the resulting phenotypes, using IMAGEJ/FIJI for quantification of worm length and fluorescence microscopy for GFP analysis of an intestinal cell fate marker. Students then isolate RNAs using Trizol RNA extraction and perform cDNA synthesis. Students then design primers to test if *dcaf-13* knock down affects the expression of different cell cycle genes by performing RT-PCR. At the end of the course, students present their work orally in a poster presentation and in written communication in a primary research style paper. We have recently added a component in which students design a future experiment in the format of a small grant proposal and after peer review, two projects are chosen to perform in lab. The lab serves two primary functions: to provide students opportunities to advance their experimental techniques so they are competitive in the job market and create an experience

that mimics a typical research project by making real contributions to science. Our goals are to publish the work the students generated. Importantly, this framework can be applied to other understudied genes in *C. elegans* in which the RNAi phenotypes are not well documented, benefiting both students and the *C. elegans* community.

491B Snip-SNP mapping of a trembler *C. elegans* mutant strain with undergraduate students

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Caenorhabditis elegans is a valued model organism that is easy to maintain in the lab. We obtained a mutant strain of *C.* elegans that exhibits constant involuntary muscle spasms, a phenotype that has not been described before in the literature (MCW230, curtesy of Dr Wang, BCM, Houston, TX). The 'trembler' mutant strain has a *nre-1(hd20) lin-15b(hd126)* genetic background. We use snip-SNP mapping to seek the causal mutant gene responsible for the trembling phenotype in the MCW230 strain. MCW230 worms were backcrossed with wild-type N2 worms to eliminate the double mutant background (*nre-1, lin-15b*). For snip-SNP gross mapping, we crossed our mutant with CB4856 worms. DNA was extracted from 43 F_2 individuals resulting from the MCW230 x CB4856 cross. The F_2 samples were studied to identify the presence of specific single nucleotide polymorphisms at 18 genomic locations (3 per chromosome) and assess how the alleles segregate relative to the trembler phenotype. We completed genotyping at all genomic locations and our data supports the idea that the trembler phenotype seems to be linked to one of the markers on chromosome III. Our work is an example of how research using *C. elegans* can be done in the undergraduate environment with basic laboratory equipment.

492C Tips for early career scientists on NSF-CAREER award proposals

Cheryl Van Buskirk[1] 1) California State University Northridge.

In keeping with the focus of this year's meeting on early career scientists, this poster aims to provide guidance and encouragement to new worm faculty in writing winning NSF Faculty Early Career Development (CAREER) proposals. The NSF-CAREER program is an exceptional funding opportunity for new investigators who are equally committed to outstanding research and education. The award is NSF-wide and thus is available to most areas of basic scientific research. If you are not yet tenured and looking to authentically integrate your research with education and/or outreach, this award is for you. Having served as a reviewer for these proposals, and currently in my last year of a CAREER award, I'd like to share some DOs and DONTs of proposal preparation and discuss ways to effectively integrate research and education/outreach components.

493A Modeling *C. elegans* Protein Structures as part of Undergraduate Research Projects

Katherine Walstrom[1] 1) New College of Florida.

Since 2014, the Biochemistry Lab at New College of Florida has been a Course-based Undergraduate Research Experience (CURE) with skill-building activities during the first half of the semester and a research project during the second half of the semester. These research projects were continued by undergraduate thesis students. We have made protein expression plasmids for a variety of wild-type and mutant *C. elegans* proteins, such as malate dehydrogenase (MDH-1 and MDH-2), isocitrate dehydrogenase (IDH-1), glucose 6-phosphate dehydrogenase (GSPD-1), and various globin isoforms. Most of these projects resulted in successful enzyme purifications and characterization measurements. However, due to various factors including the COVID-19 pandemic, some students were unable to complete their lab projects during the time available. To complete their learning experiences, the students used a variety of online tools to make and evaluate structural models of *C. elegans* proteins.

SWISS-MODEL (https://swissmodel.expasy.org/) takes the input protein primary sequence and finds homologous template proteins that have known 3-dimensional structures. Various parameters can be used to select the best template protein, and the software creates a modeled structure for the input protein. The software produces a protein data bank (PDB) file of the modeled protein, which can be viewed with any protein structure visualization program. Students used these structures to explain their experimental data or to test hypotheses that they could not test in the lab. We found that while these models were of good quality, they benefited from energy minimization, using either the YASARA energy minimization server (http://www.yasara.org/minimizationserver.htm) or the UCSF Chimera program (https://www.rbvi.ucsf.edu/chimera/). To determine the quality of the models, websites such as MolProbity (http://molprobity.biochem.duke.edu/index.php) were used to check the Ramachandran plot and rotamer angles in the structures for unusual conformations of amino acids. We will present some specific examples of these analyses and point out potential problems. The students enjoyed learning these new skills, and they

improved their abilities to analyze and visualize protein structures. We hope that others will find these resources useful for their courses and research projects.

494B Integration of research ethics training in a course-based undergraduate research experience (CURE) exploring genetic incompatibilities in *C. briggsae*

Joseph Ross[1] 1) California State University, Fresno.

The importance of bolstering public trust in the scientific process and its conclusions is increasingly evident. However, training of scientists in ethics and the responsible conduct of research (RCR) is rarely incorporated into training opportunities, especially for undergraduate students. In course-based undergraduate research experiences (CUREs), students participate in the entire process of scientific inquiry, from project conception through data analysis and dissemination. Thus, CUREs likely provide students with an initial exposure to various aspects of RCR, such as research misconduct, data management, and authorship.

To integrate RCR training in a CURE, a required biology major lab course was modified to include direct instruction in RCR, followed by weekly assessments. This period also involved training in nematode biology and molecular genetic techniques. As students worked in groups to design and conduct their novel research experiment, they experienced many situations that required ethical decision-making. At the start of the course, seven percent of participants (n=95) reported never having RCR training, despite 32% participating in faculty-led research projects and 90% having taken four or more college lab courses. Despite participants being predominantly third and fourth year undergraduates, at least sixteen percent did not identify which sentences in a research report should contain citations. Separately, two-thirds of participants were not familiar with policies related to image manipulation. Perhaps most critically, the participants did not feel that students participating in research have a role in defending against research ethics violations.

These results underscore the need to provide more direct training of RCR and highlight the role that CUREs can play in meeting this need. The presentation will further describe the instructional materials and activities, as well as a quantitative evaluation of RCR student learning outcomes. Distribution of these materials should increase the quantity and quality of undergraduate courses in which RCR training is incorporated.

495C Nematode hunters: a citizen science approach to identifying new systems for the study of host-virus interactions

Catherine Byrnes[1], Jessica Sowa[1] 1) West Chester University of Pennsylvania, West Chester, PA.

C. elegans is a popular model organism that has proved very useful for studying the cell biology of intracellular infections. However, its use as a model for the study of host-virus interactions has been limited by the fact that only one natural viral pathogen of *C. elegans* has been identified to date (Félix and Wang, 2019; Franz et al., 2014). The goal of this project is to identify novel natural nematode viruses capable of infecting *C. elegans* by mobilizing ordinary citizens to collect wild nematodes.

Studying the interactions of different types of viruses with their host's cells can provide new insights into cell biology and host-pathogen interactions. To date, only four viruses 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). In the past, identification of intracellular pathogens in wild-caught nematodes has relied on detection by microscopy of morphological changes caused by the infection (Félix et al., 2011; Troemel et al., 2008). This approach is relatively low throughput and requires an expert screener. Our approach instead uses a fluorescent reporter-based method, taking advantage of a set of genes which are expressed at low levels in basal conditions but highly upregulated during infection by intracellular pathogens (Bakowski et al., 2014; Reddy et al., 2017, 2019). Co-culturing infected nematodes together with *C. elegans* expressing these intracellular infection reporters produces fluorescence which is easily detected on a fluorescence dissecting microscope. By using this method on a large sampling of wild-caught nematodes, we hope to identify novel nematode viruses which can be transmitted to *C. elegans.*

In the pilot phase of this project, we established protocols for wild nematode collection which require minimal supplies and can be performed at home by people with no particular science background after viewing a series of short training videos. We have successfully cultured wild nematodes from these samples in the lab, and have established systems for sample intake, expansion and frozen stocking of the strains, performing co-culture experiments, and sharing experimental results with the original collectors. In the fall of 2021, we hope to expand this project by partnering with educators at a variety of levels on a larger scale who would be interested in incorporating nematode hunting into their science curriculum.

496A The *Caenorhabditis* Natural Diversity Resource: expanded and enhanced

Erik Andersen[1], Kathryn Evans[1], Robyn Tanny[1] 1) Northwestern University.

Natural diversity offers a treasure trove of variants (or mutations) that can be used to understand quantitative traits and evolutionary processes. The *Caenorhabditis elegans* Natural Diversity Resource (CeNDR) has archived and disseminated wild strains, whole-genome sequenced those strains, and released variant data and effect predictions to the community. Also, CeNDR facilitates genome-wide association (GWA) mappings to connect natural differences in quantitative traits to variants and molecular mechanisms. Since 2016, CeNDR has shipped more than 4,000 strains and enabled over 3,000 GWA mappings for the community. We continue to archive and sequence new wild strains identified by collaborators and citizen scientists, offering more than 1,300 genetically unique strains. In addition to these new strains, we updated CeNDR to make it more user-friendly, created a broad-sense heritability calculator, modernized the variant browser and variant effect predictions, and created a powerful new GWA mapping portal. These additions and updates make CeNDR more accessible and able to facilitate natural variation studies in laboratories often focused on the laboratory-adapted N2 strain.

We present the evolution of CeNDR into the *Caenorhabditis* Natural Diversity Resource (CaeNDR) where we have incorporated over 1,600 *C. briggsae* and 700 *C. tropicalis* wild strains and genome sequences. The same browsers and tools created for *C. elegans* are available for these species. CaeNDR will expand comparative genomics across the three selfing *Caenorhabditis* species and enable new approaches to quantitative genetics and population genomics.

497B WormBiome: A pipeline to predict functional profiles of *C. elegans* associated microbial communities

Adrien Assie[1], Dana Blackburn[1], Fan Zhang[1], Buck Samuel[1] 1) Baylor College of Medicine.

Teasing apart the dense network of molecular mechanisms that link digestive tract microbial community members to each other and their host is challenging in most systems due to complexity and tractability. Recently, *Caenorhabditis elegans* has emerged as a powerful model system to study host-microbe interactions. The simplicity of the *C. elegans* digestive tract, together with the nematode›s genetic amenability, and the availability of relevant microbial collections make it ideal for the study of the fundamental mechanisms of host-microbiota interactions. To characterize those mechanisms, we focused on identifying genetic features important for bacteria colonizing the *C. elegans* gut. We developed «WormBiome» a pipeline that predicts the combined functional potential of defined *C. elegans* gut microbiome.

The “Wormbiome” pipeline match predicted microbial abundance from 16S rRNA amplicon datasets to functional genomic annotations and output functional profiles for each microbial community present in the submitted dataset. Our functional annotations rely on curated metabolic (Metacyc) and functional annotations (KEGG) databases build on known *C. elegans* related bacteria. Then the pipeline predicts features significantly different between user-defined sample groups.

We tested the pipeline using a defined and fully sequenced 12-member model microbiome (CeMbio) grown with and without N2 animals. With 10 replicates for each condition, we identified 1700 significantly different features, distributed across 180 KEGG and 63 Metacyc categories. The most abundant features belong to the lipid, amino acid, and cofactor metabolisms. Among genes predicted to be more abundant in worm-associated communities, we found the *de novo* synthesis of vitamin B12 and metabolic pathways for host-essential amino acids, such as proline, alanine, and arginine.

We verified the pipeline’s prediction by examining the impact of nutrient depletion on gut microbiome composition by selectively supplementing or removing amino acids individually or altogether. Our results show that a single change in amino acid can affect how bacteria interact with each other and promote the growth of certain community members and that complete removal of amino acids promotes colonization of metabolically flexible members of the microbiome like *Ochrobactrum*. This study establishes a robust framework for identifying microbial functions that govern affect host-microbe associations and beneficial interactions.

498C Single Cell Tools for WormBase

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The number of single cell RNA sequencing (scRNAseq) publications has exploded in recent years, with over 1200 studies currently available and over 350 new studies in 2020 alone. This wealth of data presents new challenges and opportunities on how to integrate, query, and display results in ways that are useful and easy to use for scientists.

Over 85% of scRNAseq studies use human or mouse samples, and the volume of data generated by these studies is so high that their integration and unified management represents a formidable engineering challenge in itself. However for other model organisms such as C. elegans, for which there are only on the order of a dozen scRNAseq studies in the literature, data integration and maintenance of tools covering most of the published data is manageable by a small team with simpler tools.

This offers a fantastic opportunity for WormBase and other model organism databases that are part of the Alliance of Genome Resources (alliancegenome.org). We are yet to be engulfed by the tidal wave of high throughput single cell data that is swamping human and mouse communities, and can learn from their efforts to create tools that will be effective and useful for our communities.

We are presenting an overview of two WormBase single cell tools that are currently in development:

1) A differential expression tool

for selecting cell groups to compare and visualizing dynamically generated results. In the backend the scvi-tools framework (scvi-tools.org) is used for Bayesian decision theory to detect differentially expressed genes.

2) A framework for visualizing static data

that takes in precomputed results in h5ad format, slices the data according to user selection, and returns a visualization. At the moment we have the following visualizations in development:

- Heatmaps & dot plots to visualize mean gene expression across select genes and cell type within an experiment.

- Ridgeline histograms to visualize individual gene abundances stratified by cell type and experiment.

- Swarm plots to visualize expression of multiple genes across all cell types relative to one cell type.

We want to hear your thoughts on these tools!

Please come to chat with us during the poster session or email eduardo@wormbase with your thoughts. You can learn more about each tool the webpage and check their current development status at https://wormbase.github.io/single-cell/

499A Updating the *Caenorhabditis elegans* Natural Diversity Resource Variant Browser

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The *Caenorhabditis elegans* Natural Diversity Resource (CeNDR), has made the natural genetic variation found among *C. elegans* strains publicly available since 2016. Over three million variants from 913 wild *C. elegans* strains can be visualized and explored using the Variant Browser for identifying evolutionary drivers or understanding the genetic basis of quantitative traits. To gauge the relative deleteriousness of these variants, we employ variant effect prediction algorithms that predict the functional effects for each variant in every gene in the N2 reference genome. Because the majority of algorithms employ a similar prediction strategy that does not consider the interaction between variants, especially variants within the same gene, we applied BCFtools csq, which bases its predictions on the collective functional effects of each haplotype. We further expanded our prediction capabilities by adding molecular characterizations of the predicted change. Previously employed annotation software only defined the significance of each variant based on its consequence, limiting the data presented when filtering for high-impact variants. With the addition of two amino acid substitution scoring matrices (BLOSUM62 and Grantham), which take into account the probability and molecular distance of each amino acid change, we can predict the significance of each protein coding variant more accurately. We also have taken into account the relative position of each amino acid change by calculating the percent protein at which each substitution occurs, which is useful for predicting the significance of nonsense and frameshift variants. Ultimately, by using a more accurate prediction algorithm and adding additional molecular characterizations, we provide users of the CeNDR Variant Browser better functional predictions of the millions of genetic variants found in the natural populations of *C. elegans*, enhancing future genetics research in this powerful model organism.

500B Novel tools for analysis of *C. elegans* gene expression data based on organism-wide ICA-derived gene co-expression modules

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Nearly half of *C. elegans* genes lack a functional annotation and there is an unmet need for a method to detect biological signals from gene expression data that is not based on pre-assigned annotations, like GO terms. To that end, using a method based on independent component analysis we call DEXICA, we have defined and optimized a worm-wide atlas of high-quality fundamental gene co-expression modules. These 209 DEXICA modules represent responses to changes in the environment (e.g. starvation, exposure to xenobiotics), genes regulated by transcriptions factors (e.g. ATFS-1, DAF-16), genes specific to tissues (e.g. neurons, muscle), genes that change during development, as well as other complex transcriptional responses to genetic, environmental and temporal perturbations.

Because genes are grouped into modules based purely on how they behave in a large number of experimental assays, modules can provide an unbiased way to probe the hard-wired structure of the animals' gene expression circuitry. We show that interrogation of DEXICA modules reveals processes that are activated in long-lived mutants in cases where traditional analyses of differentially expressed genes fail to do so, such as activity of HIF-1 and ATFS-1 transcription factors in *isp-1* respiration mutants. These results indicate that DEXICA modules can reveal functionally relevant signals within transcriptomic data that otherwise would be missed due to incomplete knowledge of gene function, subtle gene expression changes or noisy data.

Additionally, we show that gene co-expression modules can inform the strength of the association between a gene and an annotation (e.g. GO term). Analysis of "module-weighted annotations" improves on several aspects of traditional annotation-enrichment tests and can aid in functional interpretation of poorly annotated genes.

We provide an online interactive resource at http://genemodules.org/, with tutorials, in which users can find detailed information on each module, check genes for module-weighted annotations, and use both of these to analyze their own transcription data or gene sets of interest.

501C Caenorhabditis Genetics Center (CGC)

Ann Rougvie[1], Aric Daul[1], Julie Knott[1], Theresa Stiernagle[1], Marcus Vargas[1], Liz Fox[1] 1) Univ Minnesota.

The Caenorhabditis Genetics Center (CGC) promotes *C. elegans* research by curating important, genetically characterized nematode stocks and distributing them upon request 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 nominal user fees. We strive to have at least one allele of every published gene and all useful chromosome rearrangements, duplications and deficiencies. Our catalog of more than 23,000 different strains also includes selected multiple-mutant stocks, tagged endogenous loci, and genetic tool strains for various applications such as inducible gene expression. Despite a university-mandated 7-week closure due to the Covid-19 pandemic, we shipped over 21,500 strains in 2020. A small research component has enhanced the genetic tool-kit available to *C. elegans* researchers, in part by labeling existing balancer chromosomes with fluorescent markers and deleting or tagging miRNA loci. Requests for generation of specific miRNA deletions or balancer modifications are considered. A searchable list of strains, including information about each stock, is accessible through the CGC website (cgc.umn.edu) and WormBase. Orders should 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 retain our funding by remembering to acknowledge the CGC in your publications!

502A WormCat 2.0: improving annotations and visualization for RNA seq, genetic screens, or proteomics data

Daniel Higgins[1], Dominque Lui[2], *Amy Walker*[2] 1) Georgia Tech; 2) UMASS Medical School.

Evaluation and interpretation of large data sets requires tools to identify statistically enriched characteristics of the data and visualize the results. We developed WormCat as a web-based tool to find categories of enriched genes in RNA sequencing (RNA seq) data and to produce graphs that allowed for comparison of multiple datasets in an intuitive manner. This tool utilizes a grouping of nested categories to annotate nearly all C. elegans genes. Annotation is based first on physiological function, and if that can not be assigned, then each gene is assigned a molecular or cell-location based category. If genes do not have a predicted function, they are placed in an "Unassigned" category. This is in contrast to commonly used Gene Ontology (GO) servers which commonly exclude around 30% of C. elegans genes that lack GO annotations. This alters category enrichment statistics by eliminating genes of unknown function relative to genes that are functionally annotated. We previously found that WormCat identified enriched gene sets not predicted by commonly used GO servers.

In WormCat 2.0 we have enhanced the capabilities of the web-based tool, updated the annotation list and included an annotation list specific to protein-coding genes for proteomics searches. We have also performed validation of additional datasets comparing inclusion or exclusion of the "Unassigned" genes. First, we found that while the categories with the highest enrichment values were not changed by the exclusion of the "Unassigned" genes, enrichment scores closer to the significance threshold were lost. Enrichment tools such as WormCat are used to predict gene sets of interest for further experimental analysis. Thus, including all genes in the hypothesis space, regardless of our ability to functionally annotate them, is important to provide the most appropriate enrichment scores. Finally, in the analysis of published tissue-specific RNA seq data sets, we found that enrichment in "Unassigned" genes was not uniformly distributed among tissues. This suggests that identification of these genes sets through enrichment scoring may stimulate exploration of their function.

503B Discovery of new small molecule inhibitors of methyltransferase G9a for Alzheimer's disease treatment

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Alzheimer's disease (AD) is the most important neurodegenerative disorder, with no effective cure. The epigenetic control of gene expression might be affected by the addition and/or removal of post-translational changes. Alterations of these modifications are linked with altered gene expression, resulting in cognitive decline. G9a is a lysine methyltransferase that forms a heterodimeric complex able to mono- and di-methylate lysine 9 of histone H3 (H3K9me1 and H3K9me2) of the N-terminal tail. Those epigenetic modifications lead to transcription repression, synaptic plasticity reduction, autophagy dysfunction, increased OS, and neuroinflammation. Hence, we hypothesize that overexpression of the G9a and its repressive mark serves as a driver of the repression of learning memory genes. Novel strategies are based on the synthesis of epigenetic drugs, regulating gene expression and translation modifications. While several small-molecules have been published to inhibit G9a, one of the most common problems of the firsts inhibitors, as BIX01294, is that the concentration values of the toxicity range and the last activity were akin one to the other, strongly limiting its uses. Moreover, these inhibitors were not or low effective in *in vivo* studies.

In our study, several compounds were identified as a potential inhibitor through structure-based virtual screening of the Cambridge CNS MPO library of more than 4,50,000 compounds against G9a (PDB id: 5TTF) Maestro software (Schrodinger), and further *in vivo* screenings. Firstly, we assessed the IC_{50} of eleven compounds using AlphaLISA® technology. To identify the best new candidates G9a inhibitors for AD, a compound screening method using transgenic *Caenorhabditis elegans (C. elegans)* was used to characterize and select the most promising results for further studies. Among all the compounds, four were confirmed as the best candidates through different in vivo assays, such as food clearance and motility assays, which revealed that toxicity/function ranges were safe in *C. elegans*. The treatment with all G9a inhibitors recovered the age-dependent paralysis presented by the CL2006 strain, like the well-established G9a inhibitor, UNC0638.

Thus, this work presents 4 candidates (called "CAB1, CAB2, CAB3 and CAB4") as inhibitors of G9a with novel structures, providing both leads in G9a inhibitors design and demonstrating the participation in AD pathology.

504C The effect of age on epigenetic transgenerational reprogramming in the *C. elegans* germline

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In 1972 Beguet observed that parental age affects fertility of the following generation in wild type worms (*C. elegans*). Offspring from the older hermaphrodites had a smaller brood size compared to their siblings from younger parents. To determine if this effect of age on the fertility of progeny is due to defects in maternal epigenetic reprogramming, I performed the analogous experiments in *spr-5* mutant worms. SPR-5 (LSD1/KDM1A) is a histone demethylase that removes the methylation on histone 3 lysine 4 (H3K4me2) from actively transcribed genes between generations. Our lab has previously shown that *spr-5* mutant *C. elegans* have a transgenerational sterility phenotype, due to increasing H3K4me2. Remarkably, I found that progeny of *spr-5* mutant worms have a further compromised fertility with advanced maternal age (AMA) compared to wild type. In addition, I found that progeny of *spr-5* mutant worms from young maternal age (YMA) also have reduced fertility. To further address whether these defects may be due to increasing H3K4me2, I determined whether they are exacerbated in later generation *spr-5* mutants that have increased H3K4me2. Consistent with this possibility, the difference between the YMA and AMA groups from the peak maternal age progeny (PMA) is exacerbated progressively in later

generations. My results confirm Beguet's original findings and suggest that the effect of parental age on reduced fecundity in the offspring may be through compromised H3K4me2 reprogramming at fertilization.

Understanding the potential link between maternal age and maternal epigenetic reprogramming is important because a) the effect of age on fecundity that I observe is reminiscent of the maternal age effect on the rate of autism, b) mice that are mutant maternally for the SPR-5 homolog LSD1 have autism-like behavior, and c) patients with LSD1 mutations display autism-like symptoms. Thus, these experiments may provide a foundation for understanding these potential connections.

505A The transgenerational accumulation of repressive H3K9me2 affects health and lifespan in *C. elegans*

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In *Caenorhabditis elegans*, 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 aspects of *wdr-5* mutant longevity require the H3K9me2 methyltransferase MET-2 and are recapitulated by removal of the putative H3K9me2 demethylase JHDM-1. In both *wdr-5* mutants and *jhdm-1* mutants, the transgenerational acquisition of longevity is associated with a generational increase of the repressive modification H3K9me2. These results suggest that repressive chromatin facilitates the transgenerational establishment and inheritance of a complex trait. Intriguingly, we find that although both mutants eventually attain longevity, they do so with different generational dynamics and striking differences in health. Therefore, the genomic accumulation of repressive H3K9me2 may target pathways that will shed light on the complicated relationship between health and lifespan.

507C Understanding the role of SAM synthases under heat stress

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S- adenosyl methionine (SAM) donates methyl groups to histones modified during gene regulatory processes. SAM is produced by the 1-carbon cycle and this interaction provides a key link between metabolic status and transcriptional control. We previously found that RNA interference with *sams-1*, one of the four SAM synthases in *Caenorhabditis elegans,* reduces SAM levels as well histone H3 lysine 4 methylation (H3K4me3) in intestinal cells. *sams-1* deficient animals also have altered stress responses. They are unable to accumulate H3K4me3 at promoters of bacterial response genes, upregulate these genes or survive pathogenic challenge. In contrast, *sams-1* animals have enhanced survival during heat shock and regulate heat shock gene expression normally, although metabolic gene expression is broadly downregulated. Several lines of evidence suggest SAM from a distinct synthase may be important. First, interference with the H3K4me3 transferase *set-16* had reduced survival in heat shock. Second, H3K4me3 levels recovered in heat shock in *sams-1* animals, suggesting SAM from a distinct synthase and/or activity histone modifying enzymes could contribute to this stress response. Here, we show that *sams-1* and *sams-4* have distinct roles in the heat shock response, showing that the enzymatic source of SAM is a critical predictor of SAM function. Loss of *sams-4* is distinct from *sams-1* in several critical areas. *sams-4* animals survive poorly after heat shock and is necessary for the recovery of H3K4me3 after heat shock. Next, *sams-4(RNAi)* animals do not exhibit the same reductions in metabolic gene expression during heat shock. Finally, *sams-1* and *sams-4* RNAi animals have distinct patterns of H3K4me3 in both basal and heat shocked conditions in CUT&Tag assay measuring genome wide H3K4me3, with *sams-1* animals losing enrichment at metabolic genes, similar to reductions in these genes in RNA seq. Taken together, this shows that the enzymatic source of SAM influences H3K4me3 patterns, gene expression patterns and physiological response to stress.

508A Horizontal and vertical transmission of transgenerational memories via the *Cer1* transposon

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Animals face both external and internal dangers: pathogens threaten from the environment, and unstable genomic elements threaten from within. Previously, we discovered that a single exposure to purified small RNAs isolated from pathogenic *Pseudomonas aeruginosa* (PA14) is sufficient to induce pathogen avoidance in the treated worms and in four subsequent generations of progeny. The RNA interference (RNAi) and PIWI-interacting RNA (piRNA) pathways, the germline, and the ASI neuron are all required for avoidance behavior induced by bacterial small RNAs and for the transgenerational inheritance of

this behavior. A single *P. aeruginosa* non-coding RNA, P11, is both necessary and sufficient to convey learned avoidance of PA14, and its *C. elegans* target, *maco-1*, is required for avoidance. Here we found that these memories can be transferred to naïve animals via *Cer1* retrotransposon-encoded capsids. *Cer1* functions at the step of transmission of information from the germline to neurons, and is required for *C. elegans*' learned avoidance ability and for mothers to pass this information on to progeny. The presence of the *Cer1* retrotransposon in wild *C. elegans* strains correlates with the ability to learn and inherit small RNA-induced pathogen avoidance. Together, these results suggest that *C. elegans* has co-opted a potentially dangerous retrotransposon to instead protect itself and its progeny from a common pathogen through its inter-tissue signaling ability, hijacking this genomic element for its own adaptive immunity benefit.

509B Repressive histone marks-associated reproductive defects in *Caenorhabditis elegans* exposed to chemical additives in plastics

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Histone modifications, one of the central epigenetic process, have been proposed as the toxic mechanism of various environmental chemicals due to its role in transcription regulation and many other biological processes. In this study, we aimed to figure out the role of repressive histone marks in environmental chemicals-induced toxicity in *C. elegans*. To this end, reproductive toxicity and alteration of histone methylation status under exposure to prevalent chemical additives in plastics (Bisphenol A, Di(2-erhylhexyl) phthalate, 3.3'-5.5'-Tetrabromobisphenol, Hexabromocyclododecane, Triclosan) were examined in *C. elegans* model. Then, we observed whether the adverse effects by chemicals were restored in combinational exposure with histone methyltransferase (HMT) or histone demethylase (HDM) inhibitors, which target to H3K9 and H3K27 methylation. Our results showed the higher toxicity of triclosan and 3.3›-5.5›-Tetrabromobisphenol than other three chemicals and increased protein expression level of H3K9me3 and H3K27me3 with exposure to these two additives. Interestingly, the recovery of reproductive defects induced by two additives occurred following target HMT inhibitor exposure, suggesting that repressive histone modification play a key role in adverse outcome of certain additives. To further understand epigenetically-regulated transcriptional responses, gene expression analysis would be needed in the same manner of this study. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT, Ministry of Science and ICT) (No. 2020R1A2C3006838).

510C Roles of the histone variant H2A.Z in post-embryonic development of C. elegans

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Histone variants contribute to the structural organization of chromatin and regulation of genome functions through their deposition into the nucleosome. The histone variant H2A.Z, one of the H2A variants, is evolutionarily conserved and is suggested to be involved in various biological phenomena, including post- embryonic development and tumorigenesis. However, since H2A.Z knockout vertebrates show embryonic lethality, the function of H2A.Z in post-embryonic development remains unclear. In this study, we investigated the roles of H2A.Z in post-embryonic development with C. elegans. To avoid the effect of a load of maternal H2A.Z gene transcripts, we attempted to use the auxin-inducible degron 2 (AID2) technology (Yesbolatova *et al*., 2020). We introduced GFP and degron tags into the locus of *htz-1* (H2A.Z gene of C. elegans) using the CRISPR/Cas9 system and established animals expressing endogenous GFP::degron::HTZ-1 together with the AtTIR1(F79G) mutant. The treatment of the 5-Ph-IAA ligand for AtTIR1(F79G) induced the depletion of GFP::degron::HTZ-1 within two hours, allowing us to knockdown H2A.Z in stage- and cell-specific manners. The depletion of H2A.Z at the L1 larval stage results in the early larval arrest before or at the L2 stage. Also, we found that H2A.Z knockdown at the late L2 larval stage caused sterility. These results suggested that H2A.Z has essential functions during post-embryonic development at multiple developmental stages. Then, to reveal the cause of their sterility, we characterized the sterile animals by staining DNA with DAPI. Microscopy analysis revealed morphological abnormalities of sterile animals, including germline masculinization and endomitotic oocytes. These observations suggest that H2A.Z has essential roles in the germline sex determination and proper cell function of germ cells after the L2 larval stage. It is expected that the conditional H2A.Z knockdown animals contribute to further analyses of the stage- and tissue-specific roles of H2A.Z.

Reference: Yesbolatova, A. *et al*. The auxin-inducible degron 2 technology provides sharp degradation control in yeast, mammalian cells, and mice. *Nat. Commun*. **11**

, (2020).

511A Redundant Mechanisms of X Chromosome Repression in the *C. elegans* Male Germline

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Organization of the genome into domains of euchromatin and heterochromatin is a conserved feature of all eukaryotes and precise regulation of these domains is important for organism health and development. Proper formation of heterochromatin is crucial for transcriptional repression, chromosome segregation, and maintenance of genome integrity. Heterochromatin can be categorized as either facultative or constitutive. These two types of heterochromatin are often distinguished by their associated histone modifications: methylation of lysine 27 or lysine 9 on histone H3. H3K27me is associated with facultative heterochromatin, and its domains are found throughout genomes, often with developmentally regulated genes. H3K9me is associated with constitutive heterochromatin, and is generally concentrated in gene-poor, repeat-rich regions such as pericentric regions. Thus, anticorrelation of H3K27me and H3K9me domains is observed in many model organisms. However, in *C. elegans*, H3K27me and H3K9me domains show a surprising amount of positive correlation, suggesting a species-specific mechanism for organizing facultative and constitutive heterochromatin.

In the *C. elegans* germline, H3K27me is enriched on the X chromosome, and its loss leads to sterility in the F2 generation. Interestingly, H3K27me(-) F2 males that inherit a paternal X chromosome (Xp) and no maternal X chromosome (Xm) are usually fertile. The fertility of these males is dependent on H3K9me, which is enriched on the single X chromosome in the male germline. These observations suggest a potential redundant function of H3K27me and H3K9me in repressing the X chromosome in the male germline to promote fertility in subsequent generations.

We are investigating the organization and functions of H3K27me and H3K9me in the *C. elegans* male germline. By generating chimeric animals whose germlines inherit only paternal chromosomes (XpXp), we've shown that a double dose of paternal chromosomes lacking H3K27me can also support fertile hermaphrodite germline development in an H3K9me-dependent manner. We are comparing misexpression of genes and repetitive elements in hermaphrodite and male germlines lacking H3K27me, H3K9me, or both. Lastly, we are using CUT&RUN to examine the distribution of H3K27me in germ cells and to test whether H3K9me regulates this distribution, as it does in Mouse and Neurospora.

512B Chromodomain proteins CEC-3 and CEC-6 promote germ granule integrity and genome stability

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Cooperation between mechanisms that safeguard germline immortality is required for the continual inheritance of genetic information. In *C. elegans,* the interplay between small RNA and chromatin regulation pathways impacts fertility across generations, while disruption to the chromatin landscape can also lead to genome instability. The *C. elegans* chromodomain proteins, CEC-3 and CEC-6, recognize heterochromatin-associated histone H3K9 and H3K27 methylation *in vitro* and *cec-3;cec-6* mutant animals show progressive sterility or a 'mortal germline' phenotype. To understand the mechanism underlying this fertility defect, we are investigating the roles of *cec-3* and *cec-6* in germ cell fate, small RNA pathways and heterochromatin regulation. Live imaging of GFP-tagged PGL-1 suggests disruption of perinuclear RNA germline granules (P-granules) in *cec-3;cec-6* mutant adult germlines. A reporter assay also implicated *cec-3* and *cec-6* in protecting the genome against repeat instability. We are also examining the effects of *cec-3* and *cec-6* on the expression of repetitive elements, transposons and endogenous siRNA targets. 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.

513C Temporary loss of the shelterin proteins POT-1 or POT-2 alters telomeric protein localization for multiple generations

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Telomere proteins protect the ends of linear chromosomes from shortening during cell replication. Deficiency for telomerase results in loss of telomeric sequence across multiple generations. However, there is no described role for telomeres in transgenerational epigenetic inheritance. The *C. elegans* Pot1 homologues POT-1 and POT-2, members of the shelterin complex, form foci at telomeres in germ cells. These foci disappear by fertilization and gradually accumulate during early development. We find that mutation of either Pot1 gene in sperm or oocytes alters levels of telomeric foci for multiple generations, despite progeny possessing wild-type Pot1 genes. *pot-2* mutant gametes gave rise to progeny with abundant

POT-1::mCherry and mNeonGreen::POT-2 foci during early development, an effect which persisted for six generations. In contrast, *pot-1* mutant gametes gave rise to progeny with fewer foci for several generations. We investigated a range of genes involved in epigenetic inheritance. While Pot1 foci were not affected by loss of genes involved in sRNA pathways, they were greatly diminished for at least two generations by the loss of MET-2, SET-25, or SET-32, three methyltransferases that promote heterochromatin formation. We propose that POT proteins may interact with H3K9 methyltransferases during gametogenesis to induce a persistent form of transgenerational epigenetic inheritance that influences telomeric heterochromatin.

514A Investigating the role of the chromatin remodeler LET-418/Mi2 in gene regulation and chromatin landscape during post-embryonic development of *Caenorhabditis elegans*.

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Chromatin is a dynamic structure which is condensed or relaxed to allow tissue specific expression of developmental genes. Mi2 proteins are among conserved ATP-dependent enzymes that shape chromatin during development. In *Caenorhabditis elegans* the Mi2 homolog, LET-418, operates during developmental processes such as germ cell fate maintenance, blast cell proliferation and differentiation and vulval development.

To better understand the role of LET-418 chromatin remodeler, we analysed its transcriptional output at different life stages of *C. elegans*: embryo, L1 larvae and young adult. Other than an increase in the number of deregulated genes through the progression of the life cycle, a unique tissue enrichment signature was observed for each life stage. In addition, we found that a subset of upregulated genes at L1 and adult stage were organised in clusters. Most of these gene including *pals*, *math*, *clec* and *fbxa* gene families are involved in proteostasis and immune response. Using qRT-PCR we validated the upregulation of a subset of these genes, as well as additional genes located in the clusters. These results suggest that LET-418 may act globally on chromatin domains that contain transcriptionally co-regulated genes.

To determine if LET-418 is binding directly to these chromatin regions, we are currently performing DamID profiling experiments, which rely on the expression of LET-418 fused to the *E. coli* methylase in the worm. Furthermore, to test if the genome localisation of these LET-418 regulated genes is essential, we are generating transgenic strains expressing representative genes from a different region of the chromosome.

Altogether, these results will allow us to determine whether LET-418 is regulating these gene clusters as a chromatin domain located at a specific locus in the genome. Finally, to know whether this chromatin-based regulation of immune response genes is relevant to the pathogen response, we will test whether *let-418* mutants are more resistant to pathogens.

515B Regulation of embryonic cell specification by histone methylation

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In *C. elegans*, two epigenetic enzymes, the H3K4me2 demethylase, SPR5, and the H3K9 methyltransferase, MET-2, are maternally deposited into the oocyte and cooperate to reestablish the epigenetic ground state by modifying histone methylation. Progeny of worms lacking *spr-5* and *met-2* accumulate high levels of H3K4me2 in the subsequent generation. This inappropriate H3K4me2 is associated with a low level of embryonic lethality, as well as a severe developmental delay and sterility in the small number of animals that reach adulthood. In addition, the progeny of *spr-5;met-2* mutants improperly express germline genes in somatic tissues. We hypothesized that this improper expression could result in cell lineage defects. To interrogate how reprogramming defects may affect early embryonic development, we are taking advantage of the invariant *C. elegans* embryonic lineage by performing automated lineage tracing experiments in *spr-5;met-2* progeny. Thus far, we observe several stages of defects. First, in early embryogenesis, the shape of the embryos is rounder, with a diameter that is greater than Wild Type, and the mutant cells divide faster than Wild Type. Since these defects are prior to the onset of zygotic transcription, they may be due directly to the decondensed state of chromatin associated with increased H3K4me2, or indirectly due to altered maternal RNAs and proteins. Second, after the onset of zygotic transcription, we observe many changes in cell timing, along with defects in cell migration. These defects may be due to the inappropriate somatic transcription of germline genes that we observe in the progeny of *spr-5;met-2* mutants. Interestingly, those defects happened around the 100-cell stage or later. In *C. elegans,* the maternal to zygotic transition start during the early stages, but the higher peak of transition occurs around the 60 to the 100-cell stage. We hypothesized that the effect at this stage is due to the ectopic expression of germline genes, which is a consequence of the inappropriate inheritance of histone methylation. To address this question, we are currently performing single-cell RNA sequencing in the *spr-5;met-2* mutants embryos at different cell stages including the 100-cell stage.

516C Chromatin context in the regulation of germline genes by the zinc-finger transcription factor LSL-1

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Epigenetic and transcription factors control specific gene transcription profiles that instruct cells to proliferate and differentiate throughout development. Underlying this process the chromatin structure is dynamically shaped by different enzymatic complexes called chromatin remodelers. Among them, the *C. elegans* Mi2 homolog LET-418/Mi2 is required for post-embryonic development and ensures that the germline-specific transcriptional program is silent in somatic cells. By performing a genome-wide RNAi screen, we identified a *let-418* genetic interactor, *lsl-1*, which encodes a close homolog of the human ZFP57 zinc-finger transcription factor involved in imprinting.

LSL-1 is a germ cell specific protein, whose expression is first observed in the P4 blastomere and maintained in the germ cells through adulthood. Absence of LSL-1 activity leads to various defects. Germline nuclei do not progress normally through meiotic prophase and aberrant chromatin organization and nuclear morphology is observed during homologous chromosome pairing in *lsl-1* mutants. Furthermore, an increased level of apoptosis, that relies on pairing and DNA damage checkpoints, is indicative of a high level of genome instability. By analysing the transcriptome of LSL-1 together with the ChIP-seq data available from the modERN (ENCODE) resource, we found out that LSL-1 is acting as a transcriptional activator of germline genes which are involved in different aspects of germline development, including meiotic chromosomes pairing and genome stability. Finally, we show that these defects are partially dependent on the chromatin proteins LET-418/Mi2 and HPL-2/HP1. We propose a model where the transcription factor LSL-1 is antagonizing repressive chromatin formation by LET-481/Mi2 and/or HPL-2/HP1 at germline gene promoters to allow expression of the proper gene repertoire for gamete production. In the other hand, absence of LSL-1 from somatic cells ensures that germline genes remain silent. Further molecular analysis of this interaction will let us know how LSL-1 is modifying chromatin accessibility to allow transcription.

517A Independent initiation and maintenance of germline and somatic epigenetic silencing

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Transgenerational epigenetic inheritance of *sid-1* silencing (*sid-1* TEI) is one of the few well-characterized examples of TEI that manifests in both germline and somatic tissues. Somatic *sid-1* TEI requires chromatin-modifying factors, whereas germline silencing depends solely on RNAi factors indicating distinct mechanisms. To further characterize the roles and inter-dependency of nuclear and cytoplasmic RNAi machinery in the initiation and inheritance of *sid-1* TEI, we used a unigametic germline inheritance system. In this system, maternal and paternal pronuclei do not fuse at fertilization and correspondingly form AB and P1 cell lineages. In the case of an embryo produced by epigenetically silenced *sid-1* mother, the naïve paternal pronucleus that forms germline cells is exposed to maternal cytoplasmic but not nuclear TEI factors. Therefore, F2-self progeny derived entirely from the paternal genome may inherit silencing provided by only cytoplasmic TEI information. We found these animals to be fully resistant to germline *dpy-11(RNAi)* (*sid-1* silenced) for at least six generations. This result demonstrates nuclei-independent transmission of transgenerational *sid-1* germline silencing. Surprisingly, the germline-silenced F2 animals failed to show any resistance to somatic *dpy-11(RNAi).* This result implies that nuclear TEI machinery is not required to transmit somatic *sid-1* silencing. Moreover, somatic *sid-1* silencing was not observed during the six generations of persistent germline silencing. This indicates that germline silencing is not sufficient to prime somatic silencing.

Taken together, our results put forward a working hypothesis that nuclear factors from the silenced parent are required to establish somatic silencing and that somatic and germline silencing are initiated and maintained independently.

518B Defining the functional components of constitutive heterochromatin through genetic interaction screening

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Constitutive heterochromatin makes up 20-50% of animal genomes and is associated with the silencing of gene expression, including that of repetitive elements. It is enriched for repressive histone modifications H3K9me2 and/or H3K9me3, which act as a binding sites for chromodomain-containing proteins related to HP1. Heterochromatin-induced gene silencing is an important modulator of developmental transitions, and defects in heterochromatin have been linked to a variety of human diseases, including cancer. The mechanisms through which heterochromatin is established and maintained have been extensively studied in the single-celled yeast *Saccharomyces pombe,* but they are not well understood in animals.

We seek to identify and functionally dissect components of constitutive heterochromatin in *C. elegans.* Previous work in our lab identified a network of five factors that genetically interact and co-localise with H3K9me2 at repetitive elements (*met-2, set-25, let-418, lin-13 and hpl-2;* McMurchy et al, 2017*)*. These heterochromatin factors regulate transposable element repression, gene expression, DNA repair, fertility and growth in *C. elegans,* in close collaboration with small RNA pathways. In order to expand this network, we carried out multiple genetic interaction screens using RNAi to discover genes whose knockdown could enhance or suppress the phenotypes of heterochromatin-defective mutants. Strains we screened include mutants of H3K9 methyltransferases (*met-2, set-25),* HP1 orthologues (*hpl-1, hpl-2*), and HP1-interacting genes (*lin-61, lin-13, tdp-1*). We identified genetic interactions through RNAi, using a panel of 2288 RNAi clones that target genes known or predicted to encode nuclear localised proteins.

The screens identified shared as well as unique enhancers and suppressors of the heterochromatin-defective mutants and highlighted components of ubiquitylation, SUMOylation, RNA splicing and chromatin remodelling pathways, with most hits having orthologues in humans. Our results expand knowledge of the network of functional players in heterochromatin formation and function in a metazoan organism and identify targets for in depth studies in *C. elegans* and human cells.

519C Control of *C. briggsae* germline development by TRA-1-interacting co-factors

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Chromatin remodelers work with transcriptional regulators to control gene expression during development. Many studies have focused on the role transcription factors play in evolutionary change, but much less is known about the role of chromatin remodelers. Gli proteins form a conserved group of Zn-finger transcription factors that regulate many important developmental processes. In *Caenorhabditis* nematodes, TRA-1 is the sole Gli protein, and acts as the terminal regulator of the sex-determination pathway. Both the activator and repressor functions of this Gli protein can be studied in the germ line, where they regulate the sperm/oocyte decision. TRA-1 activity is influenced by several chromatin regulators that may work as co-factors. In *C. briggsae,* it interacts genetically with TRR-1 (part of the TIP-60 HAT complex), WDR-5 and the NURF complex to promote spermatogenesis. We are studying epigenetic interactions in the germ line to learn how each co-factor works with TRA-1 to control its target *fog-3*. We made these strains to allow us to alter germ cell fates: *Cbr-glp-4 (v473ts) I* and *Cbr-trr-1(v104) II*. The *glp-4* strain is temperature sensitive for germline development, just as the orthologous mutation is in *C. elegans,* and *trr-1* mutants have decreased Tip-60 activity causing 70% of the worms become female. Next, we produced alleles of *Cbr-tra-1* with either N-proximal (*Cbr-tra-1-N-OLLAS (V455) III*) or C-proximal (*Cbr-tra-1-c-OLLAS (V424) III)* OLLAS tags that retain wildtype function. We performed anti-H3K4me3 and anti-OLLAS ChIP-qPCR to amplify the TRA-1 binding site from the *fog-3* promoter at different developmental stages, focusing on the L1 stage (long before spermatogenesis), L3 stage (initiation of spermatogenesis) and L4 stage (completion of spermatogenesis). The H3K4me3 CHIP signal gradually decreased with age, so it appears to act in early stages of germline development and not in the later stages. Perhaps one WDR-5 function is to promote early germline development by marking genes for potential transcription. Preliminary results with OLLAS CHIP revealed higher levels of TRA-1 at *fog-3* when we used the N-terminal OLLAS strain (which should detect both TRA-1 activator and repressor) than the C-terminal strain (which should only detect activator).

520A H3K4me2 regulates the recovery of protein biosynthesis and homeostasis following DNA damage

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DNA damage causes cancer, impairs development and accelerates aging. Transcription-blocking lesions and transcription-coupled repair defects lead to developmental failure and premature aging in humans. Following DNA repair, homeostatic processes need to be reestablished to ensure development and maintain tissue functionality. Here, we report that, in *Caenorhabditis elegans*, removal of the WRAD complex of the MLL/COMPASS H3K4 methyltransferase exacerbates developmental growth retardation and accelerates aging, while depletion of the H3K4 demethylases SPR-5 and AMX-1 promotes developmental growth and extends lifespan amid ultraviolet-induced damage. We demonstrate that DNA-damage-induced H3K4me2 is associated with the activation of genes regulating RNA transport, splicing, ribosome biogenesis and protein homeostasis and regulates the recovery of protein biosynthesis that ensures survival following genotoxic stress. Our study uncovers a role for H3K4me2 in coordinating the recovery of protein biosynthesis and homeostasis required for developmental growth and longevity after DNA damage.

521B Towards the mechanistic understanding of H3K23me3 in transgenerational epigenetics

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We wish to understand the regulation and function of H3K23me3, an understudied histone modification that is conserved across the eukaryotic domain. The germline nuclear RNAi pathway in *C. elegans* has been used as a powerful paradigm to uncover novel mechanisms of transgenerational epigenetic inheritance. We recently discovered that nuclear RNAi induces H3K23me3, in addition to the well-known H3K9me3 and H3K27me3, at target genes. The H3K23me3 mark is deposited by histone methyltransferase SET-32 [1]. Absence of SET-32, but not the H3K9 HMTs (MET-2 and SET-25), is linked to a defect in transgenerational establishment of heritable gene silencing [2,3].

We found that *set-32* single mutation does not fully abolish H3K23me3, indicating the existence of additional H3K23 HMT(s) [1]. In this study, we took a combined approach of genetics, biochemical and genomic experimentation to identify additional H3K23 HMTs. So far, we have collected RNA-seq, sRNA-seq, and ChIP-seq data and performed initial transgenerational phenotypic analysis for some mutant animals. To date, we have found a putative H3K23 HMT, which together with SET-32, is required for nuclear RNAi and robust germline development at the restrictive temperature. Both whole-genome and phenotypic analyses for the putative H3K23 HMT will be presented at the conference. Currently, we are investigating why H3K23me3 is needed for nuclear RNAi by examining its roles in regulating other components of the pathway, including siRNA and other histone modifications. We hope these results will help us achieve the long-term goal of understanding the channels and barriers of epigenetic inheritance.

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522C Histone methyltransferase inhibitor chaetocin strongly and specifically affects metal responsive genes

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A gene pair termed *numr-1/2* (nuclear-localized metal-responsive) was previously shown to encode an SR-like protein with an RNA-recognition motif that promotes longevity and growth in cadmium. We recently used a genome-wide screen to demonstrate that *numr-1/2* is activated by disruption of RNA metabolism. To investigate *numr-1/2* regulation and identify modulators of nucleic acid metabolism, we screened over 40,000 compounds and extracts from commercial and natural product libraries for *numr-1/2p::GFP* activation. We identified six *numr-1/2* inducers; three (floxuridine, 5-fluouracil, and actinomycin D) have well-characterized effects on DNA and RNA confirming that *numr-1/2* is regulated by changes in nucleic acid metabolism. Fungal toxin chaetocin was the most potent and least toxic *numr-1/2* inducer. RT-qPCR demonstrates that chaetocin induces *numr-1/2* and another stress-responsive SR-like protein gene (*W03G1.5*) over 50-fold within 45 minutes without affecting expression of canonical heat shock, osmotic stress, endoplasmic reticulum stress, mitochondrial stress, or detoxification response genes. Chaetocin does not activate other metal-responsive genes and actually reduces expression of metallothionein gene *mtl-2* and fluorescence of *mtl-2p::GFP* consistent with repression of *mtl-2* transcription. A time-course experiment demonstrated that induction of *numr-1/2* precedes decreases in *mtl-2* by 1-2 hours. We find that *numr-1/2* is required for chaetocin to repress *mtl-2* fully, consistent with NUMR-1/2 negatively regulating *mtl-2* transcription.

Chaetocin is a promising anticancer candidate in cell culture and *in vivo* models; it was first shown to inhibit histone lysine methyltransferase (HMT) SU(VAR)3-9 and later shown to affect thioredoxin reductase. Robust activation of *numr-1/2* in *C.*

elegans provides an opportunity to harness genetic tractability to gain new insights into chaetocin bioactivity. Using a *trxr-1/2* double mutant, we find that chaetocin induces *numr-1/2* independently of thioredoxin reductase. *C. elegans* has over 35 predicted HMTs; we are now testing HMT mutants for induction of *numr-1/2*. Our results raise the possibility that histone methylation dynamically regulates stress-responsive genes in terminally differentiated somatic cells.

This work was supported by NSF grant IOS-1452948 and a UF Research Opportunity Seed Fund Grant to KPC, CWW, and HL.

523A Dissecting the structure-function mechanism of SEM-2/SoxC in *C. elegans*

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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 transcription factor, SEM-2, which is widely expressed and is essential for embryonic viability and postembryonic mesoderm development. We have recently identified a new *sem-2* allele, *jj152*. *jj152* is a proline to serine change at amino acid 158, a highly conserved residue located near the end of the DNA-binding domain of SEM-2/SoxC. Intriguingly, P158S is located close to the residues mutated in the human SoxC proteins, Sox4 or Sox11, in Coffin-Siris syndrome (CSS) patients. CSS patients have growth and intellectual disabilities. The P158S mutation thus provides us with unique opportunities to dissect the functions of SEM-2/SoxC proteins and determine the mechanisms of the disease-associated mutations in SoxC proteins in an intact living organism. We are conducting a series of biochemical and genetic experiments to determine the functional consequences of the P158S mutation on SEM-2/SoxC function.

Our studies thus far have suggested that SEM-2/SoxC may directly regulate the expression of HLH-8, a basic helix-loop-helix, Twist transcription factor in the postembryonic mesoderm: 1) both *sem-2(P158S)* and *hlh-8* null mutants are egg-laying defective; 2) P158S mutants have reduced expression of two different *hlh-8* transcriptional reporters; 3) the *hlh-8* promoter contains a canonical SoxC DNA-binding motif, which is required for *hlh-8* promoter activity. We are currently using CRISPR to generate an endogenous *hlh-8* transcriptional reporter and testing the role of the putative SoxC binding site in endogenous *hlh-8* expression. We are also testing whether SEM-2/SoxC directly binds to this DNA motif in the *hlh-8* promoter, and whether the P158S mutation affects the DNA-binding affinity of SEM-2. Results from these studies will add to the understanding of SEM-2/SoxC-mediated regulatory network in the *C. elegans* mesoderm.

524B Studying chromatin regulation at single cell resolution during *C. elegans* postembryonic development

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Despite containing identical genomes, developing cells differentiate into a plethora of diverse cell types. Distinct patterns of gene expression now form the basis for classifying different cell fates. How the combinatorial activity of transcription factors, chromatin regulators and histone modifications achieve the proper spatiotemporal patterns of gene expression is a major question in developmental biology. Biologists increasingly appreciate the need to investigate gene expression regulation at the single-cell level because much heterogeneity and complexity is lost when averaging across populations of cells. However, profiling chromatin at the single cell level is challenging due to limited input material.

Chromatin immunocleavage with sequencing (ChIC-seq) is an efficient method to study chromatin modifications from low input samples. ChIC-seq utilises antibody targeted micrococcal nucleases, leading to controlled, binding-dependent enzymatic digestion of DNA. This releases short fragments which become preferentially incorporated during library preparation and enables high resolution mapping of genomic positions. Crucially, the absence of crosslinking and immunoprecipitation steps, required in less sensitive techniques such as ChIP-seq, leads to minimal material loss. Recently, ChIC-seq was used to profile histone modifications in single human cells [1].

Adapting ChIC-seq to profile histone modifications in *C. elegans* will provide a powerful tool for studying the epigenetic regulation of development. Here, we present progress in optimising ChIC-seq for profiling chromatin modifications at single-cell level across a developmental time-course in *C. elegans*. Specifically, we combine Cre/Lox lineage tracing with cell isolation and FACS procedures in order to isolate postembryonic mesoderm cells. Following prolonged quiescence, the mesoblast precursor resumes proliferation and produces fourteen muscle cells, two scavenger cells, and two migratory bipotent myoblasts over 24-hours. By profiling chromatin modifications at high temporal resolution, we aim to reveal regulatory

processes controlling cellular proliferation and differentiation. This work will shed light on how epigenetic modifications contribute to cellular decision making in a living animal.

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525C Precise quantification of mRNAs across all *C. elegans* embryonic stages through a microscopy and machine learning-based approach

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Precise quantification of mRNA transcripts in space and time throughout embryogenesis is essential for understanding gene regulation, a process critical for embryogenesis in all animals, including *C. elegans*. We developed an imaging approach using 3D widefield microscopy-based single-molecule RNA fluorescence in situ hybridization (smFISH) to quantify mRNA transcripts. To count individual single-molecule mRNA spots, we developed RS-FISH, a fast 3D spot detection method that we implemented in Fiji that combines radial symmetry and RANSAC outlier removal. To assign each fixed, imaged *C. elegans* embryo to its developmental stage, we used advanced machine learning-based image classification that relies on the concept of auto-encoders.

Currently, we are applying our methods to understand the role of condensins in chromosome compaction and transcription regulation. In *C. elegans*, an X-specific condensin binds to and represses X chromosomes in XX hermaphrodites by 2-fold for dosage compensation. In our study, we want to understand condensin DC's effect on transcript numbers and dynamics in single embryos across development.

We obtained thousands of smFISH images for a set of condensin DC-regulated and control genes and extracted mature and nascent RNA counts in 3D, which we use to determine transcription burst characteristics throughout embryonic development. The distribution of total transcripts in wild-type and condensin DC-depleted embryos shows that single genes on the X chromosome are downregulated ~2-fold. Our machine learning approach to separate embryo images by development stage allowed us to observe the timing of condensin DC-mediated transcription repression, which occurs from the 100-cell stage on.

RS-FISH is freely available as a Fiji plugin, and details for installation can be found at https://github.com/PreibischLab/RadialSymmetryLocalization and described at https://doi.org/10.1101/2021.03.09.434205

526A An autoregulation loop in *fust-1* for circular RNA regulation in *Caenorhabditis elegans*

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Circular RNAs (circRNAs) are always expressed tissue-specifically, suggestive of specific factors that regulate their biogenesis. Here, taking advantage of available mutation strains of RNA binding proteins (RBPs) in *Caenorhabditis elegans*, I performed a screening of circRNA regulation in thirteen conserved RBPs. Among them, loss of FUST-1, the homolog of FUS (Fused in Sarcoma), caused downregulation of multiple circRNAs. By rescue experiments, I confirmed FUST-1 as a circRNA regulator. Further, I showed that FUST-1 regulates circRNA formation without affecting the levels of the cognate linear mRNAs. When recognizing circRNA pre-mRNAs, FUST-1 can affect both exon-skipping and circRNA in the same genes. Moreover, I identified an autoregulation loop in *fust-1*, where FUST-1, isoform a promotes the skipping of exon 5 of its own pre-mRNA, which produces FUST-1, isoform b with different N-terminal sequences. FUST-1, isoform a is the functional isoform in circRNA regulation. Although FUST-1, isoform b has the same functional domains as isoform a, it cannot regulate either exon-skipping or circRNA formation. Finally, I generated two mutation isoforms (R446S and P447L) of FUST-1 to mimic the amyotrophic lateral sclerosis (ALS)-related natural mutations in the nuclear localization signal (NLS) of FUS (R524S and P525L). I found that although only P447L mutation dramatically affected nuclear localization, circRNA levels were significantly altered in both mutations.

527B Mutations in the mRNA export complex NXF-1/NXT-1 affect heat-shock driven gene-expression

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The heterodimer NXF-1/NXT-1 is a conserved transport receptor essential for the export of messenger RNA (mRNA) from the nucleus to the cytoplasm for subsequent translation. The NXF-1/NXT-1 receptor facilitates this export through interactions with mRNA, the nucleoporins lining the nuclear pore complex (NPC), and interactions with components of two conserved multisubunit TRanscription-EXport complexes, TREX and TREX-2. These complexes play key roles in the biogenesis of mRNA, and their interactions with NXF-1/NXT-1 link mRNA transcription and processing to export. In *Caenorhabditis elegans*, TREX-2 may act as a tether between transcriptionally active gene promoters and the nuclear periphery to concentrate transcription and mRNA processing directly at the NPC (Rohner et al., 2013). This localization at the NPC is observed with the bidirectional heat-shock promoter *hsp-16.2/16.41* during heat-shock in *C. elegans*. In this study, we describe four new alleles found in a genetic screen for suppressors of a toxin expressed under *hsp-16.41* in *C. elegans.* We mapped three of these alleles to missense mutations within the functional domains of NXF-1 and one to the functional domain of NXT-1. These alleles confer no apparent phenotype under normal conditions but suppress the lethality of the toxin PEEL-1 when expressed under *hsp-16.41*. However, when the PEEL-1 toxin is expressed endogenously, the *nxf-1(yak65)* allele does not suppress lethality, suggesting that suppression during heat-shock is unrelated to PEEL-1's native function. We propose that NXF-1/NXT-1 influences the expression of heat-shock activated genes due to an uncharacterized role in the recruitment of the heat-shock promoter to the NPC during heat-shock. This role may be mediated by interaction between NXF-1/NXT-1, nucleoporins, and TREX-2. The novel *nxf-1*/*nxt-1* alleles described in this study may disrupt these interactions and diminish the localization of *hsp-16.41* at the NPC during heat-shock, thus altering the expression of genes driven by this promoter.

528C Condensin I organizes the *C. elegans* interphase genome

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Recent data in mammalian cells have shown the role of cohesin, a Structural Maintenance of chromosomes (SMC) complex, in interphase genome organization into loops and domains. Since these complexes are involved in cell division, it is difficult to evaluate the functional consequences of their absence in animals. Here we use *Caenorhabditis elegans*, an animal, 90% of whose cells are post-mitotic at birth. Similarly to mammals, nematodes have three SMC complexes: cohesin, condensin I and condensin II. Additionally, condensin I^{DC}, a variant of condensin I, is involved in dosage compensation (DC). To uncover which SMC complex(es) organize the *C. elegans* interphase genome and evaluate functional consequences of genome unfolding, we constructed strains in which individual SMC complexes - cohesin, condensin I/I^{DC} and II - are acutely inactivated *in vivo* in fully differentiated animals. We then assessed their phenotype and carried out chromatin conformation capture (Hi-C) and RNA-seq. Our data shows that in contrast to mammalian cells, the major determinant of genome folding in *C. elegans* is condensin I/I^{DC} and not cohesin. Its cleavage reduces short-range contact probabilities and causes genome-wide de-compaction on all chromosomes. In contrast, cohesin cleavage has marginal impact while condensin II cleavage has no consequence on genome folding. RNA-seq data show that about a third of expressed genes are significantly differently expressed upon cohesin and condensin II inactivation, however the effect sizes were very small, and a similar number of genes were up and down regulated. In contrast, cleavage of condensin I/I^{DC} leads to up-regulation of 93% of all expressed X-linked genes versus only 1% down regulated, indicating the necessity of the constant presence of condensin I^{DC} for maintenance of DC. Cleavage of cohesin and condensin II has little effect on animal post-embryonic survival, as their lifespan are similar to control animals. In contrast, inactivation of condensin I/I^{DC} causes drastic reduction in life expectancy; yet additional experiments show that lifespan reduction is due to lack of DC rather than genome unfolding. Taken together, we discovered that condensin I is the main SMC complex folding the nematode interphase genome, yet genome unfolding has no major effect in *C. elegans* in standard laboratory conditions, apart from X-linked gene up-regulation and its consequences.

530B Characterization of the Role of the Terminal Adenosine Located at the pre-mRNA Cleavage Site

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The region downstream of the STOP codon in mRNA, referred to as the 3'Untranslated Region (3'UTR), governs the length of mature mRNA. Specifically, the cleavage site located in this region determines where mRNA cleavage will occur and where polyadenylation reaction will begin, thus terminating mRNA transcription. The mRNA cleavage and polyadenylation machinery

in *C. elegans* is highly conserved to its human counterpart, with most functional domains and critical amino acids preserved. Dysregulation of 3'UTR processing has been observed in many diseases, such as cancer, Alzheimer's disease, and muscular dystrophies, but unfortunately the molecular mechanisms underlying the mRNA transcription termination remain elusive. Although the exact cleavage site is not precise, our lab has identified an adenosine consistently located at the mRNA cleavage site. It is unclear if this adenosine is maintained in the mature mRNA transcripts proceeding cleavage and/or is used as a template for the polymerization of the poly(A) tail. In order to answer this question, we developed a novel terminal adenosine RNA methyltransferase (TAM) assay that will sense the inclusion or exclusion of this terminal adenosine at the cleavage site of *C. elegans*transcripts by taking advantage of the human nuclear methyltransferase, METTL16. METTL16 methylates the underscored adenosine in its binding motif, "UAC**A**

GAGAA", in both mRNA and snRNA. We have cloned both the human METTL16 gene and its RNA recognition motif at the cleavage site of the *C. elegans* gene *M03A1.3*and co-expressed them both in the pharynx tissue. Understanding this process is crucial to identifying the main mechanisms behind mRNA cleavage site determination, further advancing knowledge in gene regulation which influences development, growth, and disease.

531C Modelling mutations in human Argonaute *AGO1* that cause neurodevelopmental disorders: Identical mutations in the *C. elegans* homolog *alg-1* impair *in vivo* microRNA function, with global gene expression perturbance.

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MicroRNAs (miRNA) are small regulatory RNAs that exist in all multicellular eukaryotes. Mature miRNAs are bound by Argonaute proteins to form the miRNA induced silencing complex (miRISC), which post-transcriptionally regulates target gene expression. Recently, multiple *de novo* coding variants in human Argonaute gene *AGO1* have been reported to cause neurodevelopmental disorders with intellectual disability (ID). Interestingly, most of the altered amino acids are conserved between the miRISC Argonautes of *H. Sapiens* and *C. elegans*, suggesting evolutionarily conserved function in the miRNA pathway. To understand how the human *AGO1* mutations may affect miRNA biogenesis and/or function, we chose a set of mutations that were identified as associated with ID in independent families, and used CRISPR/Cas9 genome engineering to introduce the identical mutations in *C. elegans alg-1* gene. We found that several mutations resulted in phenotypes known to be characteristic of *alg-1 loss-of-function*. These phenotypes were remarkably enhanced in the absence of *alg-2*, the *C. elegans alg-1* paralog, suggesting that the impaired functions in *alg-1* are partially redundant among Argonaute genes. Intriguingly, some mutations resulted in retarded heterochronic phenotypes with penetrance greater than that of *alg-1 null* mutation, indicating that these modelled human *AGO1* mutations have antimorphic effects on *alg-1* function in *C. elegans*. To characterize how the mutations impact post-transcriptional gene regulation, we performed ribosome profiling of the mutants and observed perturbation of the translational levels of dozens to hundreds of genes, depend on the mutations. Combining the ribosome profiling analysis with RNAseq, we found that these *alg-1* mutations can affect gene expression levels by perturbing translational efficiency and/or mRNA stability. Interestingly, a large proportion of the genes perturbed by the modelled mutations are known to be expressed in *C. elegans* nervous system, and many of them have human homologs whose dysfunction is known to cause neuronal disorder diseases, including the homeobox gene *alr-1/ARX* which is highly related with intellectual disability. We are currently working on understanding how the modelled human *AGO1* mutations may affect miRNA biogenesis and/or miRISC association in *C. elegans*, and exploring how the *C. elegans* modelling may help illuminate the mechanisms of the corresponding human disorders. We also anticipate that these cross-clade genetic studies may advance the understanding of fundamental Argonaute functions, and provide insights into the conservation of miRNA-mediated post-transcriptional regulatory mechanisms.

532A Cadmium hijacks the high zinc response by binding and activating the HIZR-1 nuclear receptor

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Cadmium is an environmental pollutant and significant health hazard that is similar to the physiological metal zinc. In *C. elegans* high zinc homeostasis is regulated by the HIZR-1 nuclear receptor transcription factor. To define relationships between high zinc homeostasis and the response to cadmium, we identified 145 cadmium-regulated genes. *hizr-1* was necessary for activation of a subset of genes, indicating there are at least two mechanisms of cadmium-regulated transcription. Cadmium directly bound HIZR-1, promoted nuclear accumulation of HIZR-1 in intestinal cells, and activated HIZR-1-mediated transcription via the HZA enhancer. Thus, cadmium binding promotes HIZR-1 activity, indicating that cadmium acts as a zinc

mimetic to hijack the high zinc response. To elucidate the relationships between high zinc and cadmium detoxification, we analyzed three pathways: the phytochelatin pathway strongly promoted cadmium resistance but not high zinc resistance, the *hizr-1* pathway strongly promoted high zinc resistance but not cadmium resistance, and the *mek-1/sek-1* pathway promoted resistance to high zinc and cadmium. These studies identify resistance pathways that are specific for high zinc and cadmium as well as a shared pathway.

533B Parallel genetics of regulatory sequences using induced genome editing

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Understanding how regulatory sequences control gene expression is fundamental to explain how phenotypes arise in health and disease. The functions of regulatory elements must ultimately be understood within their genomic environment and developmental- or tissue-specific contexts. Here, we used induced Cas9 expression and multiplexed guide RNAs in *C. elegans* to create hundreds of mutations in enhancers, promoters and 3′ UTRs of 16 genes in parallel. We then analyzed the resulting complex populations by either selecting for phenotypic traits or reporter expression, or by DNA- or RNA amplicon sequencing of bulk samples. We developed a software pipeline, crispr-DART, to analyze targeted sequencing and describe the characteristics of >12,000 dsDNA break-induced indel mutations. We also analyzed the *lin-41* 3′ UTR and found that the two *let-7* miRNA binding sites can function independently and that one of the sites is more important for mRNA repression and phenotype. In summary, our approach enables highly parallelized functional analysis of regulatory sequences *in vivo*.

534C Polymorphic modifiers of human α-synuclein in *Caenorhabditis elegans*

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Parkinson's Disease (PD) is a common neurodegenerative disorder in elderly humans. PD is characterized by neuronal loss in the substantia nigra and an aggregation of α-synuclein into Lewy bodies. The morbidity in humans is dependent on the genetic background, although the exact mechanisms of background effects of genetic variation are unknown. We turned to an α-synuclein aggregation model in the model nematode *Caenorhabditis elegans* to investigate the role of genetic variation on α-synuclein aggregation.

We mapped regulators of gene expression (eQTL) in Recombinant Inbred Lines (RILs) constructed by crossing *C. elegans* strains NL5901 and SCH4856 which harbor the human α-synuclein. We found three regulatory hot-spots on Chromosome V. To pin-point the causal locus, three Introgression Lines (ILs) with wild type CB4856 introgressions in an N2 background on Chromosome V were crossed with NL5901. From each cross we obtained four genotypes: i) no α-synuclein and no introgression, ii) no α-synuclein but with introgression, iii) with α-synuclein but no introgression, iv) with α-synuclein and introgression. These verified the regulatory hotspot and through mining WormNet and CeNDR we found *hcp-1* as potential polymorphic modifier influencing human α-synuclein expression in *C. elegans*.

We will follow up with updated RILs sequence and confirm the candidate modifier further with CRISPR/CAS-9 for gene function detection.

535A Post-transcriptional regulation of *egl-1*$^{BH3\text{-}only}$, the key activator of apoptosis during *C. elegans* development

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The highly reproducible pattern of programmed cell death (apoptosis) during *C. elegans* development is dependent on the central apoptosis pathway and its most upstream component *egl-1*, which encodes a pro-apoptotic member of the Bcl-2 family of apoptosis regulators. Unlike other components of the central pathway (*ced-9*, *ced-4*, and *ced-3*), the *egl-1* gene is predominantly transcribed in cell death lineages (i.e. lineages in which a cell death occurs), and this specificity is mediated by lineage-specific transcription factors. Within a particular cell death lineage, *egl-1* mRNA copy number is dynamic. Using single-molecule RNA FISH, we previously found a low number of *egl-1* mRNAs in mothers of cells programmed to die, a high number in daughters programmed to die and essentially zero in daughters programmed to survive. Although the mechanisms

underlying this dynamic pattern are not fully understood, we have evidence that it is at least partially due to microRNA-dependent control of *egl-1* mRNA turn-over. Specifically, we reported that the miR-35 and miR-58 families of microRNAs act through respective binding sites in the *egl-1* 3' UTR to increase *egl-1* mRNA turn-over in mothers, thereby preventing their precocious deaths (Sherrard et al, 2017). We have now identified additional elements in the *egl-1* 3' UTR that are required for *egl-1* 3' UTR-mediated repression of reporter gene expression. These elements include a conserved 3' terminal element (TPTE) that is necessary and partially sufficient to repress reporter gene expression. We hypothesize that RNA-binding proteins (RBPs) also play important roles in post-transcriptional control of *egl-1* expression. To identify functionally relevant RBPs, we individually knocked down 660 genes predicted to encode RBPs and analyzed the resulting animals with respect to *egl-1* 3' UTR-dependent reporter gene expression. Using this approach, we identified 37 repressor candidates and 26 activator candidates. We are currently in the process of screening these candidates for potential cell death phenotypes and plan to verify *egl-1* mRNA–RBP interactions using biochemical approaches. We propose that regulation at the post-transcriptional level through miRNAs and potentially RBPs is crucial for spatio-temporal control of the level of *egl-1* expression in cell death lineages.

536B A specific window of NHR-23 activity is required for developmental progression

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Nematode molting is a remarkable process where the animals must essentially build a new epidermis underneath the old skin and then rapidly shed the old skin. The study of molting provides a window into the developmental program of many core cellular processes, such as oscillatory gene expression, coordinated intracellular trafficking, steroid hormone signaling, developmental timing, and extracellular remodeling. Like many of these processes, molting is coordinated by gene regulatory networks, and the nuclear hormone receptor NHR-23 is a master regulator. An imaging timecourse revealed oscillatory NHR-23::GFP expression in the epithelium which closely follows the reported mRNA expression. Previous work using RNAi depletion of *nhr-23* suggested that molting defects are rarely observed at the first molt. We have previously generated *nhr-23::AID::3xFLAG* animals carrying a TIR transgene to allow auxin-dependent depletion in all somatic tissues. When we plated these animals on auxin plates directly following synchronization, 100% of L1 larvae arrested and we did not observe molting defects, such as animals trapped in the cuticle, or dragging partially shed cuticles. Using this same strain, we observed the same L1 arrest phenotype when animals were shifted onto auxin within the first six hours of release from synchronization. However, when we delayed NHR-23 depletion until 6-12 hours after release from synchronization, animals no longer arrested. Instead, we observed defects, such as entrapment in the cuticle, formation of large vacuoles, abnormal tail morphology, and worms with a flattened morphology we termed "splatter". Thus, our AID system has revealed a more significant role for NHR-23 in the L1 molt than previously reported and connected NHR-23 levels during its oscillation with two distinct classes of phenotypes upon depletion. Our data suggest a novel NHR-23 dependent decision point controlling molting.

537C Isoforms of eIF4G (*ifg-1*) are differentially expressed to modulate mRNA translation initiation mechanism in development.

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Cellular identity is critically determined by new protein synthesis in cells. Oocytes, spermatocytes and early embryos use predominantly mRNA translational control to regulate in spatial and temporal gene expression rather than transcription. Repression of mRNAs by RNA binding proteins and licensing by miRNAs have been extensively studied in *C. elegans* germ cells. But there is far less known about the positive translation mechanisms that overcome repression. Positive regulation is mediated by the translational machinery, eIF4 factors, that recruit mRNAs to ribosomes. In recent years we have shown that recruitment, like repression, is an mRNA-selective process.

Translation factor eIF4G represents the core of the mRNA recruiting complex. In all organisms there are (at least) two major forms of eIF4G that utilize different mechanisms: one brings together a complex that recognizes the m^7GTP mRNA cap (cap-dependent, CD), and the other builds a complex that recruits without cap recognition (cap-independent, CI). Worms express these two isoforms [p170 (CD) and p130 (CI)] from a single *ifg-1* gene. Here we show by fluorescent tagging of isoforms in vivo that the CD and CI isoforms vary greatly in abundance during late oogenesis and in early embryos. Using ifg-1 mutants and isoform RNAi we demonstrate that the CD:CI isoform balance plays a critical role in preventing germ cell apoptosis but is largely dispensable following fertilization. The exception is for P granule localization, where suppression of the CD isoform promotes ectopic P granules in early embryos. A Polysome-Seq survey identified a subset of mRNAs that preferentially translates by CI mechanism, a subset that strongly favors CD mechanism, and many that show little preference. Our findings

suggest that germ cells and embryos intentionally increase and decrease the CD and CI eIF4G isoforms to regulate mRNA translation by altering initiation mechanisms.

538A Identification of the biologically relevant MEC-2 isoform

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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* with 17 known splice variants according to the Ensembl database. The encoded MEC-2 is expressed in six neurons and is responsible for gentle-touch mechanosensation. Podocin and MEC-2 are members of the stomatin protein family, both contain a PHB domain, both are integral membrane porteins with intracellular C- and N-termin and form oligomers. The MEC-2a isoform is considered to be the canonical one. This isoform shares 45% identity and 83% similarity over 275aa (72%) of podocin (383aa), with primarily different C- and N- terminal regions. We aimed to identify the biologically relevant MEC-2 isoform, which can rescue the diseased phenotype of a *mec-2* mutant strain.

Methods:

Vectors encoding MEC-2 isoforms under *mec-2* promoter and a selection marker (cbr-unc) were generated. To avoid quantitative differences due to extrachromosomal expression, we implemented the MosSCI (Mos1-mediated Single Copy Insertion) technique to achieve chromosomal integration. Double (*mec-2* and *unc-119*) mutant strains were created by crossing. The *mec-2* mutant (Tu37) strain was kindly provided by the laboratory of Prof. M. Chalfie. We used microparticle bombardment to transform worms. The gentle-touch sensation was examined by cat's whiskers in a blinded experiment. RNA expression levels were examined by qualitative and quantitative PCR after total RNA isolation from worms.

Results:

Strains were successfully transformed with MEC-2a coding vectors. However, no rescue was achieved in strains expressing the MEC-2a isoform. We excluded the biological role of several isoforms based on their absent expression, as determined in total RNA isolated from wild type worms. A complete rescue was achieved with a 16 kb genomic sequence encoding 12 of the 17 isoforms with intact 3' regions. The rescue effect of several selected isoforms is currently investigated.

Conclusion:

Mutant strains can be successfully generated by the combination of the MosSCI technique and microparticle bombardment. The canonical MEC-2a isoform is not the biologically most relevant, as it cannot rescue the gentle touch sensation defect of the *mec-2* mutant strain.

539B Apoptosis in the context of autophagy and lifespan in *C. elegans*

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Apoptosis is a genetically orchestrated execution of cells that acts as a toll-gate mechanism for the development, aging, and homeostasis of the cell. In order to study cellular processes and related diseases, it is essential to understand the role of eukaryotic gene expression in the advent or evasion of cell death. *Caenorhabditis elegans* can be used to rapidly assess the functional impact of gene mutations to understand the dynamics of gene regulation. Sin-3, a co-repressor protein that controls gene transcription by acting as a scaffold for the multiprotein Sin-3/HDAC complex, is known to be involved in multiple cellular processes. Experimental results from our laboratory showed that sin-3 mutation in *C. elegans* leads to an increase in ROS-mediated autophagy along with a sharp decline in life span. In addition, sin-3 deletion abrogates normal development and growth in worms, resulting in cuticle defects, diminished muscle function, more susceptibility to stress, and extensive DNA damage surmounting to cell death. Therefore, Sin-3 may play a crucial role in the modulation of autophagy and longevity by regulating apoptosis. This was studied in our laboratory by an *in silico* analysis of the *C. elegans* Sin-3 protein interactome and it was found that certain Sin-3 interactors were involved in the regulation of cell death. In addition, sin-3 mutants showed

increased germline apoptosis. The major cell signaling pathways that might get upregulated in sin-3 mutation background in *C. elegans* are – DNA damage response (DDR), extracellular signal-regulated protein kinase (ERK), p38 mitogen-activated protein kinase (PMK), c-Jun N-terminal kinase (JNK) pathways. Our observations suggest the regulation of insulin/IGF-1 signaling (daf-16), and JNK pathway (mek-1, jnk-1) in *C. elegans* by Sin-3 may play a pivotal role.

540C Alternative splicing through m^{6}A modification at a 3' splice site for SAM synthetase homeostasis

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Alternative splicing of precursor messenger RNAs (pre-mRNAs) contributes not only to proteome diversity but also to regulation of gene expression levels by generating mRNA isoforms with a premature termination codon (PTC). Such unproductively spliced mRNAs are unstable and almost undetectable due to an mRNA surveillance system termed nonsense-mediated mRNA decay (NMD). In order to elucidate a repertoire of mRNAs regulated by alternative splicing coupled with NMD (AS-NMD), we performed long-read RNA sequencing of poly(A)$^+$ RNAs from an NMD-deficient mutant, *smg-2*, and obtained full-length sequences for mRNA isoforms from 259 high-confidence AS-NMD genes. Gene ontology (GO) analysis revealed enrichment of genes related to metabolism in addition to those related to RNA translation and processing. Among them are *S*-adenosyl-L-methionine (SAM) synthetase (*sams*) genes. SAM synthetase activity negatively autoregulates *sams* gene expression through AS-NMD. METT-10, the orthologue of human U6 snRNA methyltransferase METTL16, is required for the splicing regulation of the *sams* genes *in vivo* and specifically methylates *in vitro* the invariant AG dinucleotide at the distal 3› splice site (3›SS) used for the productive mRNAs. RNA immunoprecipitation with anti-m^{6}A antibody and direct RNA sequencing with Nanopore technologies coupled with machine learning confirmed m^{6}A modification of endogenous *sams* mRNAs. These results indicate that homeostasis of SAM synthetase in *C. elegans* is maintained by alternative splicing regulation through m^{6}A modification at the 3'SS of the *sams* genes.

541A Detection of induced gene repression using an *in vivo* protein recruitment system

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Gene repression and heterochromatinization are associated with binding of various chromatin regulatory proteins and complexes. Ectopic recruitment of a protein of interest to a reporter locus is a powerful approach used widely in mammalian cells, *Drosophila*, and fungi to study transcriptional repression and heterochromatin formation. To develop an *in vivo* tethering system for investigating gene repression in *C. elegans*, we adapted components of the cGAL GAL4-UAS system (Wang *et al*. 2017 *Nature Methods*). The GAL4 DNA-binding domain (DBD) without the activation domain was fused to a protein of interest and recruited to a 15xUAS upstream of a single copy GFP reporter. We confirmed successful tethering of Flag-tagged GAL4 fusion proteins at the UAS by ChIP-qPCR. To establish a positive control to drive gene repression in our system, we constructed GAL4 DBD fused with HPL-2, a homologue of heterochromatin protein 1, and MIG-32, a predicted homologue of a polycomb repressive complex 1 subunit. Fluorescence quantification indicated a significant reduction of GFP intensity upon HPL-2 or MIG-32 recruitment at 1.6-fold and 1.9-fold, respectively. With further optimization, our recruitment system can be utilized to investigate the initiation of gene repression by repressive complex subunits and other heterochromatin-associated proteins in *C. elegans.*

542B Modelling BAP1 malignant pleural mesothelioma mutations in *C. elegans* reveals synthetic lethality between *ubh-4*/BAP1 and the proteasome subunit *rpn-9*/PSMD13

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BAP1 is a ubiquitin C-terminal hydrolase (UCH) that presents a powerful tumor suppressor activity. There are many studies associating BAP1 mutations to certain types of cancer, grouped in the BAP1 tumor predisposition syndrome (BAP1-TPDS). However, the functional consequences of BAP1 cancer-related alterations are still largely unknown. We aimed to establish a model to investigate the impact of BAP1-TPDS-related mutations using *C. elegans* and CRISPR-Cas9.

First, we generated distinct *ubh-4* strains: two harboring missense mutations found in malignant pleural mesothelioma patients (A87D and F73V) and two with deletion alleles. None of these mutations affect animal viability. Next, to identify *ubh-4* genetic interactors, we performed an RNAi screen on an *ubh-4* null background with more than a hundred genes related to the ubiquitin proteasome system (UPS) and cancer. We uncovered a strong synthetic genetic interaction between *ubh-4* and *rpn-9*, an essential regulatory subunit of the 19S proteasome involved in proteasome assembly.

Further validation of RNAi results with mutations revealed a detrimental effect of the null allele and the A87D cancer-related mutation on animal size, lifespan, and germline development only combined with *rpn-9* mutant background. In line with these observations, we noted that UBH-4*:EGFP* and *RPN-9:wrmScarlet* endogenous reporter, generated by Nested CRISPR, showed high expression and co-expression at meiotic regions, suggesting that *ubh-4* and *rpn-9* cooperate in meiotic progression by modulating ubiquitin-mediated proteostasis.

Finally, we have preliminary results indicating that the *ubh-4* null mutant is sensitive to the proteasome inhibitor Bortezomib. Thus, our data suggest that pharmacological targeting of PSMD13/RPN-9 or the 19S regulatory complex could be utilized to compromise cell viability in BAP1-mutated tumors.

543C Defining the Roles of *lin-28* and *hbl-1* in Gonad Development

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In *C.elegans,* the heterochronic genes are responsible for specifying the timing of non-gonadal cell division patterns throughout larval development. Mutations in these genes cause developmental patterns to be skipped or repeated inappropriately.

lin-28, lin-46, and hbl-1 are three heterochronic genes that control evens of the L2 and L3. LIN-28 is an RNA binding protein that has at least two mechanisms of action, one of which is to block the maturation of the microRNA let-7.LIN-46, a protein found only in *Caenorhabditis* species, and HBL-1, an Ikaros family zinc-finger transcription factor. Our goal is to define the relationships among these factors and determine how they control development in different tissues.

To determine their regulatory relationships, we performed epistasis analysis between *lin-28*, *lin-46* and *hbl-1*. The creation of a double mutant containing a *lin-28* null allele and a hypomorphic allele of *hbl-1* lead to the discovery of a sterility defect, a novel phenotype with respect to heterochronic genes. Further analysis of this double mutant revealed gross morphological defects of the somatic gonad. The distal tip cells of the double mutant follow aberrant migration patterns causing the gonad arms to adopt atypical configurations. The double displays variant formation of spermatheca and of those developed, all are malformed. This is further observed by the irregular expression pattern of the spermatheca specific FKH-6::GFP marker in the double mutant. Due to the absence of developing embryos, pools of yoke are seen throughout the coelom of the animal. Our findings suggest for the first time a redundant role for these heterochronic genes in gonad development.

We have previously found through a yeast-2-hybrid screen that LIN-46 physically interacts with the HBL-1 protein. Remarkably, removal of *lin-46* activity from the aforementioned double mutant resulted in the restoration of fertility.

We plan to further explore this novel sterility phenotype in order to determine how these heterochronic genes effect somatic gonad development. We are also using the somatic gonad as a new tool to continue studying the relationships among these heterochronic genes and determine if their relationship is preserved in different tissues.

544A Systematically uncovering transcriptional regulation of metabolism in *Caenorhabditis elegans*

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The spatiotemporal control of gene expression helps maintain organismal homeostasis under fluctuating dietary and environmental conditions. For instance, transcriptional regulation of metabolic genes can lead to metabolic rewiring, a process where alternative pathways are utilized to meet changing metabolic demands. The majority of previous studies for transcriptional regulation of metabolism have focused on individual metabolic genes and pathways. However, the extent of transcriptional regulation of metabolism in animals on a global scale remains largely unknown. Large-scale gene expression datasets from various dietary and environmental conditions offer an opportunity to investigate transcriptional regulation of metabolism globally. We study the transcriptional regulation of metabolism in the multicellular *Caenorhabditis elegans*

at a systems level. We developed a set of *in-situ* pipelines to infer the relationship between transcription and metabolism. We have quantified the extent of transcriptional regulation of metabolism during development, across tissues and changing temperature stimulus. By applying a supervised approach, we have unraveled metabolic pathways that are transcriptionally coregulated. We have defined pathway boundaries within the existing metabolic network by combining metabolic flux relationships and the coexpression network of metabolic genes to extract potential tightly coregulated clusters of metabolic pathways. We have also mapped transcription factors (TFs) to these coregulated metabolic pathways through TF-centric pathway enrichment and clustering approaches. Overall, this study broadens our understanding of transcriptional regulation of metabolism and uncovers parts of metabolism that are transcriptionally (co)regulated with their potential regulators.

545B RNA Binding Proteins Coordinately Control Lifespan in *C. elegans*

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Regulation of gene expression affects lifespan in *Caenorhabditis elegans*. While transcription factors have been extensively studied for their role in aging, less is known about how RNA binding proteins may contribute to the aging process. We recently performed a CRISPR/Cas-9 based Synthetic Genetic Interaction (CRISPR-SGI) screen in *C. elegans* focused on conserved neuronally-expressed RNA binding proteins, and identified many double mutants with fitness defects. In one notable interaction between the ELAVL ortholog *exc-7* and the MBNL1/2 ortholog *mbl-1*, double mutants displayed a severely shortened lifespan (~70%). Both genes are required for regulating hundreds of transcripts and isoforms. The *exc-7; mbl-1* double mutant appears to develop into healthy young adults after which their health rapidly declines. *exc-7* and *mbl-1* are both neuronally-enriched genes. Initial experiments with *mbl-1* tissue specific re-expression have shown partial rescue of the lifespan phenotype with re-expression in the nervous or intestinal tissues of the double mutant but not muscle tissue. Shortly we will be conducting experiments to test whether *exc-7* expression in the nervous system is the critical tissue affecting whole-worm lifespan seen in the *exc-7; mbl-1* double mutant. We have used RNA seq data to investigate which RNAs may be uniquely dysregulated in the *exc-7; mbl-1* double mutant. We identified eight uniquely dysregulated genes and have tested six out of eight of these genes in order to investigate their effects within the double mutant. We have identified one gene that appears to partially rescue the lifespan phenotype. *nhx-6*, a predicted Na/H exchanger, which was identified from our RNA Seq data contributes to the phenotype and is expressed in the intestine. *nhx-6* partially rescues a number of *exc-7; mbl-1* phenotypes, including intestinal permeability, defecation cycle length, and pharyngeal pumping. We are currently investigating further genes of interest identified through our RNA seq analysis, and testing whether they modulate the lifespan phenotype of *exc-7; mbl-1* mutants.

546C The Role of mRNA Decay in Embryonic Cell Fate Specification

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During development, cells undergo dynamic changes in gene expression that are required for appropriate cell fate specification. Although the regulation of mRNA degradation contributes to developmental gene expression, its influence on these expression patterns is not well understood relative to that of transcriptional regulation. Like transcriptional regulation, however, the regulation of mRNA decay has the potential to be highly complex. Transcript stability is largely regulated by the binding of protein or RNA factors to *cis*-regulatory elements within the 3' untranslated region (3' UTR) of transcripts. Considering the great diversity of RNA-binding proteins and miRNAs in eukaryotes, along with differential usage of 3' UTR isoforms, a complex mRNA degradation code may contribute to precise gene expression patterns during development. One relatively well-characterized phenomenon of developmentally regulated mRNA decay occurs during early embryogenesis, in which the widespread degradation of maternal mRNAs is required for the control of development to switch from maternally to zygotically encoded products. Increasing evidence is also emerging for the importance of zygotic mRNA degradation in cell fate decisions, though these studies tend to occur on a gene-by-gene basis or in the context of cell culture systems. Thus the extent of developmentally regulated zygotic mRNA decay remains unclear. We hypothesize that the regulation of zygotic mRNA degradation contributes to appropriate cell fate decisions throughout embryogenesis. To explore this, we are examining mRNA decay rates during embryonic development in *Caenorhabditis elegans*. By sequencing embryonic cells treated with a transcription inhibition time course, we have measured mRNA half-lives globally in *C. elegans* embryos. We found that half-lives can vary by more than an order of magnitude from gene to gene, and differences in mRNA stability correlate with functional classes of genes. Furthermore, analysis of the half-life distributions of tissue- and developmental stage-enriched genes suggests that mRNA decay is differentially regulated across different tissues and stages. Future work should extend

these results to measure differential degradation of the same mRNAs in different cell types and stages. Our results highlight that zygotic mRNA degradation is a highly regulated process with tissue- and stage-specific dynamics during development.

547A The role of parental diet on progeny´s proteome and fitness

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Animals adjust their phenotype to their environment, which is thought to provide a selective benefit under changing conditions. A famous example of such phenotypic plasticity is the slow down of growth and the delay of aging under dietary restriction (DR). Interestingly, parental DR also affects phenotypes of the F1 generation, for example, DR increases the starvation resistance of progeny (Jordan et al., 2019).

Molecularly, DR is associated with a global change in proteome expression, including a reduction in ribosome levels and a proteome-wide slow-down of protein turnover (Dhondt et al., 2016; Visscher et al., 2016). Here, we ask if and how parental diet impacts the proteome of the F1 progeny. We will seek causal relationships between intergenerationally transmitted phenotypes and the inherited proteome. Finally, we aim to determine if the parental control of the proteome provides a selective benefit to their progeny.

To address these questions, we have established a tool to precisely quantify ribosomal levels by measuring the luminescence from lysis of HiBit tagged to ribosomal proteins. We plan to globally analyze parental effects on the F1 proteome by proteomics and measure the dynamics of proteome changes, growth rates, and reproduction rates in different nutritional conditions by fluorescence live imaging in microchambers. The project will likely provide important insights about the molecular mechanisms and physiological significance of inter-generational inheritance induced by parental diet.

548B Identifying the role of Pashas WW domain in primary microRNA recognition and processing

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MicroRNAs (miRNAs) are short, non-coding RNAs that together with their protein cofactors, bind to target messenger RNAs to promote mRNA decay and translational repression. miRNAs are highly conserved and are implicated in essentially all biological processes in plants and animals. In the absence of miRNAs, *C. elegans* arrest during embryogenesis. Even partial loss of miRNAs can lead to severe development defects. The biogenesis of miRNAs is similar from *C. elegans* to humans, although there are differences in how primary miRNA transcripts are distinguished from other RNAs in different species and even between different miRNA transcripts within the same species. To better understand how miRNAs are processed in *C. elegans*, we developed an mCherry-based sensor that is desilenced when primary miRNA processing is impaired. We then performed a forward genetic screen to identify mutations that desilence the sensor and which may therefore affect primary miRNA recognition or processing. We are now characterizing a line we identified in our screen that has a mutation in the WW domain of Pasha as we more broadly explore the role of Pasha in primary miRNA recognition and processing.

549C Growth Regulation Mediated by Feedback Mechanisms in the DBL-1/BMP Pathway of *Caenorhabditis elegans*

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The DBL-1 signaling pathway in *C. elegans* is homologous to the TGF-β signaling pathway in mammals. This pathway regulates a number of important biological functions such as pluripotency, growth, and body size. Our work explores feedback mechanisms between the DBL-1/BMP signaling pathway and cuticle collagens in *C. elegans*. We aim to identify the mechanism through which cuticle collagen influences DBL-1/BMP expression. Previous work has shown that the cuticle collagen genes regulate DBL-1 signaling, and we hypothesize that a mechanism through which the cuticle collagen and neuron communicate is via mechanical forces. To determine this, we used liquid submersion to increase mechanical stress on the animal and subsequently analyzed DBL-1 transcriptional levels using a strain with a *dbl-1* transcriptional reporter, *dbl-1p::gfp*. We found that liquid submersion, possibly via an increase in mechanical stress, leads to increased levels of *dbl-1* transcription. Additionally, I studied a possible upstream transcription factor of the *dbl-1* gene, EOR-1. ChIP-seq studies have identified binding sites for EOR-1 in the *dbl-1* genomic region. To investigate if EOR-1 is regulated by cuticle mechanics, I performed RNAi knockdown of cuticle collagen genes (*col-141* and *rol-6*) in a GFP-tagged EOR-1 strain and determined the subcellular localization of EOR-1::GFP. *col141(RNAi)* and *rol-6(RNAi)* worms demonstrated a clear difference in nuclear localization of fluorescence compared with control worms. Preliminary data indicate that the control worms have a more concentrated

localization of EOR-1::GFP while collagen knockdown worms have a more diffuse pattern of EOR-1::GFP. In the future, I aim to study specific mechanosensitive transcription factors and explore possible relationships between these transcription factors and DBL-1 signaling.

550A Identification of transcriptional regulators impacted by a glucose-supplemented diet in *C. elegans*

Jose Robledo[1], David Burks[1], Luhua Song[1], Isai Leguizamo[1], Rajeev Azad[1], Pamela Padilla[1] 1) University of North Texas.

A high glycemic diet (i.e. high sugar diet) is among the main factors contributing to severe and detrimental human health issues such as type 2 diabetes, cardiovascular diseases, and metabolic abnormalities. Underlying mechanisms involved in gene expression regulation and metabolism as a result of a high sugar diet continue to remain poorly understood. In our study, we use the genetic model system *Caenorhabditis elegans* to further investigate the impact a glucose-supplemented diet has on gene expression changes/regulation. Previously, we showed that a glucose-supplemented diet increases lipid composition, sensitivity to stress responses, and significantly alters the transcriptome profile (RNA-sequencing studies). We observed differential gene expression chromosomal maps and identified various clusters majorly found in Chromosome IV/V. Given that a link exists between chromosomal clustering and transcriptional regulation we further sought to identify gene regulators. The transcriptomic analysis revealed 140 gene expression regulators among the 2,370 differentially regulated genes as a result of a glucose-supplemented diet. Furthermore, we identified the *acs-2* gene is a glucose-responsive gene, as shown by RNA-sequencing analysis, the *acs-2::GFP* reporter strain, and quantitative RT-PCR. Here, we developed a targeted RNA interference (RNAi) screen to identify the transcriptional regulators that respond to a glucose diet and impact the expression of *acs-2::GFP*. From our screen, we identified 20 regulators that significantly impact the expression of acs-2 transcript in a glucose diet dependent manner; 18 regulators increase and 2 regulators decrease *acs-2::GFP* expression. We also identified 13 gene regulators that increase *acs-2::GFP* expression in standard RNAi diet. Several of the regulators identified also show homology to human gene regulators suggesting conserved gene expression regulators between *C. elegans* and Humans. Together, these studies add to our understanding of the transcriptional and molecular changes that occur in response to a glucose-supplemented diet.

551B Regulation of anterior genes in the *C. elegans* embryo

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Anterior-posterior patterning is vital for embryonic development. In animals from worms to humans, Wnt patterns the anterior-posterior axis. Wnt drives expression of several genes expressed in lineages derived from posterior daughters. Previous work in our lab and others showed that many of these posterior genes depend on Wnt pathway component POP-1/TCF either for posterior expression or anterior repression.

There are nearly as many genes expressed in lineages derived from anterior daughter cells as there are posterior genes and much less is known about how these anterior genes are regulated. Anterior genes may be regulated by Wnt indirectly through a canonical Wnt target, directly by Wnt by non-canonical means, or by non-Wnt mechanisms. I have found that several anterior genes depend on POP-1 for expression in an opposite manner to posterior genes. To determine which transcription factors regulate an anterior gene's expression, I dissected the *cis*-regulation of the anterior gene *ref-2/ZIC1*. I used enhancer-reporter strains to identify two *ref-2* enhancers including one that explains its anterior-specific embryonic expression. I examined the expression patterns driven by enhancer fragments to identify minimal fragments sufficient to drive anterior expression. A fragment of one of these enhancers bears five putative TBX-37/38 binding sites, one of which I found to be essential for anteriorly biased expression. Synthetic enhancer-reporter constructs bearing multiple copies of this t-box site show that it is sufficient to drive anterior expression. By performing RNAi knockdown of *pop-1*, I have determined that the anterior expression driven by this site is dependent on *pop-1*. Overall this work suggests that multiple transcription factors play a role in anterior patterning of the *C. elegans* embryo.

552C Defining the mechanism by which SNPC-4 and PRDE-1 regulate piRNA expression across large genomic domains in *C. elegans*

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The Piwi interacting RNA (piRNA) is a conserved small RNA pathway that protects germ cells from consequences arising from foreign genetic elements such as transposons. In *C. elegans*, >10,000 sequence-diverse piRNA genes cluster in two distinct

regions on chromosome IV. However, the extent by which clustering in the genome promotes piRNA production specifically in germ cells remains unclear. These piRNA genes are transcribed by RNA Polymerase II (RNA pol II) and the resulting short RNAs are hypothesized to be produced when RNA pol II is in its "paused" state. In addition, piRNA genes are located within repressive chromatin environment. The transcription factors, SNPC-4 and PRDE-1 form a complex that "coats" piRNA gene clusters specifically in the germ line to promote piRNA production. However, the mechanism by which SNPC-4/PRDE-1 coordinates piRNA gene expression is unknown. We are determining whether SNPC-4/PRDE-1 mediates piRNA biogenesis by affecting chromatin regulation and/or control of transcriptional activity. I aim to investigate the relationship between histone modifications and SNPC-4/PRDE-1 and to define SNPC-4/PRDE-1 interaction with RNA Polymerase II transcriptional events. Preliminarily, I have isolated germ nuclei from wildtype and *prde-1* mutants and investigated three candidate histone modifications using ChIP-seq. Although there were no major differences specifically within piRNA domains, we did observe a global change in repressive histone modifications. We will continue to investigate whether SNPC-4/PRDE-1 may play a role in regulation piRNA expression via chromatin regulation, and begin to explore a possible relationship with the core transcriptional machinery.

553A ADR-2 Cellular Localization is Highly Regulated and Affects its Functionality

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A-to-I RNA editing is a conserved and prevalent type of RNA modification in which adenosines (A) are converted into inosines (I) by Adenosine Deaminases Acting on RNA (ADAR) enzymes. ADR-2, the only enzymatically active protein in *C. elegans*, is thought to mainly localize and function in the nucleus. However, it is still not clear whether ADR-2 can shuttle in and out of the nucleus and in which tissues it is expressed. To elucidate the localization of ADR-2, we performed immunofluorescence and RNA-seq experiments. Our results show that ADR-2 is localized to the nuclei and adjacent to the chromosomes during all stages of the cell cycle. We also found that ADR-2 is expressed in all stages and cells during embryo development. Moreover, we examined whether the nuclear localization of ADR-2 would be affected if one of its known regulators, ADR-1 or ADBP-1, were absent. Interestingly, ADR-1 does not affect the localization of ADR-2; however, we validated previous research and show ADBP-1 facilitates the nuclear localization of ADR-2. We also found that ADR-2 mislocalization strongly affects the expression of edited genes in the embryo and to a lesser extent at L4 developmental stage. Taken together, our results suggest that the localization of ADR-2 is highly regulated and likely affects the function of ADR-2.

554B DREAM interrupted: Using CRISPR/Cas9-targeted mutagenesis to assess DREAM complex formation and function

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Formation of the highly conserved 8-subunit DREAM (Dp, Retinoblastoma-like, E2F, and MuvB) complex culminates in direct repression of cell cycle and developmental genes. We are interested in what determinants of DREAM complex formation are essential for transcriptional repression of target genes. Structural studies on mammalian proteins identified key interaction interfaces between the 3 major components of DREAM, the E2F-DP transcription factor heterodimer (EFL-1 and DPL-1), the Retinoblastoma-like pocket protein (LIN-35), and the 5-subunit MuvB subcomplex (LIN-9, LIN-37, LIN-52, LIN-53, and LIN-54). For example, MuvB interacts with LIN-35 via an LxCxE interacting motif on LIN-52. LIN-35 also interacts directly with the EFL-1 transactivation domain via its pocket domain. DNA-binding by the E2F-DP transcription factor heterodimer and the MuvB subunit LIN-54 coordinate DREAM complex localization to chromatin. We hypothesize that MuvB chromatin occupancy, aided and stabilized by its association with E2F-DP and the pocket protein, establishes and maintains target gene repression. Using CRISPR/Cas9-targeted mutagenesis, we disrupted the interaction between MuvB and the *C. elegans* pocket protein LIN-35. We expected that severing MuvB from the complex would destabilize DREAM component assembly on chromatin and its repression of target genes. Instead, we observed that disrupting LIN-35-MuvB association did not affect DREAM chromatin occupancy, but we did observe upregulation of DREAM target genes. To continue our evaluation of our model of DREAM complex formation, we identified the conserved amino acid residues that likely mediate the protein-protein and protein-DNA associations for *C. elegans* DREAM. This analysis lays the groundwork for future application of our CRISPR/Cas9 functional genomics pipeline to establish how the molecular events that drive DREAM complex function contribute to target gene repression.

555C A Worm's Perspective on Early Birds: Probing Links Between Conserved Biological Timing Mechanisms in Nematodes and Mammals

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During development, genes must be expressed at both the correct time and location. *C. elegans* utilizes two separate biological timing mechanisms to coordinate development during progression from larvae to adult. The heterochronic pathway ensures the correct spatial and temporal execution of developmental events through a network of protein regulators and microRNAs. The molting cycle coordinates the replacement of the old skin with a new one to allow for growth. LIN-42 has a critical role as a transcriptional regulator that links the heterochronic pathway to the molting cycle. While the mechanisms that underlie LIN-42 control of gene expression remains elusive, the general molecular mechanism of its mammalian homolog, PER, and its role in regulating circadian rhythms, is better understood. Similar to PER levels that oscillate with ~24-hour rhythmicity, LIN-42 levels oscillate in rhythm with each molt and the key domains conserved between LIN-42 and PER are essential to the circadian function of PER. Here, we leverage our knowledge of PER function to investigate the biochemical relationship between LIN-42 and KIN-20, a protein homologous to the primary kinase responsible for regulation of PER function and influence on the clock, Casein Kinase 1δ (CK1δ). We show that the C-terminus of LIN-42 contains two highly conserved kinase-binding motifs that interact with mammalian CK1δ, suggesting that the domains that anchor the kinase to PER2/LIN-42 may be utilized in a similar mechanism. We also show that LIN-42 is phosphorylated by CK1δ to a similar degree and within the same timeframe as CK1 phosphorylation of a PER substrate. *C. elegans* mutant strains containing deletions of conserved regions show drastic changes in the expression pattern of LIN-42 when compared to wild-type. By exploring the roles of LIN-42 and the *C. elegans* CK1δ homolog, KIN-20, we aim to identify the biochemical links between these evolutionarily divergent timing mechanisms.

556A SNA-3: an essential, nematode-specific protein is a novel key component of the spliced leader *trans*-splicing machinery

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We are investigating spliced leader (SL) *trans*-splicing and its key RNA and protein components as potential anthelmintic targets, using *Caenorhabditis elegans* as a model system. SL *trans*-splicing is an essential process in nematode gene expression that facilitates translation by replacement of the 5' untranslated region of most mRNAs with the spliced leader 1 (SL1). The splicing reaction involves an interaction between the SL1 snRNP, the nascent pre-mRNA and the spliceosome. Although SL *trans*-splicing was discovered more than 30 years ago, we know little about the molecular mechanism(s) by which this is achieved. To address this, we have carried out a comprehensive molecular characterisation of the SL1 snRNP.

This work expands and refines our understanding of the proteins involved in SL1 *trans*-splicing: we have analysed factors co-immunoprecipitating with the SL1-specific protein SNA-1, giving us insight into the interaction of the SL1 snRNP with the spliceosome. Proteins critical for SL1 *trans*-splicing were identified using established RNAi-based qPCR and *gfp*-reporter gene assays (https://doi.org/10.1093/nar/gkx500). This led to the identification of a novel, essential *trans*-splicing factor termed SNA-3. SNA-3 is a highly conserved, nematode specific protein containing NADAR domains, which have been linked to NAD/ADP-ribose metabolism and may have N-glycosidase activity. SNA-3 interacts with several highly-conserved proteins associated with RNA processing including the CBC-ARS2 complex components NCBP-1 and SRRT/ARS2 involved in co-transcriptional determination of transcript fate.

Together, these observations implicate SNA-3 in key steps linking SL1 *trans*-splicing to the transcriptional control of gene expression. The identification of another essential, nematode-specific protein involved in SL1*trans*-splicing strengthens the hypothesis that the acquisition of SL *trans*-splicing requires the evolution of novel machinery required to modify the activity of the spliceosome. The novelty of these proteins makes them ideal targets for the development of new anthelmintics.

557B Investigating the interplay between sRNA pathways and germ granules in *Caenorhabditis elegans*

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Small RNA (sRNA) pathways are critical regulators of gene expression, germ cell integrity, and fertility. At the core of these pathways are effector complexes composed of sRNAs and their binding partners, the Argonaute (AGO) proteins. *C. elegans* expresses 19 different AGOs. Our lab has systematically characterized all 19 AGO expression patterns and observed that eight AGOs localize to germ granules. Germ granules are conserved, phase separated RNA/protein rich organelles that are only present in germline cells. Four different types of germ granules, P granules, Z granules, Mutator foci, and SIMR foci, are present in *C. elegans* and appear to perform separate functions. Germ granules can either be distributed in the cytoplasm, or associated with nuclear pores. In fact, up to 75% of nuclear pores are associated with P granules, therefore a extensive proportion of germline transcripts pass through germ granules upon exiting the nucleus. Disruption of germ granules leads

to defects in RNA processing and stability, transgenerational RNA interference (RNAi), and fertility. We aim to identify which AGOs and germline transcripts localize to each of the granules, and how AGOs and germ granules interact to carry out their functions. For example, CSR-1b is an essential AGO which is known to localize to P granules, thus we examined the localization of one of its known target transcripts, *pgl-3*, using smFISH to identify its localization. We observed that the *pgl-3* transcript localized to germ granules with partial overlap with CSR-1, and that this localization was disrupted in sRNA pathway, germ granule, and nuclear pore mutants. In parallel, our recent quantitative co-localization studies with AGO-tagged and germ granule-tagged strains reveal distinct patterns of AGO/germ granule overlap that strongly correlate with sRNA pathway function-that is, AGOs that target overlapping sets of genes via their sRNAs also display overlap with the same types of germ granules. Collectively, our systematic studies will uncover the sRNA and gene regulatory functions of each type of germ granule that are important for epigenetic inheritance and fertility.

558C Identifying the role of BRCA1 in transcriptional regulation using *Caenorhabditis elegans*

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Breast cancer susceptibility protein 1 (BRCA1) and its heterodimeric partner BARD1 play an essential role in genomic stability by regulating DNA damage repair, cell-cycle checkpoints, and transcription regulation. Germline mutation in either of these genes exposes individuals to a higher risk of developing breast and ovarian cancer. The *Caenorhabditis elegans* orthologs, brc-1 and brd-1, also regulate DNA damage repair and cell cycle checkpoints; however, their role in regulating gene transcription is still unknown. Here, we show the transcriptional regulation function of brc-1 and brd-1 is conserved in worms using the *cyp-13A* subfamily of genes, which are the homolog of a human estrogen metabolizing gene *CYP3A4*. Using gene expression analysis, we found that knocking out brc-1 resulted in significant upregulation of four *cyp-13A* subfamily of genes, and loss of brd-1 function led to upregulation of six *cyp-13A* subfamily of genes. Our finding provides insights into how brc-1/brd-1 transcriptional regulation function is conserved in worms and further validates using *C. elegans* as a model system to investigate BRCA1 functions.

559A Dissecting interactions across gene regulatory layers: FUST-1, TDP-1, and CEH-14 are coordinately required for gonad development

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Gene expression is a multistep, carefully controlled process with distinct regulatory layers. Previous work in our lab suggested that crosstalk between these layers plays a significant role in coordinating gene expression. Recently, we embarked on a systematic reverse-genetic interaction screen with the goal of identifying functionally relevant coordination of gene expression across regulatory layers in the nervous system. We are particularly interested in crosstalk between transcription and RNA processing. We crossed previously-generated CRISPR-Cas9 RNA binding protein (RBP) deletions with existing transcription factor (TF) mutant strains, creating over a hundred RBP;TF double mutants. We screened these double mutants for unexpected phenotypes not seen in either of the constituent single mutants. A novel phenotype in the double mutant indicates a genetic interaction between the corresponding RBP and TF. The strongest double mutant phenotypes were further investigated to characterize underlying mechanisms that contribute to the phenotype. We have identified several strong genetic interactions by studying double mutants with a variety of unexpected phenotypes.

Here, we present evidence that two ALS-related RBPs, FUST-1 and TDP-1, have a similar interaction with the transcription factor CEH-14. The loss of any one of these three genes alone has no significant effect on the overall health of the organism. However, *fust-1; ceh-14* and *tdp-1; ceh-14* double mutants both exhibit strong temperature-sensitive reproductive defects. Both double mutants, when grown at 25° C, exhibit deformed gonads, resulting in reduced egg production and correlating with an increased prevalence of males. RNA-seq results suggest that *fust-1; ceh-14* and *tdp-1; ceh-14* undergo similar gene dysregulation that cannot simply be explained by their shared *ceh-14* mutation. The similarity of the double mutant phenotypes suggests that these three genes are involved in the same process. Additionally, the lack of a similar phenotype in a *fust-1 tdp-1* double mutant implies that FUST-1 and TDP-1 interact with CEH-14, but not with each other. Our findings implicate an important role for CEH-14, FUST-1, and TDP-1 in orchestrating gonad development in C. elegans.

The physiological roles of FUST-1 and TDP-1, as well as their human homologs FUS and TDP43, have not yet been fully characterized. We hope that our findings will shed light on a fundamental role that these RNA binding proteins share.

561C Transcriptional analysis of the response of *C. elegans* to ethanol exposure

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Ethanol consumption induces differential gene expression that are likely to at least partially underlie the progression to alcohol use disorder. We use *C. elegans* to study the effects of ethanol on nervous system function. To understand the gene expression dynamics that occur upon ethanol exposure, we conducted a time series experiment exposing *C. elegans* to a behaviorally intoxicating dose of ethanol for 30, 60, 120, and 480 minutes. The number of differentially expressed genes was positively correlated with exposure duration. We used K- means clustering to cluster genes based on expression over time and divided them in 5 distinct clusters. Gene ontology analysis showed enrichment for processes known to be involved in ethanol responses, for example: drug metabolism – cytochrome P450 and metabolic pathways. Furthermore, at eight hours of exposure several genes that are known to be involved in physiological response to ethanol were down regulated; some of those genes have been previously identified as direct ethanol targets, including the gene encoding the BK SLO-1 channel, the synaptic protein-encoding *unc-13* gene, as well as genes involved in GABA signaling, suggesting a simple model in which downregulation of direct ethanol targets could decrease the depressing effects of ethanol and could contribute to the development of tolerance to ethanol.

Our exposure paradigm delivered ethanol in the absence of food, and we found that eight hours of food deprivation had a significant impact on overall gene expression as assessed by principal component analysis and transcriptional development analysis. Interestingly, ethanol was able to partially rescue the effect of food deprivation at eight hours, extending previous observations that *C. elegans* can use ethanol as a calorie source.

This timeseries captured gene expression dynamics in response to ethanol over time and will be used as a resource for our follow up studies, including investigating natural variation in gene expression in response to ethanol.

562A The role of circadian rhythm homologs LIN-42 and KIN-20 in gene regulation and development

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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. We have utilized CRISPR technology to generate epitope-tagged variants of LIN-42 and KIN-20 in order to further explore the relationship between these important, conserved genes and development.

563B Genetic regulators of Integrator complex mediated snRNA processing in *C. elegans*

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Small nuclear RNA (snRNA) molecules are essential components of the spliceosome, which in eukaryotic cells is responsible for the removal of introns from pre-mRNA and ligation of exons into mature mRNA transcripts poised for translation. Approximately one third of all disease-causing mutations in humans affect the splicing of mRNA, including various types of cancer and neurodegenerative diseases. Therefore, the elements involved in splicing regulation are of interest in understanding the mechanistic origin of various human diseases. Recently, a novel protein complex called the Integrator was discovered as the principal regulator and processing factor of snRNA molecules. Integrator recognizes a "3' box" sequence element within the snRNA transcript and directs its cleavage and maturation. The matured snRNA transcripts are then directed to the spliceosome to regulate RNA splicing. In humans, malfunction of the Integrator has been implicated in neurodevelopmental disorders and cancer. However, how the Integrator is regulated remains poorly understood.

Using the genetically tractable organism *Caenorhabditis elegans* as a model system, we constructed an *in vivo* Integrator malfunction GFP reporter to monitor snRNA misprocessing and performed a genome-wide RNAi screen searching for genetic regulators of snRNA processing. This screen identified several novel genes that, when knocked down, induce snRNA misprocessing. Most of the candidate genes discovered in this screen have been shown to function in RNA interference processing and nuclear transport regulation. Using qPCR, knockdown of several candidate genes confirmed the endogenous misprocessing of U2 and U4 snRNA indicating that these genes indeed influence Integrator activity. Interestingly none of the candidate genes have previously been reported to regulate RNA splicing, suggesting they act as novel regulators of Integrator via a yet to be identified mechanism.

564C Auxin-Inducible Degradation of DREAM Proteins, LIN-9 and LIN-54, in *Caenorhabditis elegans*

Emily Washeleski[1], Karli Chosa[1], Paul Goetsch[1] 1) Michigan Technological University.

The highly conserved Dp, Retinoblastoma-like, E2F, and MuvB (DREAM) transcriptional repressor complex is best known as a negative regulator of cell cycle progression. We previously established that the 5-subunit MuvB complex innately functions as the mediator of DRM target gene repression in *Caenorhabditis elegans*. How the DREAM complex mediates transcriptional repression remains an outstanding question, primarily because MuvB complex activity is required for viability, limiting the utility of mutational analyses. To overcome such limitations, we are utilizing the Auxin-Inducible Degron (AID) system to precisely and rapidly degrade MuvB subcomplex components in *C. elegans*. Using CRISPR/Cas9-mediated genomic editing, we degron-tagged two key MuvB components, *lin-9* and *lin-54,* and crossed each into a transgenic strain expressing the *Arabidopsis thaliana* TIR1 E3 ubiquitin ligase in somatic cells. We expected that auxin-induced degradation of either LIN-9, which we hypothesize functions as a core structural component of MuvB, or LIN-54, which acts as the sole DNA-binding component in MuvB, would lead to upregulation of DREAM target genes. Interestingly, we observed that degradation of the LIN-54 but not LIN-9 caused upregulation of DREAM target genes within a 6-hour auxin treatment timecourse in L1 larvae. Our results suggest that MuvB chromatin localization mediated by LIN-54 is critical for DREAM target gene repression. These studies lay the foundation for future degron-mediated analyses assessing DREAM complex function.

565A Tissue-specific transcriptional regulation by ELT-2 and ELT-7 in the developing *Caenorhabditis elegans* intestine

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The *Caenorhabditis elegans* intestine – a clonally-derived, 20-cell organ – is a powerful model of organogenesis owing to its relative simplicity. We seek to map the gene regulatory networks that specify and differentiate the intestine. We want to understand how key transcription factors ELT-2 and ELT-7 work together to regulate intestine genes.

ELT-2 is an intestine-specific GATA transcription factor necessary for larval viability and sufficient for *C. elegans* intestinal development. ELT-7 works redundantly with ELT-2, and deletion of both enhances intestine deformities observed in ELT-2 deletion alone. Despite extensive and fruitful study, it is still not well understood how ELT-2 engage different sets of target genes over time and how they compete with, cooperate with, or stimulate other transcription factors to execute stage-specific transcriptional outcomes within the intestine. One challenge in addressing these questions is that the field lacks a clear developmental time course of intestine-specific transcriptome profiles. To characterize the changing intestinal transcriptome, we have optimized FACS purification of intestine cells at embryo and L1 developmental stages and performed RNA-seq on populations of ~90% pure intestine cells. Comparative studies between our transcriptome profiles, published ELT-2 ChIP-seq data, and published sets of *elt-2* and/or *elt-7* dependent genes are leading us to insights into ELT-2 regulatory behavior.

Though ELT-2 is generally associated with transcriptional activation, we have observed that ELT-2 directly binds and represses expression of intestine expressed transcription factors PQM-1 and ETS-4. This finding suggests that ELT-2 repression may tune or maintain proper gene expression levels for a subset of target genes. Additionally, we have observed a subset of ELT-2 repressed target genes that are intestine depleted and instead associated with neuronal or germline biology. This suggests that ELT-2 repression may play a role in protecting the intestine from inappropriate or competing cell fates. Finally, we identified over-expression of the *elt-2* promoter in the absence of ELT-2 protein, indicating that ELT-2 binding may down-regulates its own promoter through a negative feedback loop. We demonstrated that *elt-2* promoter over-expression is ELT-7 dependent. Together these data suggest that ELT-2 both represses and activates target intestine genes, with ELT-7 primarily serving as an activator.

566B Dual role of the RNA-binding protein PUF-8 -PUM1,2 in programmed cell death

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Programmed cell death, also referred to as apoptosis, is critical for the removal of 'unwanted' cells and is conserved from nematodes to humans. How programmed cell death is regulated is still not fully understood. The core programmed cell death pathway in *C. elegans* consists of four genes *egl-1* [BH3-only], *ced-9* [Bcl-2], *ced-4* [Apaf-1] and *ced-3* [caspase], which act in a simple linear pathway. The most downstream gene, *ced-3*, encodes a caspase, which triggers cell death execution once its activity has reached a certain 'lethal' threshold. We present evidence that the *C. elegans* homolog of the mammalian RNA-binding proteins PUM1 and PUM2, PUF-8, plays a dual role in the control of programmed cell death during embryonic development. First, we found that the *puf-8* gene has anti-apoptotic activity and prevents the *ced-3*[caspase]-dependent precocious death of mothers of cells programmed to die. Using single-molecule RNA fluorescent *in situ* hybridization (smRNA FISH), we show that the loss of *puf-8* causes a significant increase in the copy number of *ced-3* mRNA in the RID neuroblast, a 'mother' of a cell programmed to die, which suggests that *puf-8* prevents the precocious death of mothers by promoting *ced-3* mRNA turnover. Second, we found that *puf-8* has pro-apoptotic activity and promotes the death of cells programmed to die. However, the loss of *puf-8* does not affect the copy number of *ced-3* mRNA in cells programmed to die, which indicates that in this context, PUF-8 has targets other than *ced-3*[caspase] and/or acts through mechanisms other than mRNA turnover. Interestingly, we found that the loss of *puf-8* increases the size of the RID sister cell, which is the smaller daughter of the RID neuroblast and which is programmed to die. An increase in the size of cells programmed to die has previously been shown to compromise the ability of these cells to undergo programmed cell death. Therefore, *puf-8* may promote programmed cell death by ensuring that the sizes of cells programmed to die are below a certain 'lethal' size. Finally, based on these findings, we propose that the dual role of PUF-8 -[PUM1,2] in the control of programmed cell death is critical for the robustness with which the same 131 cells reproducibly die during *C. elegans* development.

567C Programmed DNA Elimination in the parasitic nematode *Ascaris*: Are Argonautes and their associated small RNAs involved?

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Programmed DNA elimination in the nematode *Ascaris* is a developmentally regulated process that reduces the somatic genome, while leaving the germline genome intact. It occurs in early embryogenesis at 4 to 16 cell stage in all somatic lineages. The eliminated DNA consist of repetitive sequence as well as ~1,000 germline-expressed genes. The biological role of this process appears in part to be a gene silencing mechanism. *Ascaris* DNA elimination involves specific chromosomal DNA breaks and changes in the holocentric chromosomes. The DNA breaks and loss of CENP-A in specific chromosomal regions defines the retained and eliminated regions of chromosomes. In ciliate programmed DNA elimination, small RNAs are known to mark retained or eliminated genome regions during DNA elimination. To examine if small RNAs contribute to *Ascaris* programmed DNA elimination, we identified *Ascaris* small RNAs, 10 Argonautes, and characterized *Ascaris* small RNAs associated with 7 Argonaut proteins including all WAGOs. Immunostaining of embryos during programmed DNA elimination indicated that WAGO-2 specifically stains retained DNA while WAGO-3 predominantly localizes to chromosome fragments that will be eliminated. ChIP-seq data also showed some enrichment of WAGO-3 Argonaute on eliminated DNA regions. We also carried out small RNA sequencing and analysis in embryos enriched for DNA elimination mitoses by sorting with H3S10p labeled embryos or chromatin IP with WAGO-2 and WAGO-3. Small RNA sequencing of embryos enriched for DNA elimination (sorted) did not identify any small RNAs associated with the sites of chromosome breaks or changes in CENP-A localization that contribute to DNA elimination. Chromatin IP identified specific sets of small RNAs corresponding to repeats for WAGO-2 and mRNAs for WAGO-3. Overall, however, these small RNAs do not appear to specifically target retained vs eliminated regions as anticipated. WAGO-3 small RNAs appears to be enriched for nascent RNAs associated the eliminated regions. The possible role of WAGO-2 and WAGO-3 and small RNAs in *Ascaris* programmed DNA elimination will be further discussed.

568A Integrating bulk and single cell transcriptomics for accurate detection of tissue-specific gene expression

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Advances in RNA-seq for bulk and single cell (sc) approaches have produced increasingly fine dissections of the *C. elegans* transcriptome. Although both techniques can yield transcriptomes for individual cell types, each comes with strengths and weaknesses. scRNA-Seq affords high resolution, but suffers from dropout, leading to false negatives. Bulk sequencing detects more genes, but suffers from contaminating cell types, resulting in false positives. In this work we integrated these orthogonal approaches to improve the accuracy of both methods. We used bulk samples collected for specific neuron types and sc datasets for all *C. elegans* neurons and additional non-neuronal cells (1). We used sc data to estimate contamination in each bulk sample, and developed novel methods for removing these gene counts. In one approach we used linear histogram matching to scale sc counts, and subtracted putative contamination using data from non-neuronal clusters. In another approach we used bootstrapping to estimate gene level contributions from target and contaminating tissues in sc data and apply them to bulk counts, providing a bootstrap sample distribution of corrected expression data. We assessed these approaches in two ways: 1) Measuring improvements in calling genes with known expression in all neurons; 2) Examining effects on eliminating genes expressed exclusively in contaminating tissues. We found that our analysis reduced false positives, while maintaining robust true positive detection, thus offering a unique strategy for utilizing complementary bulk and sc RNA-Seq data sets to enhance the accuracy of cell-specific expression profiling data.

1. Taylor SR, Santpere G, Weinreb A, Barrett A, Reilly MB, Xu C, et al. Molecular topography of an entire nervous system. bioRxiv. 2020:2020.12.15.422897.

569B Deciphering a cis-regulatory code for tissue-specific alternative splicing

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Alternative splicing is a major contributor of cellular complexity and it is regulated in a highly tissue- and context-dependent manner. Many studies have focused on investigating the major regulators of alternative splicing, cis-elements and trans-factors, by learning predictive models on native sequences and through *in vitro* studies or cell-based screens. However, investigating the roles of a multitude of these factors in a more systematic manner *in vivo* is challenging and remains an understudied area. The goal of our project is to develop a high-throughput system to investigate the different cis-elements and trans-factors controlling alternative splicing in different contexts *in vivo*. To this end, we have employed a parallel reporter assay (PRA) in *Caenorhabditis elegans* using reporters to monitor alternative splicing patterns in specific cell and tissue types. We have used this approach to screen thousands of random sequences and have identified 469 putative intronic cis elements enhancing or repressing alternative splicing patterns in neuronal tissue. I will further extend this approach to identify sequence features and trans-factors associated with other tissue- as well as single neuron-biased splicing patterns. Together, this work will provide insight into the mechanisms governing tissue and cell-specific splicing patterns *in vivo*.

570C A catalogue of nematode karyotypes

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Observation of the behaviour of nematode chromosomes during meiosis by Boveri was critical to the development of the germline theory of inheritance in the late 19th century. In the current era of genomics, knowledge of the karyotype of a species is very useful in conditioning expectations of the assembly. *Caenorhabditis elegans* has six chromosomes, comprising five autosomes and an X. Other *Caenorhabditis* species also have six chromosomes, but in the sister group to *Caenorhabditis*, the *Protorhabditis-Diploscapter* clade, chromosome numbers range from 6 to 1. These changes in chromosome number imply changes in gene linkage, and also therefore in gene coregulation. Previous catalogues of nematode chromosome numbers were limited in scope (for example including data from only plant parasites or only animal parasites) or contain species names that are no longer current. We have collated a catalogue of chromosome numbers, and sex determination karyotypes, from literature from 1883 to the present day. We identified the current names for species described in older literature, and placed these data in a modern taxonomy. We identified chromosome counts for 218 species from 95 genera in 44 families. As is often the case in Nematoda, free-living species from non-rhabditid Chromadoria, Enoplia and Dorylaimia are poorly represented, Haploid chromosome numbers range from 1 to over 50, with variability especially in the plant parasitic Meloidogyninae. The modal reported haploid chromosome number is 6. Sex determination mechanisms, derived from karyotypic differences between males and females, and between different spermatid nuclei, are commonly XX-XO with several independent origins of XX-XY systems. Ascaridomorpha includes many species with multiple X chromosomes. Many nematodes are parthenogens, and parthenogenesis is associated in some lineages with polyploidy (triploidy and tetraploidy). The catalogue is available via the Genomes on a Tree system (https://goat.genomehubs.org/preview).

571A Computational Analysis of UDP-glycosyltransferase Variation across Strains of *Caenorhabditis elegans*

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Caenorhabditis elegans are simple non-parasitic nematodes with a relatively short life cycle and a wealth of genomic information across multiple databases, making them ideal model organisms. However, little is known about the UDP-glycosyltransferases (UGTs) responsible for their innate detoxification response. UGTs are a large family of phase II enzymes responsible for the glycosylation of small molecules across organisms, thus interacting with small molecules such as toxins in the worms' immediate environment. The Edison Vertically Integrated Projects (VIP) Computational Team is a group of undergraduate students who are working to identify the diversity that exists in UGTs across *C. elegans* isolates from different geographical locations found in the *Caenorhabditis elegans* Natural Diversity Resource (CeNDR) database in order to make inferences about their evolutionary relationships and functions. The CeNDR database is a collection of wild isolates of *C. elegans* and their genomic data found globally used by researchers worldwide. Out of the 250 glycotransferases are responsible for transferring sugar molecules to various substrates, there are about 79 UGTs that transfer sugar molecules to small molecules including toxins. Two approaches were implemented to identify UGTs and make inferences based on their variation. First, we created a catalog of UGTs in the N2 reference strain and used them to create a phylogenetic tree that allowed us to depict the relationships between the UGT protein sequences. For our second approach, we quantified UGT variation using the strains found in the CeNDR database. The results and inferences from this research will help us explore possible functions of UGT genes and improve our understanding of UGT variation in *C. elegans*.

573C *C. elegans* transposable elements harbor diverse transcription factor DNA-binding motifs

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Transposable elements (TEs) are powerful agents of evolution that can rewire transcriptional programs by mobilizing and distributing transcription factor (TF) DNA-binding motifs throughout genomes. To investigate the extent that TEs provide TF-binding motifs in *C. elegans*, we determined the genomic positions of DNA-binding motifs for over 200 different TFs (1). Surprisingly, we found that almost all of the examined TFs have binding motifs that reside within TEs, and all types of TEs have at least one instance of a TF motif, demonstrating that TEs provide previously unappreciated numbers of TF-binding motifs to the *C. elegans* genome. After determining the occurrence of TF motifs in TEs relative to the rest of the genome, we identified numerous TF-binding motifs that are highly enriched within TEs compared to what would be expected by chance. Consistent with potential functional roles for these TE-enriched TF-binding sequences, we found that significantly more TEs with TF motifs display evidence for selection compared to those lacking motifs through the use of publicly available genome variation data (2). We also compared the locations of TE-residing TF motifs to published ATAC-seq (3) and ChIP-seq (4) data, which identify regions of open chromatin associated with TF DNA binding and regions bound by TFs of interest, respectively. Strikingly, we found that all of the TF motif types that occur in TEs have instances of residing within accessible chromatin, and the overwhelming majority of TF-binding motifs located within TEs associate with their cognate TFs, suggesting extensive binding of TFs to sequences within TEs. Additionally, TEs with accessible or TF-bound motifs reside in the putative promoter regions of ~14% of all protein-coding genes, providing widespread possibilities for influencing gene expression. Taken together, our work shows that TE-provided TF-binding sites are ubiquitous in *C. elegans* and have broad potential to rewire gene expression.

1. Weirauch et al. (2014) *Cell* 158:1431-1443.

2. Cook et al. (2016) *Nucleic Acids Research* 45:D650-D657.

3. Daugherty et al. (2017) *Genome Research* 27:2096-2107.

4. Kudron et al. (2018) *Genetics* 208:937-949.

574A Genes essentiality in various genetic backgrounds

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Essential genes can be defined as the ones which are crucial for organism survival and reproduction.

They are responsible for the functions which are most important for the organism fitness pointing out to the most fundamental biological processes. They play a significant role in evolution, as their loss leads to the organism death or inability to reproduce. But this simple definition is not sufficient as gene essentiality is context dependent and vary with genetic and environmental background. So, the same mutation might lead to cell/organism death or severe sickness in some but not all genetic and environmental contexts. Most studies on gene essentiality were done in one genetic background. That means, that many genes essential in other genetic contexts were probably unidentified. Although the number of studies analysing gene essentiality in different genetic and environmental contexts is increasing, they are mainly done with unicellular organisms or cell lines. There is noticeable underrepresentation of such studies in multicellular, whole organisms. In this study we aim to uncover context dependence of gene essentiality in model multicellular organism, *Caenorhabditis elegans*. We show how essentiality of genes is changing depending on genetic context by using three different wild type worm isolates: N2, LKC34, and MY16. Our set of targeted genes included 100 essential and 200 nonessential genes based on DEG database (www.essentialgene.org). Each query gene was inactivated by RNA interference (RNAi) and mutant fitness was measured quantitatively as the rate of bacterial food consumption. We identified new essential genes indispensable in i) all three (22), ii) two (10), and iii) only one (11) genetic background. From previously identified N2 essential genes, we confirmed their essentiality in two additional backgrounds (68 genes), or one of them (9 cases). As we see even in a study encompassing only three strains, and 300 genes, we can observe substantial context dependence of gene essentiality. This have very important practical consequences as well. When working on gene therapies researchers should consider that different strategies might be needed depending on genetic background in which they will be used.

575B New ends with new technology: chromosome diminution in Oscheius nematodes

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Chromosome diminution involves specific deletion of parts of the germline genome from somatic cells. This phenomenon, first described in ascaridid nematodes, has been found widely (in protists, plants and animals), but occurs sporadically within any given clade. Using long read genome sequencing we identified chromosome diminution in the rhabditine nematode *Oscheius tipulae*, where this phenomenon had not been reported before. Many kilobases of DNA are removed from each end of every chromosome, including deletion of expressed genes, and new telomeres synthesized (see https://doi.org/10.1093/g3journal/jkaa020). By long-read sequencing of additional species we have identified similar patterns of diminution across the genus *Oscheius* - deletion of subtelomeric sequence and addition of new telomeres. Using these sequences we have explored the genomic contexts of diminution and begun to test hypotheses of the mechanism and functions of diminution. Long read sequencing has changed our understanding of *Oscheius* genomes, and we wonder if diminution processes may be more widely distributed.

576C Using WGS to identify intragenic suppressors of *zyg-1* in *Caenorhabditis elegans* reveals the importance of genomic context in phenotypic interpretation

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Intragenic modifiers can have dramatic effects on phenotypic outcomes, and in disease, can affect attributes such as severity or age of onset. However, the focus of many modifier screens in model systems is the identification and interpretation of extragenic variants rather than understanding the link between intragenic modifiers and phenotype. Accordingly, technological advances in the field, such as the use of whole-genome sequencing (WGS) or CRISPR, tend to be reserved for the discovery and interpretation of extragenic variants, whereas intragenic modifiers still rely on more traditional methods, such as PCR and Sanger sequencing. We demonstrate that intragenic modifier discovery and interpretation following modifier screens benefits greatly from WGS and CRISPR technologies by subjecting strains generated from a chemical mutagenesis suppressor screen using a temperature-sensitive *zyg-1(it25)* allele to WGS, filtering for intragenic variants using an in-house pipeline, and validating with CRISPR and homology-directed repair. Following this method, we rapidly identify that approximately 10% of our strains contain intragenic *zyg-1* variants. By re-creating the intragenic variants in the parental strain, we show that most intragenic variants suppress the original phenotype, but this is not universal. Based on this, we encourage extending variant validation using CRISPR to intragenic variants to determine whether they are truly capable of modifying the original primary variant and to what extent. For those intragenic variants that are capable of suppressing, the position of the variant in comparison to the original primary variant matters; closer modifying variants are more likely to be true suppressors and have a higher suppression ability, which has important implications in understanding complex alleles in disease. Finally,

genomic context, as provided by WGS, plays an important role in our interpretation of the contribution of the variant to suppression dynamics. For instance, several of the strains contain extragenic variants that, in addition to the intragenic variant, are proposed to enhance suppression. In contrast, we identified a strain with substantial genetic burden, dampening the suppression ability of the intragenic variant. Altogether, this work describes a methodology for the high-throughput identification of intragenic modifiers and highlights the importance of thoroughly understanding the phenotypic contribution of intragenic modifiers.

577A Long-read sequencing and de novo genome assemblies reveal complex chromosome end structures caused by telomere dysfunction at the single nucleotide level

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Karyotype change and subsequent evolution is triggered by chromosome fusion and rearrangement events, which often occur when telomeres become dysfunctional. Telomeres protect linear chromosome ends from DNA damage responses (DDRs), and telomere dysfunction may result in genome instability. However, the complex chromosome end structures and the other possible consequences of telomere dysfunction have rarely been resolved at the nucleotide level due to the lack of the high-throughput methods needed to analyse these highly repetitive regions. Here we applied long-read sequencing technology to *Caenorhabditis elegans* survivor lines that emerged after telomere dysfunction. The survivors have preserved traces of DDRs in their genomes and our data revealed that variants generated by telomere dysfunction are accumulated along all chromosomes. The reconstruction of the chromosome end structures through *de novo* genome assemblies revealed diverse types of telomere damage processing at the nucleotide level. When telomeric repeats were totally eroded by telomere dysfunction, DDRs were mostly terminated by chromosome fusion events. We also partially reconstructed the most complex end structure and its DDR signatures, which would have been accumulated via multiple cell divisions. These finely resolved chromosome end structures suggest possible mechanisms regarding the repair processes after telomere dysfunction, providing insights into chromosome evolution in nature.

578B Evidence of loop-extrusion by X-specific condensin in *C. elegans*

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Unlike most eukaryotes that have two condensins for compacting DNA during mitosis/meiosis, *C. elegans* have evolved a third version called dosage compensation condensin (condensin DC). The condensin DC, which shares 4 out of 5 subunits with condensin I, localizes to the X-chromosome throughout interphase to repress transcription in XX hermaphrodite. *In vitro* experiments have shown that human condensin I and II perform loop-extrusion, a process by which a DNA molecule is linearly contracted resulting in formation of a loop. We are interested in whether condensin DC performs a similar function and how it is regulated *in vivo*.

We investigate a few potential regulators of condensin DC based on their requirement for the repression of transcription on X-chromosome. We performed Hi-C data in embryos and in L3 using Arima Hi-C kit, which generates high resolution interaction matrix based on utilization of two 4-base cutters. Our analysis of Hi-C data in embryo and larva suggests that condensin DC is a loop-extruder. We conclude that while loop-extrusion by condensin DC is necessary, the mechanism by which it represses transcription is unclear. Overall, we provide the basis for interrogating how loop-extrusion may have evolved to partake in chromosome-wide repression of transcription.

579C Modeling Timothy Syndrome in *Caenorhabditis elegans*

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Timothy Syndrome (TS) is a rare genetic disease arising from mutations the gene *CACNA1C,* which codes for a subunit in calcium channel $Ca_v1.2$ expressed throughout the body. Because of the gene's wide-ranging expression, TS patients have a variety of symptoms in different organ systems including arrhythmias, neurodevelopmental delays, hypoglycemia, and syndactyly. Like many rare genetic diseases, treatment options for patients are limited and there is no cure. It is not clear how

the mechanism of the disease functions to cause a complex array of symptoms in the many organ systems. The nematode *Caenorhabditis elegans* provides an excellent model system in which to study the genetic underpinnings of TS further as it contains an orthologous gene to *CACNA1C*: *egl-19*. We have used the CRISPR/Cas-9 system to create missense alleles in *egl-19* corresponding to TS patient *CACNA1C* alleles. While CRISPR is a modern gene editing technique with few off-target editing effects, a previous *egl-19* missense allele with mutation G365R was historically generated by random mutagenesis with ethyl methanesulfonate (EMS). We observed significant differences between strains with the same missense mutation created by CRISPR versus EMS, with the EMS strain demonstrating far milder phenotypes. Our CRISPR generated G365R allele is embryonic lethal, while the EMS-derived strain is viable. Through further analysis, we hypothesized this EMS strain also contains a suppressor mutation that is repressing the phenotype of the *egl-19* mutation. Here, we use a molecular mapping strategy and whole genome sequencing techniques to find both the location and identity of this unknown suppressor. We then aim to characterize this gene and uncover how it functions as a regulator of the orthologous TS phenotype. This regulator could provide insight into new molecular targets for TS therapeutics to better treat the syndrome at the cellular level.

580A Genome-wide profiling reveals a dual role for histone H2A mono-ubiquitylation

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Polycomb complexes are major regulators of chromatin state. Variant Polycomb Repressive Complex 1 (PRC1) is responsible for histone H2A lysine 119 mono-ubiquitylation (H2AK119ub), a post-translational modification associated with the regulation of gene expression and development. Histone H2A ubiquitylation is believed to work cooperatively with PRC2-mediated histone H3 lysine 27 trimethylation (H3K27me3) to repress gene expression, however recent evidence suggests that PRC1 may have H3K27me3-independent functions as well. In *C. elegans*, putative PRC1 component homologs, *mig-32* and *spat-3*, and PRC2 homologs have different associated mutant phenotypes, suggesting they play partially distinct roles. However, whether *C. elegans* PRC1-like and PRC2 complexes and corresponding histone modifications function independently is currently unknown. To investigate the relationship between H2AK119ub and H3K27me3 in *C. elegans*, we performed ChIP-seq and RNA-seq profiling in wildtype and *mig-32* mutant embryos. We discovered that the majority of H2AK119ub and H3K27me3 peaks were distinct, with co-enrichment of the two histone modifications found at only a small subset of protein coding genes. Likewise, we observed a large decrease in H2AK119ub in *mig-32* mutants, whereas the distribution of H3K27me3 was not significantly affected, suggesting that H2AK119ub and H3K27me3 are regulated independently. Surprisingly, many H2AK119ub peaks were instead co-localized with H3K4me1 and H3K27ac, suggesting a role for H2AK119ub at enhancers. We found that H2AK119ub was also enriched at promoter regions of highly expressed genes and that H2AK119ub-associated genes were enriched for functional annotations related to nervous system development, which is consistent with roles for *mig-32* and *spat-3* in neuronal migration and axon guidance (Karakuzu *et al*. 2009 *Development*). We identified many genes with H2AK119ub-enriched promoters that were significantly upregulated in *mig-32* mutants. Interestingly, while many of these upregulated genes are highly expressed in wildtype worms, a subset are normally H3K27me3-repressed, implying a potential cooperation between H3K27me3 and H2AK119ub in regulation of a subset of genes. Together, our results indicate a dual role for H2AK119ub in the regulation of *C. elegans* gene expression, with both H3K27me3-dependent and H3K27me3-independent activities.

581B Characterizing complex genomic rearrangements in *C. elegans* using short-read Whole Genome Sequencing

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Structural variants (SVs) and complex rearrangements are a significant, but understudied, part of genomic natural diversity. They can also result in various phenotypes in *Caenorhabditis elegans.* Additionally, certain strains, known as balancers, carry various types of spontaneous or induced (X-rays, gamma irradiation, chemical mutagens, or more recently CRISPR-Cas9) chromosomal rearrangements. Because those rearrangements abolish or reduce crossover events between large portions of the *C. elegans* genome, balancers have been used for decades to maintain lethal mutations, facilitate strain construction, or capture mutations. However, despite the advent of sequencing technologies, detecting SVs and complex rearrangements is challenging and thus, in most mutagen-induced balancers, the chromosomal rearrangements have not been characterized at the molecular level. High-throughput WGS sequencing, based on short-reads (srWGS), is the most economical, accurate, and widely supported technology at this time. Yet, the length of short-reads is often reported as limiting the detection of complex genetic events. Long-read technologies are then recommended as an alternative despite their increased costs. Here, we applied srWGS alongside tailored bioinformatics approaches to unravel complex genomic rearrangements in several *C. elegans*

balancer genomes. Our method successfully identified complex balancer rearrangements such as reciprocal translocation (*eT1*), large deletion (*eDf43*), chromosome fusion (*sC4*), and free duplication (*sDp3*). We were also able to identify and experimentally confirm several chromoanagenesis events. Therefore, our results show that we can maximize the possibilities of srWGS by implementing meticulous analytical approaches. Higher confidence in complex variant calls from srWGS will help to molecularly characterize *C. elegans* balancers and in the future, to decipher the full spectrum of natural genetic variation in *C. elegans.*

582C Functional Analysis of Variants in a Gene Associated with Early Onset Epilepsy

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Syntaxin Binding Protein, or STXBP1, is a human gene that is associated with neonatal and early onset epilepsy, as well as developmental delays. In this work, we replaced the C. elegans ortholog of STXBP1, unc-18, with the human coding sequence for STXBP1. We then introduced 32 pathogenic and 25 benign variants into the sequence using CRISPR. The movement and morphology of the strains were analyzed using WormLab. We utilized the resultant 25 quantified features to train two parallel machine learning classifiers, Random Forest (RF) and Support Vector Machines (SVM). The classifiers were evaluating using the area under the Receiver Operating Characteristic curve (AUROC) and the precision-recall metric (PR). Both algorithms were found to perform well (AUROC = .94 and .85 for SVM and RF, respectively) and agreed on all variants except two 2 benign and 2 pathogenic. We next introduced 24 Variants of Uncertain Significance (VUS) into the human coding sequence for STXBP1 and phenotype the resultant strains. We found 6 variants that both classifiers classified as Pathogenic, 15 variants that both classifiers classified as Benign, and 3 for which the two classifiers disagreed. We conclude that protein humanization, variant introduction, and phenotypic characterization, and machine learning classification is a viable workflow for the functional analysis of variants in the STXBP1 gene and can help to resolve VUS that are clinically relevant.

583A Improved reference genomes for *Caenorhabditis briggsae*

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Decades of research have led to the development of comprehensive genome resources that have been essential to study the *Caenorhabditis elegans* species. In parallel, the emergence of *Caenorhabditis briggsae* as a model system has been useful to make interspecies comparisons. Despite the importance of *C. briggsae* as a model, its genome resources have not been developed to the same extent as *C. elegans*. The current genome of *C. briggsae* reference strain AF16 contains thousands of unresolved gaps and numerous mis-assemblies. Because of these issues, *C. briggsae* gene models remain incomplete and have numerous structural errors in protein-coding genes. We sought to exploit the latest sequencing technologies and computational tools to provide the highest quality *C. briggsae* genome resources to date. First, we generated high-quality genome assemblies for two strains of *C. briggsae*: QX1410 (a “tropical” strain isolated in Saint Lucia that is closely related to AF16) and VX34 (a divergent strain isolated in China). These genome assemblies incorporate high coverage Oxford Nanopore PromethION long reads and chromosome conformation capture (Hi-C) data. Second, we genotyped 99 recombinant inbred lines generated from reciprocal crosses between QX1410 and VX34. Using these data, we produced a high-quality recombination map that validated the placement of scaffolds after genome assembly. Third, we sequenced the transcriptomes of each strain to high coverage using Pacific Biosciences SMRT and Illumina platforms. We developed a computational pipeline that leverages long and short RNA reads to generate a genome annotation for each strain. These new genome annotations have improved accuracy and completeness relative to the AF16 genome. Fourth, our research group currently maintains over 1,600 *C. briggsae* wild strains, comprising the largest collection worldwide. We sequenced the genomes of this entire collection to high coverage using the Illumina platform. We mapped the sequences of all wild strains to the QX1410 genome to call single nucleotide variants across the entire population. These high-quality genome resources will facilitate new avenues of research, including quantitative and population genetic studies of *C. briggsae*, and enable informative comparisons with *C. elegans*.

584B Characterization of the terminal adenosine's influence on cleavage and polyadenylation of *C. elegans* mRNAs

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The cleavage and polyadenylation of pre-mRNAs is a critical step 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), which is a conserved hexameric element located at the end of the transcript's 3' Untranslated Region (3'UTR). The CPC then performs the cleavage reaction at the polyadenylation (PS) site. Despite their importance, PS element locations in eukaryotic genomes are poorly characterized. Prior research from our lab revealed that the distance between the PAS and the PS elements is not constant and a buffer region between 12-14 nt from the PAS element is needed by the CPC in order to perform a successful cleavage reaction. Our lab recently identified 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 PS element in pre-mRNA cleavage and polyadenylation.

In order to further study the involvement of this terminal adenosine located at the PS site, we developed a novel *in vivo* cleavage and polyadenylation assay which we will use to determine the optimal buffer region length and further study the role of the terminal adenosine at the cleavage site.

For these experiments, we have used the *M03A1.3* 3'UTR since it uses only one canonical PAS element (and thus does not use alternative polyadenylation) and has a buffer region of 14 nt. We have prepared a GFP reporter construct containing a mutated *M03A1.3* 3'UTR with no adenosines between the PAS and PS elements. This GFP reporter construct was used to prepare seven new mutants containing a terminal adenosine inserted at specific locations between 17 and 29 nucleotides downstream of the PAS site. The results of this assay will provide a working framework which we will use to model PS elements in the 5546 genes in the worm transcriptome, which currently lack annotated 3'UTR data. Our work will greatly improve our understanding of pre-mRNA cleavage and polyadenylation in *C. elegans* and will allow us to provide a needed reference for PS elements in the worm transcriptome to the scientific community.

585C SLIDR and SLOPPR: Computational pipelines for the discovery and characterisation of spliced leader *trans*-splicing and polycistronic RNA processing reveal the evolutionary dynamics of SL2 *trans*-splicing across the Nematoda

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The organisation of genes into operons — clusters of genes which are transcribed as polycistronic RNAs — is a feature of all known nematode genomes. Spliced leader *trans*-splicing is essential for the expression of downstream operonic genes because the spliced leader provides the 5' cap to their otherwise uncapped transcripts. In *C. elegans*, a specialised spliced leader, SL2, is specific for transcripts derived from downstream operonic genes via a process that is mechanistically distinct from the more generic SL1 *trans*-splicing. Studies of nematodes outside of Clade V failed to detect SL2 *trans*-splicing, with downstream operonic gene transcripts being *trans*-spliced to SL1. This led to the hypothesis that SL2 *trans*-splicing is recent innovation and that SL1 *trans*-splicing is the ancestral mechanism for resolving nematode polycistronic RNAs. However, a rigorous investigation of this hypothesis requires the comprehensive genome-wide characterisation of both operons and spliced leader *trans*-splicing. This has, until recently, been challenging, and their identification has historically relied upon sequence similarity with *C. elegans*, which may bias the results.To systematically investigate spliced leader *trans*-splicing and operon organisation, we have developed two fully automated discovery and annotation pipelines, SLIDR and SLOPPR (https://doi.org/10.1186/s12859-021-04009-7), that enable the comprehensive characterisation of spliced leader *trans*-splicing and operon organisation in any organism using standard RNA-Seq datasets. Using these tools, we showed that SL2 *trans*-splicing is more broadly distributed than previous studies suggested; it is found in all Clade I nematodes that we investigated (http://www.rnajournal.org/cgi/doi/10.1261/rna.076414.120). However, we were unable to detect SL2 *trans*-splicing in any Clade III nematode, consistent with previous studies, and could only detect it in a small sub-set of Clade IV nematodes. These distributions can be explained either by loss of an ancestral SL2 *trans*-splicing mechanism in multiple lineages, with SL1 acquiring the role in processing polycistronic RNA; or by the convergent evolution of SL2 *trans*-splicing in selected lineages. I will present data that favours the former explanation provides a possible scenario to explain how SL1 might replace SL2 *trans*-splicing despite the latter's broad conservation and therefore functional importance.

586A Crispr-ing *C. elegans* genes conserved to humans

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We are continuing our community resource project to generate gene deletions of high value to those interested in human biology and disease. Our highly coordinated three-site knockout (KO) production strategy has branches in California, Minnesota, and British Columbia, with the Canadian branch transitioning from the University of British Columbia to Simon Fraser University upon the retirement of KO legend Don Moerman. We employ a dual-pipeline strategy to generate KOs, with the Minnesota and Canadian sites using a scheme based on the Calarco Lab's dual selection cassette method. It deletes all or most of the target gene, effectively eliminating function. In addition, it replaces the gene with a fluorescent reporter, allowing simple tracking of the allele in cases where the mutation results in homozygous lethality or sterility, or fails to cause a discernible phenotype, facilitating genetic manipulation of the alleles. Alternatively, the California site developed and uses a "STOP-IN" cassette that places stop codons in all three reading frames near the beginning of the coding region, eliminating activity. This method does not allow visual tracking of alleles but has the advantage in that it inserts a unique guide RNA recognition site, enabling reversion of the locus to wild-type allowing the phenotype to be reassessed. This feature is desired by some users, for example those performing metabolomics experiments requiring quantitation of phenotypes that are sensitive to strain background effects. Each gene edit is carefully confirmed, and validated strains are grossly phenotyped and promptly deposited into the Caenorhabditis Genetics Center (CGC) strain collection along with detailed strain information. To date, 1,040 of our KOs are available through the CGC. Going forward, we plan to target an additional 2500 *C. elegans* orthologs of human genes, prioritizing known or suspected human disease genes, druggable gene classes, as well as understudied genes that are conserved to humans but that have no actionable information. Finally, we will consider community requests to prioritize specific genes.

587B Genomic landscape of the obligately outcrossing *Caenorhabditis becei*

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Breeding systems determine the way genes are transmitted to the next generation, consequently driving genetic variation and genome evolution. Breeding systems may also play a role in the activity and prevalence of transposable elements. Selfing should increase the selection efficacy against TEs as a result of high homozygosity, with the majority of TEs present having reached fixation through genetic drift. Conversely, outcrossing should facilitate the spread of TE insertions throughout populations, and result in a higher number of moderately deleterious mutations. In the *Caenorhabditis* group *Elegans*, there doesn't appear to be a clear pattern of TE dynamics across partially-selfing species and obligately outcrossing species. Most species in the *Elegans* group, appear to have chromosomal heterogeneity in repeat density, with chromosome arms being more repeat rich, and gene poor, than the centers. This association is not always consistent, as is the case for the outcrossing species *C. inopinata* and *C. bovis*, where some, but not all, repeat types have a more uniform distribution across the chromosome, with little divergence between copies, which may indicate recent TE activity. It is currently unknown whether these observations are also found across species from the obligately outcrossing *Caenorhabditis* group *Japonica*. Here we present the first chromosome-scale assembly for a species belonging to the *Japonica* group, *Caenorhabditis becei*. This high-quality assembly was generated from PacBio long read data, Hi-C data, and a genetic map of an advanced intercross panel. The gene annotation for this genome was generated using Iso-Seq data and RNA-seq data from *C. becei*, as well as orthologous protein-coding and protein sequences from other *Caenorhabditis* species. We then characterized features such as synteny to other *Caenorhabditis* species, genome size, recombination rate domain structure, gene content, orthology, and distribution. We created a classified library of repetitive elements identified through *de novo* approaches. We generated TE annotations for the *C. becei* genome, and we reveal the patterns of repeat density, prevalence, distribution, and divergence.

588C CRISPR- Nanobodies from C. elegans as an therapeutic approach for Erythroblastosis Fetalis

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Erythroblastosis fetalis is a consequence of incompatibility amongst Rh-negative antigen from mother and Rh-positive antigen of foetus thus resulting in hemolysis. The hemolysis usually results in respiratory problems, neurological disorders or even heart failure. C.elegans is known to be homologous to some extent with human genome and produces monoclonal antibodies. The nanobodies from C. elegans are engineered with CRISPR Cas 9 technology where Cas 9 protein has been incorporated into nanobodies that will accelerate its efficiency. The engineered nanobodies will target genes like RHD and RhCE that are prominent in RBC membranous environment. The nanobodies with Cas-9 might block the interaction of Rh-negative antibodies with Rh-positive antibodies of foetus as by refusing the recruitment of Rh-negative antibodies in placenta. Antibodies from the C. elegans are extracted and engineered to produce nanobodies with encoded Cas 9 protein. The haemoglobin obtained from foetus are mixed with haemoglobin from mother with Rh-antigens *in vitro*. After a period of 24-48 hours, when engineered nanobodies are treated with blood by ELISA technique indicated reduced count of Rh-positive antibodies thus blocking RHD gene not allowing the interaction with Rh-negative antibodies. The aim of the study was to study molecular interaction occurring after exposure of nanobodies to Rh- antigens present in foetal blood.

Keywords:

Erythroblastosis fetalis, Rh-negative, hemolysis, C. elegans, CRISPR Cas 9, RHD , RHCE, haemoglobin, nanobodies.

589A The smallest genome in the genus *Caenorhabditis*

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Caenorhabditis elegans is a powerful laboratory model that has provided several key findings in molecular and developmental biology and neuroscience in the past decades. However, only little is known about the evolutionary history of the nematode and the relatives. Recent extensive surveys of new *Caenorhabditis* species around the world revealed that the diversity in the genus is bigger than we previously expected. Those resources are useful to get evolutionary insights for better understanding of biological phenomena identified in *C. elegans* researches and provide opportunities to perform deep evolutionary analyses on morphology, behaviors and genomes.

Here we report a new *Caenorhabditis* species *C.* sp. 36, which has the smallest genome in the genus. The new gonochoristic species was isolated from a weevil (*Niphades variegatus*) collected in the dead log of Masson's pine in Tokyo Japan. Morphologically, the species possesses the typical characteristics of the Elegans supergroup species except the body size is a little smaller. Using Illumina, Nanopore and Hi-C technologies, we assembled the *C.* sp. 36 genome into six big scaffolds accounting for the chromosomes. The genome assembly size was as small as ~58Mb, the smallest among the well-defined *Caenorhabditis* genomes. Phylogenetic analysis revealed that *C.* sp. 36 is a close relative of *C. japonica* whose genome size is one of the biggest in the genus (156 Mb). For a comprehensive genome comparison with *C.* sp. 36, we also sequenced *C. japonica* genome using aforementioned technologies and achieved a big improvement from the wormbase ver WS279. Though the two genome sizes are different by three times, similar numbers of protein coding genes (16929 and 17652 genes, respectively) were predicted for *C.* sp. 36 and *C. japonica,* which are comparable numbers with other *Caenorhabditis* species. Whereas a total CDS span dose not differ much from other spices, intron and intergenic regions showed big size differences. Compared to *C. elegans*, *C.* sp. 36 has ~19.5Mb and ~18.2Mb smaller intron and intergenic spans, respectively. In contrast, those of *C. japonica* are ~26.3Mb and ~32.8Mb larger than of *C. elegans*, respectively. A deeper intron analysis revealed that although intron birth/death trends differed depending on each lineage of *Caenorhabditis*, each-intron length rather than per-gene intron counts mainly contribute to the intron span differences. Repeat analyses showed that transposons, especially DNA transposons are the main factors involved in the intergenic region differences.

590B Genome organization of *Caenorhabditis brenneri*

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Caenorhabditis brenneri is an outcrossing species of nematodes in the ‹Elegans› supergroup (*Rhabditida*) formally described by Sudhaus and Kiontke in 2007. *C. brenneri* is one of the most genetically diverse eukaryotes, roughly every tenth nucleotide is polymorphic, which makes it comparable to hyperdiverse bacteria (Dey et al. 2013). To study such a tremendous amount of diversity on the genome-scale, we need high-quality data and a chromosome-scale reference genome. We created a super-

inbred *C. brenneri* strain VX0223 (300 generations of inbreeding) to remove the residual heterozygosity and constructed a telomere-to-telomere genome assembly using highly accurate long-reads, short-reads, and genome-wide chromatin organization data, as well as full-length transcript sequencing and RNA-seq for the genome annotation. We have shown that *C. brenneri* has a similar pattern of genome organization to other *Caenorhabditis* species, with a higher gene density in the central regions of chromosomes and the peripheral parts of chromosomes enriched with repeats. However, the percentage of the repetitive elements in the genome is lower than in other outcrossing species of *Caenorhabditis*, only 16.3% (*C. remanei* and *C. inopinata* have 23% and 30%). That is inconsistent with the previously reported higher repeat abundance in *C. brenneri* (Feschotte et al. 2009, Fierst at al. 2015), which is probably connected to the higher duplication level and redundancy in the previously available genome assemblies (caePb2 and GCA_000143925.2).

591C Genomes of 15 *Oscheius tipulae* isolates from Chernobyl

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Background ionizing radiation is a ubiquitous environmental carcinogen that is usually considered safe, due to its low levels and our cellular pathways for detecting and repairing damaged DNA. While the key players in these pathways are known, much has yet to be learned about how they vary in the wild, and how such naturally-occuring variants affect DNA repair efficiency. One approach to understanding such variation is to compare the genetics and cell biology of radiation-tolerant animals to that of closely related radiation-sensitive animals.

In search of radiation-tolerant animals, we have collected nematodes from the fruits and soils of the Chernobyl Exclusion Zone, a landscape altered by contamination from the world's largest nuclear power plant accident 35 years ago. We have recovered and cryopreserved 298 isolates, and have begun investigations into 15 such strains of *Oscheius tipulae*, isolated from sites ranging in ambient radiation levels. *De novo* genome sequencing and assembly of these 15 animals reveals multiple chromosome rearrangements.

592A Sign and reciprocal sign epistasis across different environments

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Epistasis is a phenomenon of different loci interacting with each other, ergo having non-independent effect. But even though genetic interactions are widely spread across genomes their importance is still being questioned. Depending on the perspective, epistasis is said to either play an essential role in both applied (animals and plants breeding, forensics, personalized medicine) and evolutionary biology (emergence of sex, speciation, shaping evolutionary landscapes) or to be seen as a *noise* of linear genes effect. In general, epistasis can be either positive, when double mutant has higher than expected fitness, or negative, when the fitness is lower than expected. Sign and reciprocal sign epistasis are peculiar cases of genetic interactions, where one or both mutations have effect opposite from both mutations combined. We have tested over 1000 pairwise interactions in standard conditions and in three different environmental stressors: heat shock (37°C), oxidative (H_2O_2) and mutagenic (MMS) stress. We have found that sign epistasis is a prevalent event; 40-78% of all significant interactions are in fact sign epistasis. What is even more interesting, we were able to detect few examples of reciprocal sign epistasis, however only in harsh environments; one in MMS treatment and four in H_2O_2 treatment. All of them were the examples of *positive reciprocal sign epistasis* – despite the deleterious effect of both single mutations, double mutant exhibited higher than expected fitness (higher than animals without any mutations). Both sign and reciprocal sign epistasis are a game changer when it comes to predicting fitness landscapes. As much as sign epistasis is able to slow down the process of reaching fitness optimum, reciprocal sign epistasis can actually block it, unless e.g. two mutations occur simultaneously. Our findings are notably important, since they point to the importance of considering environment in studying genetics and evolutionary scenarios.

593B Variant Discovery mapping for identification of phenotype causing mutations: case studies and a new online pipeline

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Mapping by sequencing has become an accessible method for identifying phenotype-causing mutations in *C. elegans*. The main strategy for this is bulked segregant analysis, which involves outcrossing the strain carrying the mutation of interest with a polymorphic strain and assaying the segregation of genetic markers in pooled samples as a means for mapping qualitative traits. This strategy has been broadly implemented in the Hawaiian variant mapping method, where a strain carrying the mutation of interest is outcrossed to the CB4856 polymorphic background.

Variant discovery mapping (VDM) combines bulked segregant analysis with an isogenic backcross, performed between the mutant and the non-mutagenized starting strain. VDM uses mutagen-induced variations as markers for mapping. By using the non-mutagenized starting strain to perform the mapping cross, VDM allows mapping of mutations in strains with complex genetic backgrounds or mutations with phenotypes that are affected by polymorphic genetic backgrounds.

Here, we present a new online pipeline for VDM on the public Galaxy server, which can be used as an alternative to the Cloudmap pipelines (Minevich et al. 2012) that have now deprecated. The new VDM pipeline uses Freebayes as a variant caller, along with easy-to-use tools for SNP subtraction and generation of mapping plots that support outputs from GATC, Freebayes, and MiModD variant callers. We further present case studies where we apply the VDM strategy and our new pipeline to map and clone several novel, dominant and recessive mutations, that suppress trp-4(ot337) induced dopaminergic neurodegeneration.

594C Use of *C. elegans* for investigating functional consequence of orthologous variants

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The widespread use of next-generation sequencing technologies has resulted in the emergence of millions of new genetic variants in humans and non-human species. One of the most important key tasks in genomics is to differentiate harmful genetic variants from non-harmful variants to diagnose disease better. Computational prediction tools and experimental data have helped to shed light on the functional importance of human genetic variants in this respect. However, mutants bearing human equivalent variants, known as orthologous variants (OrthoVar), have gotten less attention, even though they can be beneficial resources for unraveling the functional data for human OrthoVars. Here we used ConVarT (https://convart.org/), an OrthoVar search engine, to look for human OrthoVars in C. elegans. We selected ift-140, which encodes ciliopathy associated intraflagellar transport 140, because a null mutation causes easily detectable cilia abnormality in C. elegans. We identified conserved amino-acid positions that undergo amino acid changes within IFT-140 and discovered seven different variants that fall into this category, three of which have human OrthoVars. We obtained variant carrying mutants from CGC generated by the Million Mutation project and performed a fluorescent dye uptake assay, which indirectly assesses cilia structure. Except for V444I, none of these mutants exhibit Dye uptake defect, indicating that these variants are likely non-harmful variants. V444I mutants have a temperature-sensitive Dye uptake defect as well as IFT protein accumulations in the cilia, which is similar to the ift-140 null mutant phenotype. We are currently performing a rescue analysis for this variant. We then generated two mutants with human OrthoVars (G680S, P702A), and our thorough analysis found that the P702A variant (Human P726A, reported likely pathogenic) is a null mutant of ift-140, but not G680S (Human G704S, VUS). Our current OrthoVar-focused study has uncovered the functional implications of nine variants (5 human OrthoVars), and we believe that functional assessment of human OrthoVars can provide valuable insight into human variants.

595A CUT&Tag in *Caenorhabditis elegans*

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Chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-seq) has been the gold standard for analyzing interactions between DNA and proteins in the field of gene regulation. It does, however, require a considerable number of cells. Alternative strategies like CUT&RUN and CUT&Tag have been introduced to overcome the limitations of cell number. CUT&Tag utilizes a transposome consisting of a Tn5 transposase fused with proteins A and G, which direct the transposase to an antibody bound to its target. The transposase is also pre-loaded with sequencing adapters, which allows for antibody directed tagmentation followed by library preparation. Here, we applied CUT&Tag to purified nuclei of embryos using at least five different antibodies. We will present our progress in applying this technique to nematodes and share our data.

596B A tool for warp speed genetics in C. elegans

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What if we could rapidly and reliably homozygose every locus in the C. elegans genome during genetic manipulations? What if we could combine the full nuclear information from one C. elegans strain with cytoplasmic contents from a second strain? We can do both, and it is remarkably easy!

Besseling and Bringmann (2016) identified a molecular intervention for C. elegans in which premature segregation of maternal and paternal chromosomes in the fertilized oocyte can produce viable animals exhibiting a non-Mendelian inheritance pattern. Overexpression in embryos of a single protein regulating chromosome segregation (GPR-1) provides a germline derived clonally from a single parental gamete. We present a collection of strains and cytological assays to consistently generate and track non-Mendelian inheritance. These tools allow reproducible and high-frequency (>80%) production of non-Mendelian inheritance, the facile and simultaneous homozygosis for all nuclear chromosomes in a single generation, the precise exchange of nuclear and mitochondrial genomes between strains, and the assessments of non-canonical mitosis events.

597C ***Transgenic hph::gfp gene fusion allows streamlined screening for C. elegans gene knockouts***

Sarah Brivio[1], Rotimi Fasimoye[1], Bernadette Connolly[1], Berndt Müller[1], Jonathan Pettitt[1] 1) University of Aberdeen.

We are interested in understanding the protein and RNA components of the spliced leader *trans*-splicing machinery. Previous work has implicated a set of novel non-coding RNA components, the SmY RNAs, in spliced leader *trans*-splicing. These RNAs are encoded by 12 distinct genes and we are in the process of systematically deleting them to understand the precise mechanistic roles of their products.

To facilitate this approach we have developed a novel hph::gfp reporter transgene that allows tagging and knockout of any gene of interest in a single injection via CRISPR/Cas9 induced homology directed repair (HDR). This method generates a loss of function allele and a fluorescent protein fusion reporter, while the *hph* hygromycin resistance gene facilitates the selection process. The fusion protein is functional, successfully generating both broad cytoplasmic fluorescence and hygromycin resistance in the assayed worms.

This *hph::gfp* repair template provides a simple and flexible approach - it can be modified by oligo cloning to be flanked by homology arms corresponding to the region upstream and downstream of any gene of interest.

This approach significantly reduces the time and labour required to achieve each knockout, facilitating our goal of complete *smy* gene complement knockout. Our results to-date show that loss of ten of the twelve *smy* genes impair spliced leader trans-splicing, confirming the role of this enigmatic set of RNAs in this process.

598A **Non-transgenic Functional Rescue of Neuropeptides**

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Neuropeptides are small proteins produced in the nervous system that regulate synaptic communication by modulating the response of G-protein coupled receptor signals. Neuropeptides serve to repurpose neural circuits: a single gene can encode multiple peptidergic regulatory elements, making it difficult to identify roles of discrete and active neuropeptides. Here, we present a novel tool for the functional rescue neuropeptides that exploits an RNAi feeding-like technique using *E. coli* delivery to feed genetic loss-of-function *C. elegans* mutants active peptides. This technique allows us to tease apart unique roles of individual neuropeptides encoded by single genes. Using our rescue-by-feeding paradigm, we rescue three neuropeptides that have previously been rescued using canonical, transgenic methods – *pdf-1*, *trh-1*, and *ins-6* – to examine the efficacy of the paradigm. Our study confirms that some peptides are functionally redundant, while others that appeared to play similar roles in transgenic rescues are indeed unique. Finally, we argue that the mechanism of peptide delivery is reminiscent of the mRNA uptake observed in RNAi feeding paradigm, though we are currently exploring the mechanisms of how the peptide rescues the phenotype and altering delivery methods to work in other bacterivores. While these neuropeptide genes encode simpler pro-peptide products, we have recently employed this method to decipher the role of *flp-3*-encoded peptides– a FMRFamide-like neuropeptide gene which encodes ten unique peptides. We postulate that this rescue-by-feeding paradigm can offer the ability to dissect the functional landscape of neuropeptide genes, previously inhibited by the bandwidth limitations of combinatorial transgenic analyses.

599B A faster and more efficient RNASeq protocol supports new approaches for studying gene regulation and tissue composition in *C. elegans*

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Individuals with identical genotypes often show high phenotypic variability. To better characterize inter-individual differences in aging, we further optimized the low-input Smart-seq2 RNASeq strategy (Picelli *et al.*, 2014; Serra *et al.*, 2018) to support routine collection of high-quality *C. elegans* transcriptomes. Compared to conventional techniques that require thousands of individuals as input material, we have validated our method to show that it can produce robust results from single individuals, and scales up to pools of thirty worms at which point it replaces existing bulk methods. This combination of single and pooled RNASeq provides a quantitative means for studying variation in gene regulation and tissue composition between individuals and across populations.

Looking to increase throughput and reduce cost over existing approaches, we identified an eccentricity of the Smart-seq2 template-switching polymerase that in *C. elegans* leads to an aberrant amplification of specific ribosomal RNAs independent of the poly-dT primers used during reverse transcription. More generally, we find that Smart-seq2 template-switching oligomers bind non-specifically to multiple non-coding RNAs. We solve this problem by combining biotin-labeled primers with an additional bead purification step after reverse transcription. This procedure excludes all non-coding RNAs and increases our effective sequencing depth by 4.5 fold, providing pooled or single-worm transcriptomes at dramatically lower cost.

We demonstrate the usefulness of this new method to identify novel downstream targets of the *daf-2* insulin/IGF receptor.

References

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600C Tissue-specific transcription footprinting in *C. elegans* using RNA Pol DamID (RAPID) and Nanopore sequencing

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Differential gene expression across cell types is a major determinant of multicellular organism development and physiology. In *C. elegans*, it is notoriously difficult to characterize individual cell types due to the difficulty to obtain comprehensive tissue-specific gene transcription data. Available methods require tissue dissociation and cell sorting from large worm populations or the use of transgenic approaches to purify transcripts from the target cell type. This can be challenging when the considered cell type is hard to separate from surrounding cells or is a rare cell type.

Here, we present the RNA Pol DamID (RAPID) approach, in which the Dam methyltransferase is fused to a ubiquitous RNA polymerase subunit in order to create transcriptional footprints *via* methyl marks on the DNA of transcribed genes. We implemented a Cre-lox recombination system to target specific tissue and 3rd generation sequencing (Oxford Nanopore Technologies) to eliminate the need for an external sequencing facility and streamline data acquisition to achieve analyses from DNA extraction to sequencing results in less than a week.

To validate the method, we determined the polymerase footprints in whole animals and in different tissues from intact young adults. We obtained meaningful transcriptional footprints in line with RNA-seq studies in whole animals, muscle and intestine. To challenge the sensitivity of RAPID and demonstrate its utility to determine novel tissue-specific transcriptional profiles, we determined the transcriptional footprints of the pair of XXX cells, representing 0.2% of the somatic cell content of the animals. We identified 2362 genes potentially transcribed in XXX cells, among which the few known XXX markers, such as *daf-9* and *sdf-9*. Using transcriptional reporters for a subset of new hits, we confirmed that the majority of them were indeed expressed in XXX. Interestingly, results of a gene ontology analysis on a refined list of 275 genes, whose expression is strongly enriched in XXX cells as compared to other profiled tissues, are in line with the endocrine function of these cells, with implications in signalling and dauer formation. A transcription factor predictive tool also revealed an association with specific transcription factors playing a role in dauer formation.

Taken together, our work establishes RAPID as a valid method for the determination of polymerase footprints in specific tissues of *C. elegans* without the need for cell sorting or RNA tagging.

601A A model for partial depletion of disease gene homologs reveals dose-dependent effects of the Kabuki Syndrome-related factors SET-16/KMT2D and UTX-1/KDM6A

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Human genetic disorders often involve haploinsufficiency, in which disruption of just one copy of a gene causes disease symptoms. For example, the developmental disorder Kabuki Syndrome (KS) is caused by disruption of a single copy of the genes that encode the histone modifying factors KMT2D and KDM6A. By contrast, in laboratory organisms, loss-of-function mutations are typically recessive. For example, null alleles of the *C. elegans* KS gene homologs, SET-16 and UTX-1 respectively, appear outwardly normal as heterozygotes (50% gene dose) but are lethal as homozygotes (0% gene dose). We reasoned that more severe depletion of SET-16 and UTX-1 – for example, to 10% or 20% of normal levels – might provide a better model of the cellular defects caused by haploinsufficiency in KS. In order to systematically manipulate protein levels, we turned to the auxin-inducible degron (AID) system. We inserted tandem GFP and AID tags at the endogenous SET-16 and UTX-1 genes. Consistent with previous reports, we find that SET-16 and UTX-1 are both expressed in all, or nearly all, cells beginning in mature gametes and continuing through adulthood. We find that titrating auxin levels causes a dose-dependent depletion of SET-16 or UTX-1 as assessed by GFP intensity, using either a somatic- or germline-specific TIR1 ubiquitin ligase. For example, in somatic cells of animals treated with 0 mM, 0.01 mM, 0.1 mM, or 1.0 mM auxin, we observed relative GFP intensities of 100%, 74%, 24%, and 1% for SET-16 and 100%, 14%, 3%, and 0% for UTX-1. To determine how partial depletion affects cellular phenotypes, we performed transcriptional profiling of SET-16 or UTX-1 somatic depletion strains grown under each auxin condition, in triplicate. We identified ~3000 and ~1500 genes whose expression levels are significantly correlated to the extent of SET-16 or UTX-1 depletion, respectively, across conditions. Overall, the gene lists were highly concordant, for example, 95 of the 100 genes whose expression is most highly correlated with SET-16 levels are also significantly correlated with UTX-1 levels. Intriguingly, 24 of the top 100 affected genes are members of the *pals* gene family, which has been implicated in gene silencing and pathogen-driven stress responses. Overall, this study provides a general strategy for partial depletion of disease-relevant genes that may better reflect the cellular changes that occur in human disorders involving genetic insufficiency.

602B Whole Gene Humanization as Platform for Disease Diagnostics and Therapeutics Discovery.

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Disease research is being greatly impacted by advances in genome-editing. CRISPR and its related techniques now allow the creation of precision animal models of human disease. With the ease of genetic manipulation in C. elegans, the fast lifecycle, and the wealth of phenotypic assays, they are a great genetic model, however, not all human disease variants can be inserted into C. elegans due to sequence divergence. We have developed a method of Whole Gene Humanization to overcome this drawback. First a human gene coding sequence is inserted as a gene replacement of its ortholog sequence in the C. elegans. When this rescues the abnormal function present in the locus null, the system becomes confirmed for conservation of biological function between the worm and human genes. Next, a test clinical variant is installed into the humanized locus and a set of deep phenotyping assays are performed to determine the landscape of phenotypic activity. The variant's phenotypic profile is referenced against the phenotypic profiles of training-set comprising of at least 11 strains (5 established benign, 5 established pathogenic variants, and one wt-humanized control). A receiver operator characteristic (ROC) curve is applied to the euclidean distance of the established benign and pathogenic variants from the wt-humanized control control. The harmonic mean separating benign from pathogenic, is then applied to the test clinical variant's euclidean distance from wt-humanized control. When the clinical variant shows a phenotypic cosegregation with the pathogenic that is above the harmonic mean, an assessment of abnormal function is achieved. We are applying this methodology to over 15 disease-gene targets. Functional rescue of 86% of the test disease loci was achieved which will enable disease modeling in a humanized locus.

603C High-contiguity genomes from single nematodes

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Nematodes are one of the most abundant phyla in the animal kingdom. Along with *Caenorhabditis elegans*, several other soil nematodes and some parasitic species have been established as laboratory model organisms. For these species well-annotated and high-quality reference genomes have been established. Usually a huge number of animals are pooled to generate libraries for sequencing resulting in a final genome sequence of the species. However, the information about the diversity within the species is usually lost and only a fraction of nematode species can be cultivated and studied in laboratories. Small size and cryptic anatomical features makes the isolation of sufficient nematodes of the same species within a meiofaunal community extremely difficult.

We are developing a low-input method for generating high-quality annotated genomes and can generate long-read HiFi genomic DNA libraries and transcriptome libraries from a single isolated nematode. We are working towards also generating Hi-C chromatin conformation capture libraries and linked-read genomic libraries for single individuals. Initial studies using single individuals of the soft-bodied flatworm *Stenostomum* confirmed that preparing all four libraries from single animals is indeed achievable, despite the limited number of cells. We optimised different tissue disruption methods to recover intact nuclei from individual animals. With these intact nuclei in suspension we can isolate RNA, high molecular weight DNA and take a fraction of the nuclei for Hi-C library preparation.

Through optimizing the extraction protocol and different library preparation steps we will be able to gain sequencing data of long-read, proximity-read, linked-read and transcriptome sequencing which will provide us with a high-quality genome. Thus, it will be possible to explore the genomic diversity within populations, among populations and across species at the resolution of single individuals. Further, this low-input method will be of great benefit for identifying and studying these and other meiofaunal species, and understanding the ecology of their communities.

604A Single Nucleotide Substitutions Effectively Block Cas9 and Allow for Scarless Genome Editing in *Caenorhabditis elegans*

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In *C. elegans*, direct germline delivery of Cas9 ribonucleotide protein complexes along with single-stranded DNA repair templates is reliably used to induce genome editing through homology-directed repair (HDR). As Cas9 generates DNA breaks based on complementarity to a guide RNA sequence, additional blocking mutations are often required to prevent Cas9 from continuing to create DNA breaks after homology-directed repair has occurred. Current practices prioritize creating a silent mutation within the PAM sequence (NGG) that is essential for Cas9 activity. In cases where the PAM cannot be silently mutated, it is recommended to design several silent mutations within the 20 nucleotide guide portion of the donor to minimize the risk of Cas9 targeting DNA after HDR. While this approach works well for protein-coding sequences where silent mutations can be easily designed, it remains challenging to predict how extraneous blocking mutations will affect non-coding sequences such as non-coding RNAs, untranslated regions, and other regulatory sequences. To overcome this challenge, we tested whether single-nucleotide substitutions within the guide sequence were sufficient to block Cas9 and allow for efficient genome editing. Our results show that single nucleotide substitutions are sufficient to allow for efficient genome editing and recovery of edited animals. We propose that when the intended mutation is located within the guide sequence, additional blocking mutations are not essential to recover edited animals. Surprisingly, we found that edited animals can be readily recovered without introducing any Cas9 blocking mutations, albeit with reduced efficiency, suggesting a temporal block might restrict Cas9 from continuing to target DNA after HDR. We show that single nucleotide guide substitutions effectively, though not completely, block Cas9 from targeting repaired DNA. We found that editing of the maternal genome occurs at much greater frequency than the paternal genome, and that maternal editing appears to have increased rates of HDR. We suggest that mating to balancer chromosomes before injection can be used to select for maternally-provided haplotypes that are more likely to be edited through HDR, and substantially reduce screening efforts post-injection. Collectively, our findings expand the repertoire of genome editing strategies in *C. elegans* and allow for scarless genome editing in cases where incorporating extraneous blocking mutations are not possible.

605B Recombination-based approaches for efficient knock-ins, robust transgene expression, and modular strain construction

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The ability to introduce exogenous sequences into the genome at specific sites has transformed *C. elegans* research. However, two major areas still limit this technology. First, both the accuracy and efficiency of creating large insertions is sub-optimal.

Secondly, expression from single copy inserts often limits the utility of these reagents. Recently, I introduced a Recombination-Mediated Cassette Exchange (RMCE) method for creating large-DNA single copy transgenes at defined landing sites at improved frequency and fidelity over current MosSCI and CRISPR/SEC methods (Nonet, 2020 Genetics). The system integrates DNA using FLP and excises a selection cassette using Cre. Insertion events occur at 1 per 3 injected animals. Furthermore, the insertions are faithful since they result from recombination rather than repair.

To address expression issues, I used RMCE to improve bipartite reporter systems which have not been exploited by the *C. elegans* community. I introduced an improved GAL4/*UAS* system, a lexA/*lexO* system, a tet OFF tetR-QF/*tetO* system and a QF2/*QUAS* system that all function in single copy and provide for more robust expression.

Bipartite expression systems improve expression, but the complexity of genetic manipulation of these multi loci systems limit their practical utility. To overcome these limitations, I have developed additional methods that permit the creation of bipartite insertions at a single locus. First, I have developed a tandem RMCE method, in which the initial insertion includes not only the first insert, but also an additional mini-landing site. After isolating the initial integration event, a second insert can be integrated at the same position.

Second, I developed Recombination-Mediated Homolog Exchange (RMHE) which permits recombining two RMCE insertions integrated at the same landing site from a *trans* to a *cis* configuration. Single insertions are created containing either an *attP* site 5› of the insertion or an *attB* site 3' of the insertion. After crossing into a germline phiC31 expression background, *trans* heterozygotes recombine to *cis* in approximately 15 percent of the progeny. I outline a new approach in which a panel of appropriately *attB*- or *attP*-tagged lines can be easily recombined permitting flexible mixing and matching of driver and reporter lines to rapidly create novel complex bipartite reporter strains.

606C Self-Selecting Clone-Free Transgene Integration in *Caenorhabditis elegans*- Expanding the Toolkit

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The integration of transgenes in *Caenorhabditis elegans* has traditionally utilized transposable elements or bombardment, with current protocols adopting CRISPR-based integration. CRISPR also allows for the creation of custom-designed integration loci. Here, we take advantage of this experimental freedom to design such loci, or 'landing pads,' to facilitate transgene integration. These landing pads are designed for integration-specific selection, thereby avoiding the selective benefit of array formation and consequent false positives, eliminating the need for anti-array selection or co-CRISPR methodologies. Within the genome, landing pads are built with a partial Hygromycin B resistance gene missing the promoter and the 5'coding sequence. In addition, a synthetic unique guide RNA target sequence is present upstream for targeting by Cas9. Six synthetic guides were pre-tested to choose an optimal guide target, and four were identified with similar integration efficiencies. Donor homology plasmids are constructed with the transgenic cargo plus the *rps-0p*::HYGRΔ 5' coding sequence. As the Hygromycin B resistance gene has been split, there is no selectable advantage in the array. Only upon integration is the Hygromycin B resistance restored. A landing pad locus was initially constructed on Chr. II, and we recently expanded the toolkit, creating landing pad strains and plasmid donors for Chr. I and III. For each landing pad/donor pair, the Hygromycin B resistance gene is flanked by unique Lox sites, allowing for optional removal of the resistance gene without inducing interchromosomal rearrangements. Additional donor plasmids have been constructed to allow for self-excision of the resistance gene, similar to Dickinson et al. 2015.

In addition to streamlining the integration process, we bypassed the requirement for pre-constructed plasmids. It is known that *C. elegans* can stitch together linear dsDNA fragments with homology-directed repair (HDR), which has been used for clone-free transgenesis. We tested two and six-part transgenic insertions for *sqt-1*(e1350) and found functionally integrated transgenes for both. We couple this strategy with our synthetic landing pads to streamline the transgenesis process from PCR to confirmed single copy integration in as little as five days. Overall, the combined approach provides an economical and rapid method to generating single-copy transgenes for *C. elegans*.

607A RNA Pol II binding changes in auxin-inducible degradation system in *C. elegans*

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Techniques to study protein function in living cells is useful in the investigation of biological regulatory mechanisms. Among the *Caenorhabditis elegans* transgenic toolkits, the Auxin-Inducible Degradation (AID) system provides spatial and temporal

control of protein degradation in both a conditional and reversible manner. The AID system utilizes the F-box related protein TIR1, which upon auxin addition, leads to the degradation of AID degron tagged target protein. In *C. elegans*, the usage of AID system is commonly in transgenic lines that express the modified *Arabidopsis thaliana* TIR1. Here, we report that lifelong exposure to 0.01mM, 0.1mM, and 1mM auxin does not significantly affect the morphological quantified phenotypic features or embryo lethality in TIR1 expressing strain CA1200. However, Pol-II ChIP-seq enrichment profile upon 60 minutes of auxin treatment in CA1200 worms shows a dramatic shift within coding regions, which is notable for researchers who are using this strain doing any ChIP-seq analysis.

608B Minimal PAM nucleases and expanded nested CRISPR tools to facilitate CRISPR-Cas genome editing

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Many *C. elegans* CRISPR-based genome editing protocols have been developed in the last few years, ranging from plasmid-based approaches to cloning-free methods. We contributed to this collection of methods with nested CRISPR (Vicencio et al., *Genetics* 2019), facilitating the efficient and cloning-free generation of knock-in strains. We have also investigated the relationship between fragment length and efficiency to determine insertion limits given a single double-strand break. Our data demonstrate that insertion efficiencies decrease as the inserted fragment›s length increases from 600 bp to 1600 bp. In terms of expanding our tools, we have developed new nested CRISPR sequences for the insertion of SL2::mCherry, GFP::H2B, and GFP::degron::3xFLAG tags.

We are interested in testing the efficiency of nucleases aside from Cas9 to overcome the NGG PAM limitation. Our comparative studies of Cas9 and Cas12a (TTTV) PAM indicate that both nucleases are equally efficient for inserting a fluorescent tag. We have also explored the use of Cas9 variants with minimal PAM requirements, namely SpG (NGN) and SpRY (NRN > NYN). Our preliminary results using ribonucleoproteins (RNPs) and a strain endogenously producing SpG Cas9 validate their use in *C. elegans*, albeit with adjusted conditions, to produce both imprecise indels and precise knock-ins. As examples, we observe up to 80% efficiency for knocking out a wrmScarlet reporter and up to 20% efficiency for knocking in a 100-bp fragment using SpG (NGC PAM). Overall, these tools will further expand genome editing possibilities in *C. elegans* with new nucleases that allow editing of previously inaccessible sites and an expanded catalog of nested CRISPR sequences that require little to no additional optimization.

609C Novel approaches to studying maternal transcript regulation in *C. elegans*

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In most model organisms of sexual reproduction, transcription is interrupted before the end of oogenesis, only to resume in the early embryo, after several rounds of mitosis. In the meantime, during the maternal-to-zygote transition (MZT), the fully differentiated oocyte must clear away maternal transcripts before the zygote becomes totipotent and autonomous. In the absence of transcription, post-transcriptional gene regulation is the dominant form of gene expression. RNA regulation is therefore critical for early embryogenesis during the MZT.

Recent single molecule imaging methods have provided great insight into different aspects of RNA biology. One such method images nascent peptides on individual transcripts. Briefly, a reporter transcript is comprised of a coding sequence containing repeated SunTag motifs and a 3'UTR containing 24 tandem MS2-stem-loop hairpin structures. A GFP-tagged antibody fragment that binds to SunTag motifs is co-expressed, identifying sites of translation. An MS2 binding protein tagged with mCherry is also co-expressed, allowing for visualization of the transcript. The multiplexed nature of the SunTag and stem-loop motifs amplifies the corresponding signals, allowing for single-molecule resolution. Various features of translation can be dissected from the quantification of the signal intensities, the number of corresponding foci, and their localization.

We are adapting this approach to study post-transcriptional control of maternal transcripts in germlines and embryos during the MZT of *C. elegans*. First, we reduced the repetitive nature of the 24 SunTag cassette sequence while optimizing codons for expression in *C. elegans*. Next, this cassette was inserted upstream of an endogenous coding sequence of interest (*nos-2*), using a CRISPR approach. In these tagged strains, single molecule FISH and immunofluorescence will be used to label transcripts and nascent SunTag-containing peptides, respectively, to characterize translation across tissues and developmental stages. Combined, this system will provide new perspectives and tools to dissect the dynamics of maternal transcripts and deepen our understanding of RNA regulation in the germline and the early embryo in *C. elegans.*

610A Investigating the Role of sRNA and Argonautes in Intercellular Communication

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The nematode *Caenorhabditis elegans* uses sRNA and Argonaute proteins (AGOs) to degrade, inhibit the translation of, or upregulate target transcripts in a process called RNA interference (RNAi). Remarkably, the worm is capable of taking up dsRNA from the environment via its intestine and transporting to distant tissues to elicit RNAi systemically. While some of the mechanisms of systemic RNAi are understood, one question that remains is: do AGOs themselves move throughout the animal? There is precedent for mobile AGOs in multiple species. For example, the pathogenic nematode *Heligmosomoides bakeri* secretes sRNAs and an AGO called exWAGO during infection to manipulate expression of mouse immunity genes. There are three homologs of exWAGO in *C. elegans* that localize to the apical membrane of the intestine (the intestinal Secondary AGOs, iSAGOs). Their apical intestinal localization places them at an interface with the environment. Our lab used IP/MS to identify protein interactors of iSAGOs and found interactors are involved in membrane and vesicular transport. I hypothesize that the localization of iSAGOs to the intestinal apical membrane allows them to take up dsRNA and sRNA from the environment, and transmit RNAi signals to other tissues in the worm. To test this, I will determine how iSAGOs are localized to the intestinal apical membrane, define the roles of iSAGOs in mediating host-pathogen interactions, and determine whether iSAGOs are necessary for systemic RNAi. This research will illuminate the mechanisms by which the iSAGOs are involved in intercellular communication.

611B Sensitized piRNA reporter identifies multiple RNA processing factors involved in piRNA-mediated gene silencing

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Metazoans guard their germlines against transposons and other foreign transcripts with small RNAs, in particular with PIWI-interacting RNAs (piRNAs). Due to the robust heritability of piRNA initiated silencing in *C.elegans*, previous screens using *Caenorhabditis elegans* were strongly biased to uncover members of this pathway in the maintenance process but not in the initiation process. To identify novel piRNA pathway members, we have utilized a sensitized reporter strain which detects defects in both initiation and downstream processes. Using our reporter, we have identified pre-mRNA splicing factors, Integrator Complex subunits, protein import components, and nuclear pore components as essential for piRNA-mediated gene silencing. Remarkably, only four out of the 30 nuclear pore components tested were required to silence our reporter. We have shown that one of these components, NPP-7, exists both in a membrane-bound form at the nucleoplasmic-facing nuclear pore as well as in a soluble form that accumulates in germ cell nucleoli. The loss of nucleolar NPP-7 through disruption of the nucleolus itself also triggered desilencing of our reporter, indicating that nucleolar NPP-7 is essential for piRNA pathway function. Additionally, we showed that two Importin class genes – *ima-3* and *imb-2* – which trigger reporter desilencing when depleted are necessary for the nuclear localization of the piRNA pathway Argonaute HRDE-1. Further, we have shown that the splicing factors, Importin genes, and nuclear pore components which triggered desilencing of our reporter are all essential for the maintenance of silencing but largely dispensable for piRNA biogenesis itself. In contrast, the snRNA processing cellular machine termed the Integrator Complex is required for piRNA production. Intriguingly, although the Integrator Complex contains over a dozen subunits that are critical for 3' end resolution of snRNAs, only four of these components are required for piRNA biogenesis. Knockdown of these components results in depletion of both mature piRNAs and piRNA precursors, and in the 3' elongation of piRNA precursors in adult worms. This finding is consistent with the recently reported role of the Integrator Complex in piRNA biogenesis. Using our sensitized piRNA reporter, we have shown that multiple facets of piRNA silencing are dependent on extant RNA processing machinery that has been co-opted to function in the piRNA mediated genome surveillance pathway.

612C Reverse complementary matches simultaneously promote both back-splicing and exon-skipping

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Circular RNAs (circRNAs) play diverse roles in different biological and physiological environments and are always expressed in a tissue-specific manner. Tissue-specific circRNA expression profile can help understand how circRNAs are regulated. Here, using large-scale neuron isolation from the first larval stage of *Caenorhabditis elegans* (*C. elegans*) followed by whole-transcriptome RNA sequencing, I provide the first neuronal circRNA data in *C. elegans*. I show that circRNAs are highly expressed in the neurons of *C. elegans* and are preferably derived from neuronal genes. More importantly, reverse complementary matches

(RCMs) in circRNA-flanking introns are not only required for back-splicing but also promote the skipping of exon(s) to be circularized. Interestingly, one pair of RCM in *zip-2* is highly conserved across five nematode ortholog genes, which show conserved exon-skipping patterns. Finally, through one-by-one mutagenesis of all the splicing sites and branch points required for exon-skipping and back-splicing in the *zip-2* gene, I show that exon-skipping is not absolutely required for back-splicing, neither the other way. Instead, the coupled exon-skipping and back-splicing are happening at the same time.

613A Characterization of a potential gene interaction between *spr-5*, *met-2*, and *mep-1* in determining germline versus soma in *C. elegans*

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In *C. elegans*, histone modifying enzymes aid in the activation and repression of genes that are required to distinguish germline from soma. For example, SPR-5 is a histone demethylase that removes the activating histone modification H3K4me1/2 and MET-2 is a histone methyltransferase that adds the repressive histone modification H3K9me2. Together, these two histone modifying enzymes work synergistically to establish a totipotent ground state by shutting down the transcription of germline specific genes that were expressed in the germline of the parents. Previous studies have shown that *spr-5;met-2* double mutants produce progeny that experience a severe developmental delay at the L2 larval stage. Transcriptomic analysis of these animals indicated that the delay is largely due to the inappropriate expression of germline expressed genes in somatic tissues. Here we explore the possibility that the chromatin state established in the early embryo by SPR-5 and MET-2 is reinforced in the later embryo by ATP-dependent chromatin remodeling activity. MEP-1 is a component of the ATP-dependent epigenetic deacetylase complex, MEC. Similar to *spr-5;met-2* double mutants, *mep-1* mutants fail to suppress germline genes in somatic tissues, as indicated by the misexpression of PGL-1 and GLH-3 germline proteins in the soma of L1 larvae. We tested a potential interaction between these two pathways by subjecting *spr-5*, *met-2* and *spr-5;met-2* mutants to *mep-1* RNAi. *mep-1* knockdown when combined with any of these mutant backgrounds, results in an exacerbated developmental delay phenotype. The “triple” mutant is most severe, displaying a full L1 arrest. To fully understand the molecular basis of this synergy, we performed RNA-sequencing on these arrested L1 larvae versus controls. We find very little evidence of new expression changes induced in the triple mutants compared to controls. Instead, we find that expression changes in the *spr-5;met-2* double mutants are exacerbated in triple mutants. This suggests that MEP-1 specifically reinforces the repressed chromatin state established by SPR-5 and MET-2 reprogramming at fertilization. These results help establish how chromatin modifying enzymes work together to establish developmental cell fates.

614B piRNAs regulate transcriptional programs during germline development

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The RNA-guided targeting of nucleic acids is an ancient and conserved mechanism of cellular immunity that has been evolutionary adapted and diversified to regulate eukaryotic gene expression. In animal germ cells, PIWI-interacting small RNAs (piRNAs) have been extensively characterized as a defense mechanism targeting transposable elements (TEs) to promote fertility and genome integrity. In a nutshell: loaded into PIWI effector proteins, piRNA sequences provide mRNA targeting specificity by antisense-complementarity, promoting gene silencing through a variety of mechanisms. Yet, piRNA sequences do not necessarily match TEs, pointing to extended possibilities in gene regulation.

In *C. elegans,* piRNAs do not require perfect complementarity for targeting and PIWI/piRNA complexes promiscuously interact with most of the germline transcriptome without necessarily affecting mRNA levels. In fact, several mechanisms have been proposed to confer resistance to piRNA-mediated silencing of endogenous germline genes, including the targeting and licensing of mRNAs by the Argonaute protein CSR-1. Therefore, whether piRNAs regulate endogenous gene expression programs remains largely unexplored.

Studying piRNA pathway functions in the context of the developing *C. elegans* germline we show that spermatogenic genes are susceptible to piRNA-mediated transcriptional silencing during late spermatogenesis, a function required to ensure proper germ cell differentiation.

This nuclear piRNA signaling requires the activity of two spatially distinct biomolecular condensates present in the perinuclear environment of germ cells, also known as germ granules. Our results show that the organization of germ granules changes dynamically during development, and a particular configuration enable nuclear piRNA silencing at a specific time and location in the germline tissue, allowing the spermatogenic piRNA-dependent small RNA biosynthesis and loading onto the downstream nuclear Argonaute HRDE-1. These results suggest that changes in germ granule composition directly influence nuclear processes through modulation of small RNA related activities. We demonstrate that the silencing capacity of piRNAs on spermatogenic genes is also determined by the targeting of the Argonaute CSR-1, which preferentially targets spermatogenic mRNAs and antagonizes piRNAs silencing in early phases of spermatogenesis, suggesting that the targeting of mRNAs by different small RNA pathways is flexible and dynamic and depends on the developmental context.

Overall the results of our work show that the function of piRNAs can be co-opted to regulate endogenous transcriptional programs during development, and might contribute to expand the notion that piRNAs do not only function as a cellular immune system but also act as extremely versatile regulators of gene expression programs in animals.

615C RNA polyphosphatase PIR-1 regulates endogenous small RNA pathways

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Eukaryotic 5'-triphosphorylated RNAs (ppp-RNAs) are tightly regulated to promote cellular functions and prevent recognition by antiviral RNA sensors. For example, RNA capping enzymes utilizes their triphosphatase domains to remove the γ phosphates of ppp-RNAs during RNA capping processes, generating capped RNAs for RNA protection, nuclear export and translation. PIR-1 (phosphatase that interacts with RNA and ribonucleoprotein particle 1) is closely related to RNA capping enzymes. However, some members of PIR-1 family may serve as an RNA polyphosphatase, removing both the β and γ phosphates from ppp-RNAs. Here, we show that *C.elegans* PIR-1 is indeed an RNA polyphosphatase, converting ppp-RNA to p-RNA. Although WT PIR-1 dephosphorylates ppp-RNA and then rapidly releases product p-RNA, the catalytic-dead PIR-1(C150S) selectively binds and remains tightly bound to ppp-RNA substrates without dephosphorylation. Our proteomics analyses revealed that PIR-1 interacts with ERI (Enhanced RNAi) complex components, including DCR-1, RRF-3, DRH-3, ERI-1b and RDE-8, all of which are required for the biogenesis of 26G-RNAs. We showed that PIR-1 is also required for the biogenesis of 26G-RNAs. For example, 26G-RNAs that depend on the ALG-3/4 Argonautes, were ~10-fold less abundant in *pir-1* mutants than in WT worms. We showed that 26G-RNAs are produced in a semi-phased manner along mRNA templates via a coordinated action of RRF-3, which generates short triphosphorylated double-stranded (ds) RNAs and Dicer, which dices blunt-ended dsRNAs in a special mode. PIR-1 plays at least two roles in this process:1) promoting recruitment of Dicer to triphosphorylated dsRNAs; and 2) dephosphorylating 26G-RNA precursors. Moreover, we showed that PIR-1 may be required for the biogenesis of CSR-1-dependent small RNAs either directly or indirectly. Our findings suggest that PIR-1 likely modulates both Dicer-dependent and Dicer-independent small RNAs and provide insight into how cells utilize a conserved RNA phosphatase to respond and regulate cellular and viral ppp-RNA species.

keywords

: RNA phosphatase; RNAi; germline gene regulation; regulation of triphosphorylated RNA; spermatogenesis; embryogenesis; germline small RNAs; mRNA regulation; double-stranded RNAs; RNA binding proteins.

616A Developmentally regulated microRNA decay of the *mir-35* family is seed sequence dependent

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The *mir-35* family of microRNAs (miRNA) in *Caenorhabditis elegans* are maternally contributed as well as zygotically expressed in early embryos. Expression of the *mir-35* family, which consists of 8 miRNAs, *mir-35-42*, is essential for viability; complete loss of the *mir-35* family results in embryonic lethality. *mir-35* family abundance is developmentally regulated and sharply decays at the end of embryogenesis. While there is much known about the biogenesis and functions of miRNAs, very little is known about the decay mechanisms of miRNAs. Because of this tight regulation of the *mir-35* family during development, this family of miRNAs is an interesting model for studying the mechanisms of miRNA decay. The *mir-35* family has two defining characteristics: the first is a shared, family-specific seed sequence (nucleotides 2-8 at the 5' of the miRNA), and the second is preferential loading into the Argonaute protein ALG-2 (rather than the better studied ALG-1). We are interested in ascertaining

whether either of these characteristics play a role in the regulated decay of the *mir-35* family. To examine if the *mir-35* family turnover is seed sequence-specific, we mutated the seed sequence of the *mir-35* miRNA via CRISPR and monitored levels of the mutant *mir-35* as it compares to wild type *mir-35*. We detected perdurance of mutant *mir-35* past embryogenesis, demonstrating the necessity of the seed sequence in regulating *mir-35* family decay. We found that the *mir-35* seed mutants were loaded into ALG-2 similarly to wild type *mir-35,* indicating that altered Argonaute loading is not the cause of the altered turnover of the *mir-35* seed mutants. Therefore, ALG-2 loading is not sufficient for regulated turnover since mutant *mir-35* is misregulated despite its normal loading into ALG-2. Having shown that the seed sequence is necessary for *mir-35* decay, we next interrogated whether the seed is sufficient for the regulated decay of the *mir-35* family. We mutated all residues of *mir-35* outside of the seed region and observed that these miRNAs are decayed fairly similarly to wild type *mir-35*. Thus, the seed is largely sufficient to elicit developmentally timed decay. Overall, our findings contribute to a better understanding of the regulation of *mir-35* family abundance in early development. By elucidating the mechanisms of *mir-35* family decay, this research will offer insight into the broader mechanisms that couple miRNA decay to developmental progression.

617B PRG-1 and ZNFX-1 act in parallel to regulate small RNA-mediated transgenerational epigenetic inheritance

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The PIWI-interacting RNA (piRNA) pathway in *Caenorhabditis elegans* engages thousands of endogenously encoded piRNAs within perinuclear nuage (P-granules) to scan RNAs exiting the nucleus. Sufficient base-pairing between a piRNA and a target RNA elicits a silencing response that is maintained transgenerationally by worm-specific Argonaute (WAGO) pathways following recruitment of RNA-dependent RNA polymerases (RdRPs) which synthesize 22 nt RNA species with a bias for a 5’ guanosine (22G-RNAs). We previously described ZNFX-1 as a factor involved in piRNA-triggered RNA-induced epigenetic silencing (RNAe); ZNFX-1 was also found to engage the CSR-1 pathway which targets endogenous genes to protect them from silencing. Our study identified ZNFX-1 as being involved in positioning of RdRPs along small RNA-targeted transcripts to promote uniform 22G-RNA production to prevent bias for 5’ targeting of mRNAs. Here we report that ZNFX-1 acts in parallel with PRG-1 to affect epigenetic inheritance of signals for both CSR-1 and WAGO target genes; small RNA sequencing shows us that 22G-RNAs targeting WAGO-target genes are strongly reduced in *prg-1;znfx-1* double mutants relative to either single mutant or wild-type, and the double mutant sees increased expression of transposases for transposons Tc1 and Tc3 as shown by quantitative PCR (qPCR). Some small RNA targets whose targeting 22G-RNAs are depleted in a *prg-1* single mutant see increased small RNA production in *prg-1;znfx-1*, which may be the result of these genes becoming targets for CSR-1 rather than WAGOs. Furthermore, we see that unlike *znfx-1* single mutants which are defective in RNAi inheritance, *prg-1;znfx-1* double mutants can inherit RNAi, consistent with the idea that loss of endogenous small RNAs frees up small RNA machinery to respond to an exogenous RNAi trigger, also suggesting that PRG-1 activity negatively regulates, and ZNFX-1 function is dispensable for, RNAi inheritance. Moving forward, we will immunoprecipitate Argonautes in the CSR and WAGO pathways and sequence the small RNA populations being loaded into these Argonautes to better understand how loss of PRG-1 and ZNFX-1 activity affects endogenous small RNA pathways, and we will sequence small RNAs in animals grown on RNAi to develop a clearer picture of how these factors act in RNAi inheritance. We will also use DNA and RNA fluorescence *in-situ* hybridization (FISH) to look at spatial regulation of RNAs undergoing desilencing in these mutants.

618C RNA helicase RHA-1 safeguards thermosensitive sperm fertility by promoting small RNA-mediated mRNA clearance

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Male fertility and sperm development are thermosensitive processes whereby germline exposure to high temperature reduces viable sperm. Small RNAs and Argonaute proteins play critical roles in male fertility, particularly at high temperature conditions. In *C. elegans*, several small RNA pathways function in spermatogenic germlines and target germline expressed mRNAs. Argonaute CSR-1 targets many spermatogenesis genes and recent evidence highlights a dual role for CSR-1 in mRNA protection and mRNA clearance, the second requiring CSR-1 catalytic activity to slice mRNA targets. Whether and how these distinct functions of CSR-1 promote male fertility remains largely unkown. Intriguingly, a germline enriched helicase RNA helicase A (RHA-1) is also required for male thermosensitive fertility. Here we show that, in *rha-1* mutants, CSR-1 small RNAs exhibit a slight decrease in overall abundance. The CSR-1 small RNAs are preferentially depleted from the coding sequence rather than the 3’ UTR of target mRNAs, similar to the reported CSR-1 small RNA defects found in CSR-1 slicer mutant. When analyzing the mRNA levels of CSR-1 target genes, *rha-1* mutants show increased CSR-1 mRNA targets, including

spermatogenesis genes. Taken together, our results suggest RHA-1 facilitates CSR-1 slicing of mRNA transcripts to promote mRNA clearance. Our study further highlights the critical role of small RNA-mediated mRNA clearance in sperm fertility.

619A Comparative analysis of nematode small-RNA pathways using Gametocyte specific factor-1 (GTSF-1)

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Evolutionary arms race with transposable elements has greatly diversified the sRNA pathways in individual species. This resonates in the GTSF-1 proteins, which are essential for sRNA pathways in many species but show evolutionary plasticity by acting at different steps of the pathway in different species. In *D. melanogaster*, GTSF-1 is a downstream factor where it binds a nuclear Ago and drive transcriptional silencing of transposons. However, in *M. musculus*, GTSF-1 acts upstream of the pathway by binding a cytoplasmic Ago and enabling the biogenesis of sRNAs. Surprisingly, in *C. elegans*, GTSF-1 is not involved in transposon silencing and does not bind Ago proteins. It instead forms a protein complex with an RNA Dependent RNA polymerase (RdRP) called RRF-3. Together they facilitate the biogenesis of siRNAs (26G RNAs), which ultimately targets pseudogenes and other recent gene duplications.

This striking functional plasticity of GTSF-1 led us to hypothesize that even closely-related species have very customized sRNA populations. We are curious whether biochemical building blocks that together make a sRNA pathway can be differentially used to better meet the specific needs for genome defense in individual species

To address this, we are studying GTSF-1 proteins in a set of nematodes i.e. *C. inopinata*, *C. briggsae* and *P. pacificus*. GTSF-1 is well conserved and these nematodes each carry a single homologous *gtsf-1* gene. We wish to characterize GTSF-1 in these nematodes using transgenic techniques, transcriptomics and proteomics. The study will describe a general molecular function of GTSF-1 like proteins in small RNA biology. Further, it will reveal how small RNA pathways have evolved during nematode evolution in relation to genome characteristics and gene-regulatory needs of the individual species.

620B Distinguishing between self and non-self siRNA encoded in the C. elegans genome

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RNAi plays an evolutionarily conserved function in silencing of transposons and other foreign DNA to protect genomic integrity, especially in the developing germline. *C. elegans* provides a highly tractable system to study this pathway and its role in transgenerational epigenetic inheritance. Despite recent progress, the biogenesis and regulation of the endogenous siRNAs remain elusive. To address this question, we recently developed the “siRNA generator” approach using CRISPR, by inserting various DNA sequences from protein-coding genes, which normally produce low levels of endo-siRNAs, into a LTR retrotransposon that produces abundant endo-siRNAs. The objective of this approach was twofold: 1) to determine how this insertion affects the siRNA profile of the host retrotransposon and 2) to determine whether the siRNAs produced from the inserted sequence are sufficient to silence the target gene in *trans*. To our surprise, we found that C. elegans germline can distinguish between siRNAs that target germline-expressed protein-coding genes (self siRNAs) and siRNAs that target the host transposon (non-self siRNAs) even though they are produced from the same locus. We are currently conducting analysis to determine the sequence and genetic requirement of the “self siRNA suppression” phenomenon, and its implication in transgenerational epigenetic regulation. Our work will provide insights into the regulation of siRNA homeostasis and how an eukaryotic genome distinguishes between self and foreign nucleic acids.

621C A convenient strategy for generating small RNA/mRNA high-throughput sequencing libraries

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High-throughput sequencing has become a standard and powerful tool for analyzing nucleic acids in this big data era. To sequence RNA/DNA, a cDNA/DNA library is usually generated with target sequences ligated with specific 5’ and 3’ linkers. Unlike mRNA, small RNA often contains multiple modifications including 5’ cap or triphosphate and 2’-*O*- methyl, requiring additional processing steps before linker additions during the cloning processes. And due to low expression levels, it is difficult to clone small RNA starting with a small amount of total RNA. To simplify the cloning process, we have developed a new strategy to clone 5’ modified or unmodified small RNA in a one-pot reaction (inside a single PCR tube) with all the steps carried out sequentially using liquid manipulation and as little as 20 ng total RNA (at a single worm level). The 7-hour cloning

process only needs ~1-hour labor, a mutiple-fold labor reduction as compared to the canonical cloning methods. Moreover, our method can efficiently clone purified mRNA, simplifying the need to prepare two cloning systems, one for small RNA and the other for mRNA; the barcoded PCR primers utilized in the cDNA amplication process (PCR) are also compatible with those in the genomic DNA cloning protocols, basically unifying all the PCR primers required for amlifying cDNA and DNA (a significant cost reduction since multiple barcoded primers are needed in PCR for pooling samples in the final step). Our method is more convenient for cloning modified RNA, sensitive, versatile and cost-effective than available methods. Moreover, we are developing a low cost home-made Ribo-minus method for enriching mRNA to replace the cost-prohibitive commercial kits. These methods will greatly promote nucleic acid related research.

622A Antisense ribosomal siRNAs inhibit RNA polymerase I-directed transcription in C. elegans

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Eukaryotic cells express a wide variety of endogenous small regulatory RNAs that function in the nucleus. We previously found that erroneous rRNAs induce the generation of antisense ribosomal siRNAs (risiRNAs) which silence the expression of rRNAs via the nuclear RNAi defective (Nrde) pathway. To further understand the biological roles and mechanisms of this class of small regulatory RNAs, we conducted forward genetic screening to identify factors involved in risiRNA generation in Caenorhabditis elegans. We found that risiRNAs accumulated in the RNA exosome mutants. risiRNAs directed a NRDE-dependent silencing of pre-rRNAs in the nucleolus. In the presence of risiRNAs, NRDE-2 accumulated in the nucleolus and colocalized with RNA polymerase I. risiRNAs inhibited the transcription elongation of RNA polymerase I by decreasing RNAP I occupancy downstream of the RNAi-targeted site. Meanwhile, exosomes mislocalized from the nucleolus to nucleoplasm in suppressor of siRNA (susi) mutants, in which erroneous rRNAs accumulated. These results established a novel model of rRNA surveillance by combining ribonuclease-mediated RNA degradation with small RNA-directed nucleolar RNAi system.

623B Annotation of primary microRNA transcripts using conditional depletion of Drosha *drsh-1*.

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microRNAs (miRNAs) are a class of small noncoding RNAs that interact with many developmental and cellular pathways and are critical for the regulation of gene expression. Most miRNAs are transcribed by RNA Pol II and undergo successive enzymatic processing step by nucleases Drosha and Dicer. Mature miRNAs load into Argonaute proteins to form the miRNA induced silencing complex (miRISC). The first enzymatic processing step, performed by the endonuclease Drosha is responsible for the cleaving the pre-miRNA structure from the primary miRNA transcript, a process that often occurs co-transcriptionally or shortly thereafter. The speed of Drosha processing means that primary transcripts are poorly represented in total RNA samples and thus difficult to characterize. To overcome this hurdle, we utilized an auxin-induced degron system to conditionally deplete Drosha and greatly reduce processing of primary transcripts, leading to their accumulation. We then performed RNAseq to map the primary miRNAs and allow for their annotation. Furthermore, we analyzed the effects of Drosha depletion on the mature miRNA population and the transcriptome as a whole. In addition to annotating a fraction of primary miRNAs, we uncovered previously unannotated Drosha-dependent transcripts. This analysis furthers miRNA gene annotations and will facilitate future research on miRNA biogenesis.

624C *hrpa-1* coordinates with miRNAs to regulate gene expression in *C. elegans*.

Shilpa Hebbar[1], Ganesh Panzade[1], Anna Zinovyeva[1] 1) Kansas State University.

Regulation of gene expression is essential for normal physiology and development. One of the ways cells regulate gene expression is through a class of non-coding RNAs called microRNAs (miRNAs). miRNAs associate with Argonaute proteins to form miRNA Induced Silencing Complexes (miRISCs), which post-transcriptionally repress gene expression by targeting mRNA 3'UTRs through partial sequence complementarity. We previously identified HRPA-1 (Heterogenous nuclear RibonucleoProtein (HnRNP) A homolog) as a potential interactor of miRISC. Using an endogenous GFP tag, we show that HRPA-1 is ubiquitously expressed throughout *C. elegans* development with a strong expression in the nucleus. To determine whether HRPA-1 is functionally important for miRNAs, we knocked down *hrpa-1* in sensitized miRNA mutant backgrounds. *mir-48 mir-241(nDf51)* animals show a partially penetrant heterochronic phenotype including delayed expression of the adult collagen *col-19::gfp*. Deletion of *hrpa-1* in *mir-48 mir-241(nDf51)* background enhanced these defects suggesting that *hrpa-1* is important for *let-7* family miRNA activity. Similarly, loss of *hrpa-1* strongly enhanced developmental defects associated with other miRNA sensitized mutants, *lsy-6(ot150)* and *let-7(n2853).* Small RNA sequencing revealed depletion of *hrpa-1* results in an increase of mature miRNA levels for some miRNAs and a decrease in mature miRNA levels for other miRNAs. Surprisingly, let-7 miRNA

levels increased upon *hrpa-1* depletion, while miR-84 and lsy-6 miRNA levels remained unchanged. Overall, our data suggest that HRPA-1 activity is important for regulation of gene expression and directly or indirectly interacts with miRNAs, potentially by affecting miRNA biogenesis. To identify the effects of loss of *hrpa-1* on global gene expression, we performed RNAseq and identified 143 genes that were significantly disrupted. Interestingly, a predicted interactor of HRPA-1, R06C1.4 and a homolog of a yeast component of cleavage and polyadenylation factor I (CF I), was found to be significantly downregulated. RNAi knockdown of *R06C1.4* partially recapitulated the enhancement effects on miRNA sensitized mutant developmental phenotypes observed with *hrpa-1* loss. We propose that *hrpa-1* could be affecting miRNAs by (i) influencing biogenesis or processing, and (ii) potentially affecting miRNA targets through mRNA processing mechanisms.

625A Intrinsically disordered protein PID-2 modulates Z granules and is required for heritable piRNA-induced silencing in the *Caenorhabditis elegans* embryo

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Argonaute proteins (AGOs) are involved in many gene regulatory processes and use small RNAs as guides to find their target. *C. elegans* employs several small RNA mediated pathways to initiate and maintain proper gene regulation throughout development. In germ cells, 21U RNAs and associated Argonautes are particularly important to maintain an immortal germline. 21U RNAs trigger a silencing mechanism that is dependent of amplification of 22G RNAs by RNA dependent RNA polymerase. Strikingly, silencing responses can become independent of triggering 21U RNAs and maintained across generations, a mechanism called RNA epigenetic memory (RNAe). Components of small RNA pathways often localize to perinuclear phase separated condensates termed germ granules. The exact functions of germ granules are not clear, but it is known that they are necessary for proper function of small RNA pathways and inheritance of small RNAs. Intrinsically disordered regions (IDRs) can mediate multivalent protein-protein interactions and are often important for phase separation.

We show that maternally provided 21U RNAs are sufficient to initiate RNAe in embryos and that the IDR containing protein PID-2/ZSP-1 is required for this process. PID-2/ZSP-1 localizes to and affects the appearance and number of Z-granules[1][2]. Furthermore, we found that PID-2/ZSP-1 mutually exclusively interacts with PID-4 and PID-5. Mutants lacking PID-4 and PID-5 partially phenocopy *pid-2* mutants. Interestingly, even though PID-4 and PID-5 interact with PID-2, they localize to the adjacent P-granules rather than Z-granules.

We identify distinct interacting proteins for PID-4 and PID-5. For instance, PID-5 interacts with the X-prolyl aminopeptidase APP-1 and PID-5 itself has an APP-1 related domain that is likely catalytically inactive. Several AGOs contain intrinsically disordered N-terminal tails and are predicted substrates for APP-1, suggesting a role for N-terminal processing in small RNA pathways and germ granule dynamics.

Taken together, we have found a protein complex that affects small RNA mediated regulation and germ granules, potentially providing a mode of communication between different granules, and are on the route to understand the functional relationship between small RNA mediated gene regulation, epigenetic inheritance, and phase separation.

[1] Placentino *et al.* EMBO J, 2021

[2] Wan *et al.* EMBO J, 2021

626B Deletion of circRNAs derived from the *crh-1* CREB locus increases mean lifespan of *C. elegans*

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Circular RNAs (circRNAs) are an abundant class of non-coding RNAs with largely uncharacterized functions. CircRNAs are formed via backsplicing, whereby a downstream splice donor of an exon covalently bonds to an upstream splice acceptor, linking the 3' and 5' ends in a continuous circle. Hundreds of circRNAs increase in expression during aging, a phenomenon found across phyla, but their function in the aging process is mostly undiscovered. Previously, we identified two highly abundant circRNAs arising from exon 4 of *crh-1*, an ortholog of human CREB1. Within *crh-1*, two large reverse complementary

motifs (RCMs), that can facilitate backsplicing, flank the circularizing exon of *crh-1* (exhibiting 83% base complementarity), giving evidence that these RCMs could drive circularization. Using CRISPR/Cas9, we deleted the downstream RCM sequence of *crh-1* to uncover its effect on age-accumulated circRNA formation, and impact on lifespan. In four independent *crh-1* circRNA (circ-*crh-1*) CRISPR-generated alleles, RCM deletion resulted in complete loss of circ-*crh-1* expression in both young and old adults without disruption of linear *crh-1* expression or activated phospho-CRH-1 protein levels. Introduction of exon 4 from *crh-1* with flanking RCM sequences into circ-*crh-1* mutants restored the loss of circ-*crh-1*. Interestingly, mutants of *adr-1*, a double-stranded RNA-specific adenosine deaminase (ADAR), resulted in increased circ-*crh-1* expression levels in both young and old adults. These results suggest that RCMs likely drive circ-*crh-1* formation, which is depended on ADAR function. Remarkably, two independent RCM deletion alleles of circ-*crh-1* showed a small but significantly increased mean lifespan (~11% change) but decreased thermotolerance, suggesting these mutants may extend the period of frailty. Finally, we performed RNA sequencing on wild-type and circ-*crh-1* mutants and identified 17 significantly downregulated genes, which may contribute to the observed lifespan phenotype. Our findings represent the first circRNA-specific knockout in *C. elegans*, and this strategy for circRNA specific deletion can be applicable to study the functions of other circRNAs in *C. elegans.* Furthermore, these results highlight a new role for the *crh-1* circRNA in the regulation of lifespan and suggest that circRNAs may be a target for further understanding healthy aging.

627C Characterizing the regulatory role of uridylation on small RNA activity in *C. elegans* germline development

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RNA regulation, from RNAi networks to maternally provided mRNAs, is essential for proper germline development and identity. Modifications to RNAs, such as the addition of nucleotides to the 3' end, can alter their function and affect biological activity. Poly(U) polymerases (PUPs), also known as terminal uridylyl transferases (TUTases) in vertebrates, place uridines on the 3' end of RNAs, an event called uridylation. While the presence of uridylation is well-documented on small non-coding RNAs (sRNAs) and mRNAs in many model organisms, the functional significance of uridylation is less clear. The uridylation catalyzed by PUPs usually mark the RNA for degradation, but other functions are being discovered. Identifying which sRNAs are being uridylated will provide a better understanding of how RNA tailing influences development.

The roles of *pup-1*, *pup-2*, and *pup-3* have previously been described in the *C. elegans* germline (Li and Maine 2018). Under temperature stress, the combined loss of *pup-1* and *pup-2* resulted in ectopic somatic expression in the germline, disorganized P-granules, and sterility by the third generation. In addition, *pup-1* and *pup-2* act redundantly to suppress *pup-3* expression. In this work, we aim to connect the developmental defects of *pup-1*, *pup-2*, and *pup-3* mutants to 3' uridylation activity on sRNAs. We performed sRNA-seq experiments to examine changes in sRNA expression and 3' tailing in *pup-1*, *pup-2*, and *pup-3* deletion mutants. Uridylation is the most prevalent tail type, followed by adenylation, among siRNAs, miRNAs, and piRNAs. The overall proportion of uridylated sRNAs in *pup-2(0)* and *pup-3(0)* mutants are comparable to wildtype, while it is decreased in *pup-1(0), pup-1/-2(0)*, and *pup-3(0);pup-1/-2(0)* mutants. When examining total sRNA abundance, *pup-2(0)* and *pup-3(0)* have few differentially expressed sRNAs compared to wildtype whereas *pup-1(0), pup-1/-2(0)*, and *pup-3(0);pup-1/-2(0)* have many. Among ~2,670 differentially expressed sRNAs, 41% are shared between *pup-1(0)* and *pup-1/2(0)* while <1% are shared between *pup-2(0)* and *pup-1/-2(0)*. However, 935 sRNAs are differentially expressed only in *pup-1(0)* and another 630 only in *pup-1/-2(0)*, indicating that the combined loss of *pup-1* and *pup-2* has unique effect on sRNA expression. Future analysis will determine if uridylation is responsible for maintaining correct gene expression in the germline and how it affects different Argonaute-based classes of 22G-siRNAs.

628A An alternative ERGO-1 pathway in a sibling species of *C. elegans, C. inopinata*

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The recent discovery of *Caenorhabditis inopinata*, a sibling species of *C. elegans*, provides an opportunity to investigate the conservation and diversification of small RNA (sRNA) pathways in two closely related species. We report that the microRNA, PIWI and male-expressed ALG-3/4 siRNA pathways are highly conserved in *C. inopinata* and *C. elegans*. However, *C. inopinata* has lost key components of the female-expressed ERGO-1 siRNA pathway associated with the clearance of transcripts. *C. inopinata* do not express the ERGO-1 associated 26G primary siRNAs and have similar sRNA expression profiles to *C. elegans ergo-1* and *eri-6* mutants. However, expression of the downstream secondary 22G siRNAs is retained in *C. inopinata*. We have

identified a class of 22U siRNAs that are candidates for an alternative ERGO-1 siRNA. The 22U loci overlap with 22Gs and target similar genes but, unlike *C. elegans* 26Gs, are not dicer-processed. We also identified an Argonaute protein with an expression profile similar to the *C. elegans* ERGO-1, as a candidate for an alternative ERGO-1 Argonaute protein. Our results indicate that there has been a partial loss of the ERGO-1 pathway in *C. inopinata* and an alternative ERGO-1 pathway has evolved.

629B The role of MORC-1 in regulating CSR-1 germline gene licensing

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In *C. elegans*, germline gene expression is tightly regulated by endogenous small RNA (endo-siRNA)-mediated gene regulatory pathways. Nuclear RNAi pathways function to silence pseudogenes, transposons, and protein-coding genes not intended for germline expression by inducing heterochromatin formation. An opposing small RNA-mediated pathway also exists to promote germline gene expression. This pathway is governed by a distinct subset of endo-siRNAs that interact with the essential Argonaute protein, CSR-1, to facilitate transcription of target genes. This atypical "gene licensing" RNAi pathway is mechanistically poorly understood. Our lab identified a role for the conserved Zinc-finger ATPase MORC-1 in maintaining silencing downstream of nuclear RNAi. Unexpectedly, we uncovered a novel role for MORC-1 in the germline licensing pathway. By purifying germline nuclei from *C. elegans* and using ChIP-seq to identify the endogenous germline binding sites of MORC-1, we found that MORC-1 binds the transcriptional start sites of germline-expressed genes, significantly overlapping with CSR-1 regulated targets. CSR-1 targets are specifically upregulated in the *morc-1(-)* mutant by mRNA-seq. Further, *in vitro* data using purified *C. elegans* MORC-1 demonstrates that MORC-1 efficiently condenses DNA. Additionally, we show that *morc-1* expression is directly regulated by CSR-1. Mutations of *morc-1* and *csr-1* genetically suppress each other. While *morc-1(-)* mutants display a germline mortal (Mrt) phenotype, *morc-1(-)* mutants on *csr-1* RNAi maintain fertility beyond either single mutant alone. This rescue of fertility is accompanied by restoration of gross germline morphology. Taken together, we propose a novel mechanism for *csr-1*-mediated gene licensing: in a *csr-1* mutant, its target, *morc-1*, is overexpressed, resulting in enhanced condensation of MORC-1 target loci and repression of these germline-expressed genes. Therefore, we posit a novel role for MORC proteins in regulating *csr-1*-dependent germline gene licensing.

630C Target-dependent requirements of regulators for gene silencing during RNA interference

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RNA interference (RNAi) is a widely used method for regulating gene expression, whereby the addition of double-stranded RNA (dsRNA) results in the silencing of genes with matching sequence. Although initial processing of dsRNA is generic, our observations suggest target-specific requirements of downstream regulators for silencing in *C. elegans*. After ingestion of dsRNA, all tested target genes require import of dsRNA into the cell, processing by an endonuclease, and loading of the primary Argonaute RDE-1 with small interfering RNAs (siRNA) that is used to bind complementary mRNAs. However, the pathways required for the downstream production of secondary siRNAs and the secondary Argonautes required for silencing can vary based on the target. All silencing of the hypodermal gene *bli-1* was eliminated in animals that lack either the intrinsically disordered protein MUT-16 or the Maelstrom domain-containing protein RDE-10, which have both been implicated in the production of secondary siRNAs. However, silencing of the muscle gene *unc-22* was only eliminated upon loss of both MUT-16 and RDE-10. Similarly, all *bli-1* silencing, but not *unc-22* silencing, was eliminated in animals that lack the secondary Argonaute NRDE-3. Thus, branches of the RNAi pathway can be selectively required for silencing specific target sequences. These selective requirements could reflect a qualitative difference in the kind of regulators needed or a quantitative difference in the amount of regulators needed for silencing of the two targets. Consistent with a quantitative difference, the requirement for NRDE-3 to silence *bli-1* can be overcome by loss of the exonuclease ERI-1 or by the overexpression of the dsRNA-binding protein RDE-4, which are both perturbations thought to enhance the processing of long dsRNA. Changing features of the two targets using genome editing and then quantifying secondary siRNA accumulation along with mRNA localization could suggest an explanation for the observed selectivity. Understanding these gene-specific requirements will aid the development of effective strategies to silence any gene of interest for therapeutic and agronomic applications.

631A Studying the tissue-specific functions of the conserved *mir-51* family of microRNAs

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MicroRNA (miRNA) are small non-coding RNAs that regulate gene expression post-transcriptionally by targeting mRNAs for translational inhibition and degradation. MiRNAs are functionally categorized into families based on nucleotides 2-7 at their 5' ends, which is referred to as the "seed" region. Due to the length of the seed sequence, a single miRNA is capable of regulating many target transcripts, and identifying functional miRNA targets can be difficult. Members of a miRNA family share the same seed sequence and are believed to have largely overlapping targets.

The *mir-51/100* family of miRNAs is widely conserved across metazoans and is essential for embryonic development in *C. elegans*. In the worm, this miRNA family consists of six mature miRNAs, *mir-51* through *mir-56*, and loss of the entire *mir-51* family leads to embryonic lethality with a pharynx unattached phenotype. Experiments with extrachromosomal transgenes indicate that the *mir-51* family members are functionally redundant when rescuing the embryonic lethality. The redundancy between family members indicates that the *mir-51* family probably shares a core set of essential targets; however a comprehensive list of the essential targets regulated by the *mir-51* family remains elusive.

The *mir-51* family is expressed beyond embryonic development, and hypomorphic *mir-51* family mutants that bypass embryonic lethality display post-embryonic phenotypes, which include developmental delays, reduced brood size, and food avoidance, suggesting that the *mir-51* family has functions beyond embryonic development. In this study, we will attempt to identify the post-embryonic roles of the *mir-51* family using tissue-specific techniques. We have generated transgenic strains expressing *mir-52* tissue-specifically in the intestine, body wall muscle, pharynx and neurons in a *mir-51* family hypomorphic background and are testing these strains for rescue of post-embryonic phenotypes. By combining these tissue-specific requirements with bioinformatic predictions of miRNA targets and tissue-specific transcriptome data, we will identify candidate miRNA targets. We will then use CRISPR-Cas9 to perform deletions in the predicted miRNA binding sites of these candidate genes and attempt to recapitulate the post-embryonic phenotypes caused by deficiency of the *mir-51* family. The overall goal of this study is to identify the essential targets of this conserved miRNA family and to investigate the significance of these interactions for development.

632B The role of the microRNA miR-71 in amyloid β plaque formation in a *C. elegans* model

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Alzheimer's Disease is an aging-associated neurodegenerative disease associated with plaque formation and Aβ(1-42) peptide aggregation in the brain. Previously, we have shown that the aging-associated microRNA (miRNA) miR-71 delays pathogenicity caused by human Aβ(1-42) expression in *C. elegans*. To examine if miR-71 might reduce the aggregation of Aβ(1-42), we used the Congo-Red derivative X-34, which allows for the observation of amyloid aggregation *in vivo*. We measured Aβ(1-42) aggregation in mutants that either deleted or overexpressed miR-71. Surprisingly, the greatest number of aggregates formed in strains that overexpress miR-71 suggesting that miR-71 might actually facilitate Aβ(1-42) aggregation. We hypothesize that miR-71 activates stress response pathways that modulate the proteotoxicity caused by Aβ(1-42) either by increasing the aggregation of soluble, but toxic, Aβ(1-42) or by decreasing the size of each plaque, thereby reducing the proteotoxicity caused by each. Our current experiments intend to confirm our initial findings and to test these hypotheses.

633C Functional analysis of HRPK-1 domains reveals domain and subcellular localization requirements for miRNA-mediated gene expression regulation.

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microRNAs (miRNAs) are small non-coding RNAs that post-transcriptionally regulate gene expression. Heterogeneous nuclear ribonucleoproteins (hnRNPs) have recently emerged as factors important for miRNA processing and/or activity. We previously showed that *C. elegans* HRPK-1 (an hnRNPK homolog) physically interacts with *C. elegans* Argonaute ALG-1 and genetically interacts with multiple miRNA families, suggesting that HRPK-1 coordinates with miRNA in regulating gene expression. Disruption of HRPK-1 function alters mature miRNA levels, suggesting that HRPK-1 contributes to miRNA processing. To better understand the molecular mechanisms by which HRPK-1 influences gene regulation, we performed a comprehensive functional domain analysis of HRPK-1 protein using CRISPR/Cas9 genome editing. These mutations include inactivation of predicted nuclear localization and exporting signals, PLD domain deletion, and KH domain mutations disrupting HRPK-1 RNA binding activity. We found that all HRPK-1 mutations contribute to HRPK-1 function in coordinating with miRNAs to some extent. For example, mutating the predicted NLS motif abolishes endogenous HRPK-1 expression in the nucleus and enhances the cell fate defect phenotype of *lsy-6(ot150)* and the heterochronic phenotype of *mir-48 mir-241(nDf51)*, suggesting that the nuclear localization is required for HRPK-1 function with regards to miRNA-mediated gene regulation. We will describe our

data on HRPK-1 domain requirements and present models on how HRPK-1 may modulate miRNA activity. Our investigation of HPRK-1 functional domains furthers our understanding of how hnRNP proteins may coordinate with miRNAs in gene expression regulation.

634A DOT-1.1 histone methyltransferase loss leads to lethality dependent on RNAi pathways

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DOT-1.1 is a *C. elegans* homolog of yeast Dot1 (disruptor of telomere silencing-1) and mammalian DOT1-like (DOT1L). DOT1 enzymes catalyze mono-, di-, and tri-methylation of histone H3 lysine 79 and their loss leads to lethality in metazoans.

Previously published research from our lab described enhanced antisense and non-coding RNA transcription under conditions of reduced DOT-1.1 occupancy on chromatin. We also found that *dot-1.1(lof)* embryonic and larval lethality is completely suppressed by the RNAi-deficient *rde-4* and *rde-1* mutants, but not by mutations unrelated to RNAi. Since RDE-4 and RDE-1 are required for siRNA production, our results suggested that ectopic endogenous dsRNA and siRNAs cause *dot-1.1(lof)* lethality.

Next, we tested whether mutation in the enhanced RNAi mutant *eri-1* could suppress *dot-1.1* lethality, and it did. We are still considering the possibility of ectopic *rde-4*, *rde-1*, and *eri-1*-dependent siRNAs playing a direct role in silencing of a specific gene(s) causing lethality. However, it is equally possible that ectopic dsRNA generated in *dot-1.1(lof)* saturates Dicer and does not allow proper expression of miRNA(s). In this scenario, both *rde-1/4* and *eri-1* deficiency would free Dicer.

We are conducting more suppression experiments with RNAi-related mutants and will present our new results at the conference.

635B Functional analysis of male gonad-enriched microRNAs in *Caenorhabditis elegans*

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Gene regulation mediated by microRNAs (miRNAs) at the post-transcriptional level has been suggested important for producing functional sperm in diverse species including *C. elegans*, however, the specific regulatory roles of miRNAs in this process are largely unknown. To address this question, I proposed to study function of miRNAs enriched in male gonads, which are approximately 95% germ cells and 5% somatic cells. I isolated adult gonads of males and hermaphrodites for small RNA sequencing and the analysis revealed a differential miRNA expression profile between hermaphrodite and male gonads, which also indicates the different miRNA profile between oogenesis and spermatogenesis. Specifically, 29 miRNAs were found to be higher in the male gonads compared to the hermaphrodite gonads, with two miRNA clusters, *mir-2209.2-2209.3* and *mir-4807-4923.1*, overrepresented among male gonad-enriched miRNAs. These 29 miRNAs have potential to function in regulating normal spermatogenesis. Many of them did not have available loss of function mutations. Therefore, we used CRISPR/Cas9 genome editing to generate miRNA mutants with single miRNAs or a miRNA cluster deleted. To identify miRNAs that regulate sperm formation or function, male and hermaphrodite fertility in miRNA mutants were assessed by mating assay, brood size assay, and sperm quantification. For male fertility, while the results suggested that single miRNA or miRNA cluster is dispensable for sperm function, four miRNA mutants produced fewer mature spermatids, suggesting possible defects in spermatogenesis. And two of these miRNAs mutants also had lower brood size in hermaphrodites, likely due to defects in spermatogenesis. Computational analysis using male gonad-enriched miRNAs, published gonad transcriptome data, and miRNA target predictions was used to reveal miRNA-target regulatory network in male gonads, which suggested complex genetic miRNA interaction to regulate germ cell development and differentiation during spermatogenesis. Our analysis on multiple mutants further suggested genetic interaction between these miRNAs. Future studies will further characterize the molecular mechanism accountable for the spermatogenesis defect in these four miRNA mutants. Taken together, this study will further our understanding of specific roles of miRNA in the male germline and somatic gonad that promote male fertility and fecundity.

636C mRNA Splicing Promotes Polyadenylation and Counteracts Novel Default Argonaute Silencing in the Germline of *Caenorhabditis elegans*

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Mechanisms that safeguard the germline genome are essential for species preservation. For example, in metazoans the PIWI Argonaute pathway promotes genome integrity by silencing transposons and newly acquired foreign genes. PIWI Argonautes engage genomically-encoded piRNAs to detect their targets and to trigger gene regulation at both the mRNA

and transcriptional level. However, the question of how foreign or aberrant gene expression is detected in the germline is still poorly understood. In yeast, genes with suboptimal mRNA splicing signals can trigger Argonaute silencing, and a similar connection has been made in the silencing of *C. elegans* early embryo transcripts with weak and abnormal splicing by the ERGO-1 ERI small RNA pathway.

Here we show that the removal of introns from a gene and replacement with a cDNA-derived open reading frame results in complete silencing of the gene in the *C. elegans* germline. For example, a transgenic copy of *gfp*-tagged *cdk-1* gene (*cdk-1::gfp*) yields robust germline and somatic expression. However, an intronless *cdk-1::gfp* transgene fails to express in the germline and can also transitively silence other intron-containing *gfp* transgenes. Transitive silencing depends on the WAGO Argonaute pathway and is correlated with robust production of RNA-dependent RNA Polymerase (RdRP)-derived antisense small RNAs from the unspliced RNA. Silencing and small RNA production were also observed when introns were removed from the endogenous *oma-1* gene. Surprisingly, although transitive silencing requires the WAGO Argonaute pathway, the intronless genes (themselves) remain silenced in WAGO-pathway mutants, suggesting that an additional, cis-mode, of silencing occurs on intronless genes.

The known primary Argonautes including *prg-1, ergo-1, alg-3/4,* and *rde-1* are not required for the initiation of "intronless" silencing. Northern blot analysis revealed that intronless genes yield only a shorter mRNA isoform that appears to lack polyadenylation. These findings suggest mRNA splicing serves as a licensing step in germline gene expression promoting both polyadenylation and protein translation in cis, while preventing mRNAs from becoming default templates for RdRP-dependent transitive Argonaute silencing.

637A piRNAs prevent runaway amplification of siRNAs from ribosomal RNAs, histone mRNAs, and other coding gene mRNAs

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Piwi-interacting RNAs (piRNAs) are a largely germline-specific class of small RNAs found in animals. Although piRNAs are best known for silencing transposons, they regulate many different biological processes. Here we identify a role for piRNAs in preventing runaway amplification of small interfering RNAs (siRNAs) from certain genes, including ribosomal RNAs (rRNAs) and histone mRNAs. In C. elegans, rRNAs and some histone mRNAs are heavily targeted by piRNAs, which facilitates their entry into an endogenous RNA interference (RNAi) pathway involving a class of siRNAs called 22G-RNAs. Under normal conditions, rRNAs and histone mRNAs produce relatively low levels of 22G-RNAs. But if piRNAs are lost, 22G-RNA production is highly elevated. We show that 22G-RNAs produced downstream of piRNAs likely function in a feed-forward amplification circuit. Thus, our results suggest that piRNAs facilitate low-level 22G-RNA production while simultaneously obstructing the 22G-RNA machinery to prevent runaway amplification from certain RNAs. Histone mRNAs and rRNAs are unique from other cellular RNAs in lacking polyA tails, which may promote feed-forward amplification of 22G-RNAs. In support of this, we show that the subset of histone mRNAs that contain polyA tails are largely resistant to silencing in piRNA mutants. Despite hyperproduction of 22G-RNAs in piRNA mutants, the effects on histone and rRNA expression are modest and may have a negligible impact on germline development.

638B Exploring Argonaute Loading Mechanisms and Unloaded Argonaute Stability

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Argonaute proteins catalyze RNA-guided search to direct RNA interference (RNAi) and micro-RNA based gene silencing. These natural programmable search mechanisms have fundamental roles in gene regulation and development. In addition, a new class of precision medicines function by artificially programming Argonautes with synthetic guide RNAs (siRNAs) designed to target disease-related gene expression. Despite a great deal of knowledge and research on Argonaute mediated silencing, surprisingly little is known about how Argonaute proteins normally acquire and load their RNA guides. Understanding how Argonautes are properly loaded could provide novel insight into aberrant loading events in human diseases. Transgenerational silencing in the C. elegans germline depends on a constant cycle of de-novo Argonaute synthesis and loading necessary to generate robust supplies of Argonaute guide complexes that transmit silencing via the sperm and egg to offspring. Our previous investigation of this silencing process has identified protein candidates for factors that stabilize and promote the loading of unloaded Argonautes. One of these is GLH-1, where perturbation of the ATP binding domain increases affinity to unloaded WAGO-1. Here we design and validate unloadable mutations of WAGO-1 to further probe the role of the GLH-1 ATP binding cycle in loading WAGO-1. We report that unloadable mutations of WAGO-1 are stably expressed, with no signs

of innate instability despite previous reports. We also find that inhibition of WAGO-1 loading and perturbation of GLH-1 ATP binding cycle work synergically to reveal a soluble, cytoplasmic complex of WAGO-1 and GLH-1. Future work will aim to leverage the unloadable mutations of WAGO-1 and other germline Argonautes to identify loading co-factors.

639C Annotation of isomiR dynamics across the *C. elegans* developmental stages

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microRNAs (miRNAs) are short, ~22 nucleotides, small RNAs that repress genes through mRNA destabilization and translational repression. During canonical miRNA biogenesis, several miRNA species, or isoforms, known as isomiRs, are produced from a single precursor miRNA. The isomiRs are thought to be generated through Drosha or Dicer cleavage at alternate positions on either the primary or the precursor miRNAs, generating truncated or extended 5' or 3' miRNA ends. Because mature miRNA sequence modifications can alter their gene target repertoire, we wished to investigate the extent of isomiR prevalence and dynamics across *C. elegans* developmental stages. We performed small RNAseq from staged animals to assess isomiR variability across developmental stages. Using isomiR-SEA (Urgese et al 2016) for isomiR identification and quantification, we provide an isomiR profiling map against the miRBase annotated miRNAs at all stages of *C. elegans* development. We found that many miRNAs display isomiR level variability at different developmental stages, suggesting that the functional specificity of isomiRs to the developmental stage may exist. Not surprisingly, 3' end miRNA alterations were more frequent than the potentially seed-altering 5' end extensions or truncations. Some miRNAs had isomiRs that were just as, or more abundant than their annotated canonical mature miRNAs. These isomiRs included those with 5' end truncations and deletions, predicted to target new, potentially distinct sets of genes. Overall, we will present annotation of isomiR dynamics across *C. elegans* developmental stages, which we hope can provide us with insights into miRNA biogenesis and the intriguing potential of isomiR function.

640A Investigation of the role of the protein complex PETISCO in *C. elegans* embryonic viability

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The PIWI/piRNA pathway is a highly conserved small RNA pathway. Central to the pathway is an RNA silencing complex made of an Argonaute protein of the PIWI clade and PIWI-interacting RNAs (piRNAs), which target transposons in animal germlines. In *C. elegans*, piRNAs also target endogenous protein-coding genes, making the PIWI/piRNA pathway an important regulator of gene expression. *C. elegans* piRNAs are referred to as 21U RNAs since they are 21 nucleotides long and have a 5' bias for uridine monophosphate.

We recently identified a protein complex driving 21U RNA biogenesis, which we named PETISCO (Cordeiro Rodrigues et al., 2019). However, we found that PETISCO can have different functions depending on whether it interacts with the proteins PID-1 (PiRNA Induced silencing Defective) or TOST-1 (Twenty-One U antagoniST). PETISCO:PID-1 complex participates in the 21U RNA biogenesis by interaction and stabilization of the 21U RNA precursors. While being important for gene regulation, the loss of PID-1 is not embryonic lethal. In contrast, PETISCO:TOST-1 is not involved in 21U RNA biogenesis but is nevertheless essential for embryonic development of the subsequent generation (Cordeiro Rodrigues et al., 2019; Zeng et al., 2019). The molecular function of this complex as well as the observed lethality effect is not yet understood.

Based on preliminary data, we hypothesize that the PETISCO:TOST-1 complex regulates replication-dependent histones mRNA expression. In this study we want to characterize the PETISCO::TOST-1 complex and dissect its role in embryogenesis. This study would highlight the role of 21U RNA pathway factors in gene expression and *C. elegans* embryonic development.

641B The Germline KH Protein, TOFU-7, engages the HSP-90 chaperone and PRP-19 spliceosome components to promote piRNA-dependent epigenetic silencing.

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Genetic studies in eukaryotes have implicated spliceosome components in Argonaute-mediated silencing. Little is known however about how this occurs and whether or not it involves direct participation in small RNA biogenesis and downstream silencing, or more indirect effects for example on the expression of Argonaute pathway proteins. Here, we show that the C. elegans germline KH protein TOFU-7, previously reported as a piRNA-related factor, interacts by yeast 2 hybrid with SFTB-2, CDC5L and M03F8.3., components of the PRP-19 splicing complex. TOFU-7 also interacts with HSP-90, a chaperone required for

loading piRNA guides onto PIWI. These factors are all required for piRNA-mediated establishment of silencing, yet their genetic behavior suggest they may function at different steps of the piRNA pathway. In tofu-7 mutants PRG-1 and mature piRNAs are completely absent. In contrast, depletion of HSP-90 or CDC5L using an auxin-inducible system results in rapid disappearance of TOFU-7 protein and a mobility in shift in PRG-1. The tofu-7 phenotype is epistatic to the degron depletion of HSP-90 or CDC5L resulting in absent rather than mobility shifted PRG-1. Our findings support a model in which TOFU-7 engages HSP-90 and PRP-19 components to enable PRG-1 loading and stability. Future studies will also explore the possibility that PRP19 complex recruits the spliceosome to promote piRNA biogenesis.

642C Germline inherited small RNAs facilitate the clearance of untranslated maternal mRNAs in *C. elegans* embryos

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Inheritance and clearance of maternal mRNAs are two of the most critical events required for animal early embryonic development. However, the mechanisms regulating this process are still largely unknown. Here, we show that together with maternal mRNAs, *C. elegans* embryos inherit a complementary pool of small non-coding RNAs that facilitate the cleavage and removal of hundreds of maternal mRNAs. These antisense small RNAs are loaded into the maternal catalytically-active Argonaute CSR-1 and cleave complementary mRNAs no longer engaged in translation in somatic blastomeres. Induced depletion of CSR-1 specifically during embryonic development leads to embryonic lethality in a slicer-dependent manner and impairs the degradation of CSR-1 embryonic mRNA targets. Given the conservation of Argonaute catalytic activity, we propose that a similar mechanism operates to clear maternal mRNAs during the maternal-to-zygotic transition across species.

643A Regulation of antiviral responses in *C. elegans* embryos

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C. elegans embryos utilize various endogenous RNA interference (RNAi) pathways to modulate signaling and ensure normal development. One such pathway is the 26G endogenous RNAi pathway, where certain transcripts are processed by a complex which includes Dicer (DCR-1), RDE-4, and the RNA-dependent RNA polymerase, RRF-3. Using the transcript as a template, RRF-3 synthesizes an antisense strand, and DCR-1 cleaves the resulting dsRNA into small-interfering RNAs (siRNAs) that are 26 nucleotides long with a bias for guanosine at the 5′ end (26G siRNAs). Deleting *rrf-3* disrupts the 26G pathway and makes the worms hypersensitive to extracellular dsRNA. Recently, the Bass lab discovered that genes upregulated in *rrf-3(-/-)* embryos overlap with a set of genes known as the Orsay virus-induced genes (OVIGs). OVIGs are genes that are upregulated during infection with Orsay virus (OV), a naturally infecting *C. elegans* pathogen. Interestingly, worms that lack *rrf3* evade the virus better than wildtype and have significantly less viral load relative to wildtype. Upon OV infection and OV genomic RNA replication, the viral replication intermediates are recognized by DRH-1, a Dicer-related helicase, which leads to the upregulation of OVIGs. We observed that two-thirds of the OVIGs upregulated in *rrf-3(-/-)* embryos are only DRH-1-dependent during OV infection. We show that DRH-1 is not necessary for induction of OVIGs in *rrf-3(-/-)* embryos. These results suggest that other pathways are involved in the regulation of OVIG expression. We propose that the resulting accumulation of 26G target transcripts and proteins upon disrupting the 26G RNAi pathway leads to the induction of an OVIG response. We are developing a fluorescent reporter to identify the triggers that induce an antiviral response in *rrf-3(-/-)* embryos.

Keywords:

26G RNAi pathway, *rrf-3*, OVIGs, DRH-1

644B Father knows best: Small RNA-mediated regulation of male fertility and paternal epigenetic inheritance in *Caenorhabditis elegans*

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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 by associating with effector Argonaute proteins (AGOs) to either degrade, inhibit translation, or promote expression of target transcripts. With a robust developmental program and an expanded group of 19 AGOs, *Caenorhabditis elegans* is a superb system to study these highly conserved regulatory mechanisms. Our lab has systematically characterized the expression and sRNA populations associated

with all 19 AGOs in *C. elegans*. In doing so, we identified four AGOs expressed in the gonad only during spermatogenesis, and nine AGOs expressed constitutively in the germline. Although it is well established that sRNA pathways are essential for fertility in multiple organisms, the bulk of worm sRNA research has focused on roles in oogenesis. Therefore, we aim to understand how these 13 AGO/sRNA pathways contribute to proper sperm development and paternal epigenetic inheritance. By assessing trans-generational fertility in single and multiple 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. Our current efforts are focused on understanding the molecular mechanisms by which the spermatogenesis AGOs contribute to the development and differentiation of fertile sperm. Because mature spermatids can carry several potential epigenetic couriers to progeny, including chromatin modifications and sRNAs, we also aim to understand which AGOs and sRNAs are passed from father to progeny via sperm. Using our set of GFP-tagged AGOs, we determined that only two AGOs are packaged into mature sperm, one in the nucleus and one cytoplasmically. Collectively, our work will define new pathways and gene regulatory modes that contribute to male fertility and epigenetic inheritance.

645C A small RNA-mediated feedback loop maintains proper levels of 22G-RNAs in *C. elegans*

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Proper functioning of RNA interference (RNAi) is essential to regulate gene expression, maintain chromatin structure, and protect against expression of foreign genetic elements in all animals. In *C. elegans*, several classes of small RNAs act to silence or license expression of mRNA targets. ERI-6/7, which is formed via trans-splicing of *eri-6* and *eri-7* pre-mRNAs, is a helicase required for the biogenesis of endogenous ERGO-1-class 26G-siRNAs and acts as a negative regulator of the exogenous RNAi pathway because the two pathways compete for access to the downstream 22G-siRNA amplification pathway. We found that the genomic locus encoding *eri-6* contains two distinct regions that are targeted by endogenous 22G-siRNAs. Loss of these 22G-siRNAs disrupts expression of the *eri-6/7* trans-spliced mRNA and therefore production of ERGO-1-class 26G-siRNAs, ultimately freeing up the downstream 22G-siRNA amplification pathway for increased production of 22G-siRNAs from other small RNA pathways. In this way, the pathway acts like a negative feedback loop, to ensure homeostasis of gene expression by small RNA pathways. Recently, similar feedback loops that maintain chromatin homeostasis have been identified in yeast and *Drosophila melanogaster*. Our findings suggest that autoregulatory mechanisms are employed across multiple gene regulatory pathways, and that these mechanisms are likely evolutionarily conserved.

646A An RNAi Screen to Identify Factors that Enhance microRNA Activity After Dauer

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Proper execution of developmental events and their timing is crucial for animals to develop normally. Many animal species can pause their development by entering a stress-resistant and developmentally arrested diapause stage. This interruption can disrupt the timing of important biological processes such as cell fate specification and differentiation. Despite this disruption, wild-type animals develop normally after diapause. The mechanisms by which developmental pathways accommodate diapause can be studied using *C. elegans* dauer larvae. Dauer occurs after the second larval molt in response to unfavorable conditions. During dauer, progenitor cells such as seam cells pause their development. Seam cells divide in a particular pattern and sequence at each larval stage, called stage-specific cell fate, and differentiate at adulthood. After dauer, seam cells complete development normally. MicroRNAs act as a molecular switch to regulate seam cell fate by downregulating target genes that specify early cell fate. MicroRNAs regulate their targets as a part of the microRNA-Induced Silencing Complex (miRISC) that includes the core Argonaute proteins ALG-1 and ALG-2. In *alg-1(0)* mutants, stage-specific cell fates are reiterated. Interestingly, seam cell fates occur normally in *alg-1(0)* mutants that have experienced dauer diapause. This observation suggests that miRISC function is enhanced after dauer to allow cell fates to occur normally. Here, we are using RNAi to screen for factors that potentiate miRISC function in post-dauer animals. Specifically, we are screening for reiterative phenotypes in post-dauer *alg-1(0)* adults. We are focused on conserved kinases and RNA-binding proteins as factors that are most likely to regulate miRISC function. Thus far, we have screened 25% of the genes in our list. We have identified *nekl-3* as a potential candidate gene to enhance microRNA function after dauer. *nekl-3* encodes a kinase that promotes molting. We are currently investigating the mechanism underlying the *nekl-3(RNAi)* phenotype observed in our screen. Once complete, we expect our work to provide insight into the mechanisms by which microRNA pathways can be modulated to allow normal development after diapause. Because we are focusing on conserved genes, these findings may be relevant across animal species.

647B Natural genetic variation in multigenerational non-genetic phenomena in *C. elegans*

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While heredity mostly relies on DNA sequence, other molecular and cellular features are heritable across generations. This non-DNA based memory could be of importance for adaptation of organisms to varying environments. Here we test whether and how non-genetic inheritance systems are modulated by natural genetic variation using two experimental paradigms: the mortal germline phenotype and memory of RNA interference.

The mortal germline (Mrt) phenotype is a progressive onset of sterility over multiple generations. Temperature-sensitive Mrt mutations are known to affect small RNA pathways, histone methylation and thereby multigenerational inheritance. We previously showed that some *C. elegans* natural isolates display a temperature-sensitive Mrt phenotype. Using recombinants between two isolates, we had identified a globally rare causal polymorphism in the *set-24* gene.

To identify polymorphisms explaining a greater part of natural variation in the Mrt phenotype, we performed a genome-wide association study using 95 isolates. A strong association on chromosome III was found, and replicated across two labs. Introgressions of chromosome III from the Mrt isolate JU775 in non-Mrt genetic backgrounds confirmed the association. These results show that a genetic variant underlying the multigenerational phenotype is found at intermediate frequency at the species scale.

The sterility phenotype is likely suppressed in nature, by variation in temperature or potentially other interactions. We indeed find that naturally associated bacteria or infection by microsporidia strongly suppress the Mrt phenotype. Thus, the Mrt phenotype is likely revealed by unnaturally homogenous standard laboratory conditions, thereby providing an experimental handle on natural variation in transgenerational inheritance pathways. Importantly, we also reveal a positive, condition-dependent effect of an intestinal “pathogen”, affecting the *C. elegans* germline.

In addition to the Mrt phenotype, we turned to directly assay the memory of RNA interference, using a *pie-1::GFP* silencing paradigm. *C. elegans* isolates also differ greatly in the number of generations of silencing memory of an RNAi trigger provided only in the first generation; some isolates do not show any memory. Moreover, genetic variation on chromosome III also underlies the short RNAi memory of JU775 compared to N2.

Overall, we show that multigenerational memory is widely modulated by natural genetic variation in *C. elegans*.

648C Characterizing the PASH-1-independent *mir-1829* family in *C. elegans*

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MicroRNAs (miRNAs) must be processed in order to mature and successfully bind to target mRNAs to exert translational repression and/or mRNA degradation. Through the canonical miRNA biogenesis pathway, miRNAs are transcribed from intergenic or intronic loci by RNA polymerase II. The resulting primary miRNAs are then processed by the Microprocessor, an enzyme complex that consists of Drosha, a catalytic RNase III enzyme, and a homodimer of the RNA-binding protein DGCR8 (known as PASH-1 in *C. elegans*) to produce a precursor miRNA. Subsequent processing by Dicer, another catalytic RNase III enzyme, produces the miRNA that is loaded into Argonaute to regulate gene expression. However, there are many noncanonical pathways that bypass one or more steps in the canonical processing pathway. Notably, the biogenesis of mirtrons bypasses Microprocessor cleavage and instead relies on mRNA splicing. In this Microprocessor-independent pathway, splicing of small introns generates miRNA precursors suitable for Dicer processing. Recent data from our lab using a temperature-sensitive allele of *pash-1* in *C. elegans* revealed a unique family of PASH-1-independent miRNAs, the *mir-1829* family. The *mir-1829* family is derived from unusually long introns (ranging from 844-1861 nucleotides in length) of three host genes that have no apparent overlapping functions. We aim to characterize the biogenesis of the *mir-1829* family in *C. elegans*, first determining whether they require Dicer for maturation and if they are splicing-dependent. We will also examine if they function in repression and play a role in physiology. Thus far, we have generated multiple mutant strains with various combinations of *mir-1829* family members deleted to determine if they display any mutant phenotype. Understanding the biogenesis and function of the *mir-1829* family will provide further insight into noncanonical miRNA processing pathways and the biology of miRNAs.

649A Exploring the function of an ancient miRNA family that is essential for *C. elegans* embryogenesis

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Biogenesis of microRNAs (miRNAs) is essential in animals, as disruption of this pathway leads to developmental arrest in multiple species. Interestingly, in most studied animals, developmental arrest during embryogenesis is mainly caused by the absence of functionally redundant groups of miRNAs, called miRNA families. In the case of *C. elegans,* embryonic arrest in the absence of miRNAs seems to be primarily due to loss of two miRNA families, both highly expressed in the embryo: the miR-35 and miR-51 families.

Here, we set out to investigate the function of the miR-51 family, evolutionarily related to miR-100 (the most conserved animal miRNA). The members of this family, miR-51 through 56, share the same seed sequence, but differ in their remaining sequence. Only simultaneous deletion of the six members leads to arrest during embryogenesis, while presence of at least one of them is sufficient to allow for completion of the process. However, the contributions of the different family members to larval development differ and it is unclear whether this is due to their differences in sequence, in expression pattern or in dose. Moreover, despite the importance of this miRNA family, we have no knowledge about its functionally-relevant targets or its cellular impact on embryogenesis.

Using the extensive genetic toolbox available in *C. elegans*, we have shown that, unlike what happens with other miRNA families in the worm (e.g. the *let-7* family), the sequence beyond the seed is not responsible for the differences observed during larval development. However, using a quantitative assay to explore dose-dependency in the miR-51 family function, we found quantifiable differences upon changes in miR-51 family concentration. Our results suggest that the miR-51 family regulates, as a whole, a common target or set of targets in a dose-dependent manner.

To find the target/s of the family, we performed RNAseq in wild type and miR-51 family deficient embryos, at different timepoints of embryogenesis. Our data show that several genes seem to be regulated by this family -including the homolog of the NOTCH receptor, *lin*-12. We are currently validating our hits by generating endogenous reporters of gene expression,and will report our updated efforts in the search for functional targets of this miRNA family.

650B Developing a Comprehensive Tissue-Specific miRNAome by Nuclear Isolation and Small RNA Sequencing in *C. elegans*

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Tissue and cell identity relies heavily on the 3' untranslated region (3'UTR) of mRNAs, which contain several regulatory elements required for proper gene expression. Mechanisms including alternative polyadenylation (APA), which produces distinct 3'UTR isoforms, and downregulation through microRNAs (miRNAs), are essential in establishing tissue identity through modulating gene expression. The networks formed between these distinct mechanisms spin a complex web around tissue and cell identity, which are poorly understood in metazoans. Previous experiments in our lab utilized an immunoprecipitation-based approach which identified APA allows mRNA transcripts to evade miRNA targeting in a tissue-specific manner in the model organism C. elegans. In addition, we identified miRNA targets in the intestine and body muscle tissues, but this approach is unable to identify specific miRNA populations, which are essential pieces in the post-transcriptional regulation puzzle. Identifying tissue-specific miRNA populations will provide a better understanding of how gene regulation modulates identity.

With the ultimate goal of producing a comprehensive tissue-specific miRNAome in C. elegans, we developed a novel approach in which RNA is isolated from tissue-specific nuclei using FACS sorting then sequenced. The miRNAs identified with this method are validated using a second unbiased RT-qPCR -based approach. To develop strains expressing fluorescent tissue-specific nuclei, the mCherry fluorochrome was fused to the C. elegans histone H2B ortholog, his-58. Six worm strains were prepared expressing this construct specifically in the intestine (ges-1p), body muscle (myo-3p), hypodermis (dpy-7p), seam cells (grd-10p), and excitatory (nmr-1p) and

GABAergic neurons (unc-47p). Briefly, the worms are homogenized in a tissue grinder with a clearance slightly wider than the diameter of the nuclei, then the lysate is sequentially filtered and centrifuged before FACS isolation. Finally, RNA is isolated and the library is prepped with the Nextera XT kit. Our initial results support the validity of this methodology which will be over-

imposed to tissue-specific miRNA targets and the tissue-specific 3'UTRome datasets available in our lab. This will ultimately provide the first comprehensive tissue-specific miRNA and 3'UTR Interactome in a living organism.

651C *De novo* damaging variants in microRNA processor *DROSHA* are associated with a severe progressive neurological disorder

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DROSHA encodes a ribonuclease that is a subunit of the Microprocessor complex and is involved in the first step of microRNA (miRNA) biogenesis. To date, *DROSHA* has not been associated with a Mendelian disease. *DROSHA* is constrained for missense variants and moderately intolerant to loss of function. Sequencing of a human patient with profound intellectual disability, epilepsy, brain white matter atrophy, microcephaly and dysmorphic features revealed a *de novo* heterozygous variant p.D1219G in the conserved Ribonuclease III domain of *DROSHA.* In worms, CRISPR knock-in of the orthologous *drsh-1* (p.D943G) variant, when homozygous, results in loss of miRNA expression and heterochronic phenotype. A separate mutation targeting the catalytic site of the RNAase III domain of *drsh-1*(E946Q), phenocopies the heterochronic phenotype of the patient variant *drsh-1* mutant. The existing *drsh-1* deletion alleles used in the field, when homozygous, result in normal somatic development through adulthood, but the adult germlines display sterility. To determine whether the heterochronic phenotypes in the CRISPR generated alleles were arising due to off-target effects, we generated trans-heterozygotes between the CRISPR alleles and the *drsh-1*(tm654) allele. The trans-heterozygous mutants display the heterochronic phenotype of the CRISPR alleles. These data suggest that (a) the phenotypes observed in the *drsh-1*(p.D943G) and the *drsh-1*(E946Q) are because of a loss of *drsh-1* function. (b) the alleles may be maternally dominant, despite being wild-type in a heterozygous state. Together, our data show that the *DROSHA* variant found in the patient presented here is likely maternally dominant and loss of function, and results in a severe developmental phenotype in both worms and humans.

652A Translation and codon usage regulate Argonaute slicer activity to trigger small RNA biogenesis.

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In the *Caenorhabditis elegans* germline, thousands of mRNAs are concomitantly expressed with antisense 22G-RNAs, which are loaded into the Argonaute CSR-1. Despite their essential functions for animal fertility and embryonic development, how CSR-1 22G-RNAs are produced remains unknown. Here, we show that CSR-1 slicer activity is primarily involved in triggering the synthesis of small RNAs on the coding sequences of germline mRNAs and post-transcriptionally regulates a fraction of targets. CSR-1-cleaved mRNAs prime the RNA-dependent RNA polymerase, EGO-1, to synthesize 22G-RNAs in phase with ribosome translation in the cytoplasm, in contrast to other 22G-RNAs mostly synthesized in germ granules. Moreover, codon optimality and efficient translation antagonize CSR-1 slicing and 22G-RNAs biogenesis. We propose that codon usage differences encoded into mRNA sequences might be a conserved strategy in eukaryotes to regulate small RNA biogenesis and Argonaute targeting.

653B Understanding the spatial organization of the somatic RNAi response

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RNA interference (RNAi) was first discovered in *C. elegans* and is a well conserved mechanism that regulates gene expression and fights pathogens like viruses. Gene expression is tightly controlled in the nuclear space and the genome has been shown to spatially segregate into active "euchromatin" and silenced "heterochromatin". Heterochromatin is often localized at the nuclear periphery, while euchromatin is more likely to be found in the nuclear space. Our preliminary results indicate that the RNAi silencing response is able to initiate a reorganization of the nuclear space of the germline. Upon silencing initiation, RNAi silenced mRNA and DNA localize to one germ granule at the nuclear periphery, which can be visualized by smRNA and

DNA FISH. Mutants deficient for RNAi fail to concentrate the silenced DNA and/or mRNA at the granule, suggesting that the reorganization has functional implications. We are currently analyzing whether the somatic RNAi silencing response leads to a similar restructuring of the nuclear space.

Somatic cells possess somatic granules that can be visualized by GFP tagged to the DEAD box RNA helicase, RDE-12, which also localizes to germ granules. DEAD box RNA helicases trigger the unwinding of RNA molecules, which is required for many RNA processing steps. In an effort to uncover functional properties of somatic granules, we mutated different RDE-12 DEAD box helicase motifs by CRISPR. Mutations in these motifs lead to RNAi dysfunction and RDE-12 mislocalization in germ granules. One mutation in the DEAD box domain, which likely prohibits RNA substrate release, provokes an aggregation of RDE-12 specifically in somatic granules. Our results suggest that RDE-12 plays distinct roles in RNAi processing in the germline and somatic tissue.

654C Exploring the role of small RNA- and sumoylated NuRD complex-mediated silencing in germline identity maintenance

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Due to the germline's responsibility to pass on genetic information in all sexually reproducing organisms, it is not surprising that multiple layers of control maintain germline totipotency. However, the identities and mechanisms of these layers have yet to be fully elucidated. Multiple lines of evidence point towards the importance of repressive epigenetic modifications in maintaining germline identity. In embryos, the essential CCCH zinc finger protein PIE-1 maintains germline specification by preventing somatic differentiation programs. Our group has shown that PIE-1 also functions in the adult germline to facilitate the sumoylation and formation of a nucleosome remodeling and histone deacetylase (NuRD) complex containing the histone deacetylase HDA-1/HDAC, zinc finger protein MEP-1, and ATP-dependent chromatin remodeler LET-418/Mi2. Further work has shown that this sumoylated NuRD complex promotes fertility and piRNA-mediated epigenetic silencing. Notably, recent work from our group suggests that loss-of-function of key piRNA and WAGO-class 22G pathway components, *prg-1, rde-3,* or *wago-9,* or expression of unsumoylatable HDA-1 K444/459R results in increased expression of a subset of spermatogenic and soma-specific genes in the germline. Therefore, we hypothesize that the recently described sumoylated NuRD complex promotes germline identity. To determine whether the sumoylated NuRD complex and upstream piRNA and WAGO-class 22G pathways contribute to safeguarding germline identity, we have employed an assay described by the Tursun lab that partially primes the conversion of germ cells to neuron-like cells. Preliminary data suggests that knockdown or knockout of core components of the PIE-1-dependent sumoylated NuRD complex or the piRNA and WAGO-class 22G pathways result in loss of germline identity in this assay, further implicating these pathways in germline identity maintenance. Future studies will determine whether other components of this complex and its associated pathways are also required and aim to further elucidate the intricate mechanisms underlying germline identity maintenance.

655A pre-piRNA trimming and 2'-O-methylation protect piRNAs from tailing and degradation

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The Piwi/piRNA pathway suppresses transposable elements and promotes fertility in diverse organisms. Maturation of piRNAs involves pre-piRNA trimming followed by 2'-O-methylation at their 3' termini. We show that 3' termini of *C. elegans* piRNAs are subject to nontemplated nucleotide addition and piRNAs with 3' addition exhibit extensive base-pairing interaction with their target RNAs. Animals deficient for PARN-1 (pre-piRNA trimmer) and HENN-1 (2'-O-methyltransferase) accumulate piRNAs with 3' nontemplated nucleotides. In *henn-1* mutants piRNAs are shortened prior to 3' addition whereas long isoforms of untrimmed piRNAs are preferentially modified in *parn-1* mutant animals. Loss of either PARN-1 or HENN-1 results in modest reduction in steady-state levels of piRNAs. Deletion of both enzymes leads to depletion of piRNAs, desilencing of piRNA targets, and impaired fecundity. Together, our findings suggest that pre-piRNA trimming and 2'-O-methylation act collaboratively to protect piRNAs from tailing and degradation.

656B High-resolution microscopy reveals *C. elegans* germ granule organization

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RNA interference is a widely conserved mechanism of gene regulation and silencing wherein small RNAs, 18-30 nucleotides in length, target and downregulate deleterious transcripts. In *C. elegans* germ cells, RNA silencing is coordinated through perinuclear nuage containing at least four compartments: P granules, Z granules, *Mutator* foci, and SIMR foci. Multiple of these compartments are phase-separated condensates and maintain distinct structures within the surrounding bulk cytoplasmic phase, similar to the immiscibility of oil droplets in water. Fluorescent widefield microscopy reveals that these compartments are closely adjacent to one another, yet also appear as separate and distinct puncta. It remains unclear 1) how these compartments are organized on the nanomolecular scale, 2) what constitutes the interface between compartments, and 3) how these compartments exchange small RNAs, proteins, and transcripts to facilitate RNA silencing. Our work aims to address the unknowns of nuage organization and interaction to model RNA silencing through phase-separated condensates.

We use ectopic protein expression, aliphatic alcohol, and heat stress to probe the interactions and characteristics of P granules and *Mutator* foci, and have discovered that these condensates remain separate yet adjacent in ectopic environments, respond differentially to perturbation of hydrophobic interactions and, after disruption, can re-establish adjacency in a dynamic manner. The re-establishment of granule interaction may indicate the separate and adjacent nature of these condensates is essential for proper siRNA routing and silencing.

Using 3D-STORM imaging, we reveal the nanomolecular scale of *Mutator* foci and capture a preliminary view of protein density and distribution within the condensate. To image multiple condensates in high resolution we use 3D-Structured Illumination Microscopy and find previously uncharacterized P granule “pocket” formations that house Z granules, *Mutator* foci and SIMR foci, alluding to a more interesting granule organization than previously suspected. Ultimately this work on understanding RNA silencing through *C. elegans* germ granules may also provide fundamental biological insight to how phase-separated condensates coordinate cellular processes.

657C Extending immunity through small RNA inheritance

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Invertebrate immune systems are most often characterized by a “lack of x” compared to mammalian immune systems (Pradeu & Du Pasquier, 2018). “Lacking” processes akin to clonal selection and expansion of B- and T-cells, *C. elegans* is believed not to display an adaptive immune response. Ideas and concepts guiding the description of immunity-related phenomena, such as self/nonself, biotic/abiotic, and innate/adaptive, are developed from a mammalian perspective. This perspective is, however, epistemically pernicious, as it excludes other possible instantiations of immune systems. Ideally, a framework that resists biases is also a framework that describes the processes studied more accurately (Longino, 2008).

I will give a conceptual talk, discussing the possibility of reinterpreting *C. elegans* small RNA inheritance as a transgenerational adaptive immune system. Upon viral infection, *C. elegans* produces virus-derived RNAs (viRNAs), which immunize offspring against repeated infection (Rechavi et al., 2011). Similar heritable responses are launched against abiotic stressors (e.g., Houri-Ze’evi et al., 2016).

I will discuss *C. elegans* small RNA inheritance as an intersection of genetic- and immune systems. Considering small RNA inheritance as part of the C. elegans immune system requires redefining immune systems in several ways. An immune system is, first and foremost, a system that immunizes. Any step further needs to avoid presupposing particularities based on what works in mammals. On the one hand, the differentiation between biotic and abiotic stressors needs to break down. On the other hand, the idea of self/nonself, organism/environment, needs to be reconsidered: Apart from small RNAs recognizing what is “foreign,” other small RNAs transmit information on what is “self” (Rechavi, 2014). Inheriting self- and nonself-recognizing small RNAs results in an intricate equilibrium, shifted by environmental stimuli (Ishidate et al., 2018), and thus, what the organism will recognize as “self” and “nonself.” The environment continuously “blends into” the organism.

In conclusion, I will not only show how examining C. elegans small RNA inheritance helps overcome biased accounts of immune systems but also how it contributes to addressing current theoretical questions in immunology that debate the adequacy of self-nonself-, danger-, and continuity theory (Matzinger, 1994; Pradeu, 2012) as overarching theoretical frameworks.

658A Functional interplay between microRNAs, RNA binding proteins, and alternative polyadenylation in *Caenorhabditis elegans.*

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Regulation of gene expression is fundamental for proper development, homeostasis, and adaptation to the environment for all living organisms. Post-transcriptional gene regulation, which takes place between the transcription and the translation of a gene, is largely controlled by two classes of regulators, microRNAs (miRNAs) and RNA-binding proteins (RBPs). MiRNAs are short non-coding RNA molecules that hybridize to complementary sequences on target mRNAs, usually located in the 3'UTR region, and repress their translation or mediate their degradation. RBPs also perform their function by binding to mRNAs; however, their binding sites can be located in various regions including 5'UTRs, coding sequences, and 3'UTRs. Unlike miRNAs' repressive role, the regulatory activity of RBPs may be positive or negative, depending on the protein, the mRNA, and the biological context. In recent years, a wide repertoire of functional connections between miRNAs and RBPs has been discovered, uncovering a new level of complexity in gene expression regulation. These connections include mutual regulation of the same target mRNAs by both miRNAs and RPBs.

Since both miRNAs and RBPs bind to the 3'UTR region of mRNAs, its length may add a layer of complexity to the interplay between miRNAs and RBPs. 3'UTR length is regulated by alternative polyadenylation (APA). By changing the position of polyadenylation, APA can generate transcripts with multiple 3' UTR isoforms, each containing distinct regulatory elements (e.g., miRNA and RBP binding sites). In this study, we used *C.elegans* UTRome.org data that provides 3'UTR variants across different tissues, to study the coordination between miRNAs and RBPs in tissue-specific gene regulation that happens through binding to 3'UTRs. We performed a comprehensive analysis to explore regulation by individual miRNA and RBPs, as well as mutual regulation by both miRNAs and RBPs. We identified miRNA-RBP pairs that show statistically significant differential co-targeting across tissues. Our results provide evidence for complex gene regulatory networks that involve multiple factors such as miRNAs and RBPs.

659B Regulation of *C. elegans* Argonaute proteins by Arginine Dimethylation

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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 the evolutionarily conserved small RNA pathway. 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 modified with dimethylarginine within arginine/glycine-rich regions (RG/RGG motifs). In *D. melanogaster*, dimethylation of the Argonaute protein Aub is required for assembly of the ping-pong complex and ultimately for piRNA mediated transposon silencing. However, it remains unclear whether there are any general principles underlying the function of Argonaute modification by dimethylation.

First, to determine if any of the 27 *C. elegans* Argonaute proteins are dimethylated, we identified Argonaute proteins containing RG/RGG motifs. The proteins with the highest incidence of these motifs are the cytoplasmic Argonautes ERGO-1, CSR-1, ALG-3 and PRG-1, and the nuclear Argonaute HRDE-1. These five Argonaute proteins were tagged using CRISPR and immunoprecipitated. Following mass spectrometry analysis, we identified multiple dimethylation sites within each of these Argonaute proteins.

Next, to understand the physiological role of these dimethylarginine modifications, we used CRISPR to generate methylation-defective mutant Argonaute proteins. We are currently focusing on the PRG-1 RG-motif mutant, and assessing how mRNA and small RNA expression change compared to wild-type and a *prg-1* null mutant. To further probe PRG-1 function in the absence of dimethylarginine, we will next determine whether the PRG-1 RG-motif mutant activates a sensor for piRNA silencing activity and whether it has transgenerational fertility defects. This work will define the functional relevance of Argonaute protein methylation in the *C. elegans* germline.

660C Neuronal control of maternal provisioning in response to social cues

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Mothers contribute cytoplasmic components to their progeny in a process called maternal provisioning. Provisioning is influenced by the parental environment, but the

molecular pathways that transmit environmental cues between generations are not well understood. Here we show that in C. elegans, social cues modulate maternal

provisioning to regulate gene silencing in of 5 fspring. Intergenerational signal transmission depends on a pheromone-sensing neuron and neuronal FMRF (Phe-Met-

Arg-Phe)-like peptides. Parental FMRF signaling dampens oxidative stress resistance and promotes the deposition of mRNAs for translational components in progeny, which

in turn reduces gene silencing. This study identifies a new pathway for intergenerational 10 communication that links neuronal responses to maternal provisioning. We suggest that loss of social cues in the parental environment represents an adverse environment that stimulates stress responses across generations.

661A *In vivo* CRISPR screening for biologically important *mir-35* targeting sites in *C. elegans*

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microRNAs play fundamental roles in maintaining appropriate gene expression levels. They repress multiple target genes through complementarity between the “seed” sequence at position 2-7 of the 5’ end of the microRNA and the 3’UTR of the target gene. Identifying miRNA target genes is difficult, and delineating which targets are the most biologically important is even more difficult. Therefore, we devised a novel strategy to test the phenotypic impact of individual microRNA–target interactions by disrupting each predicted miRNA-binding site by CRISPR–Cas9 genome editing in *C. elegans*. We developed a multiplexed negative selection screening approach in which edited loci are deep sequenced, and candidate sites are prioritized based on apparent selection pressure against mutations that disrupt miRNA binding. Importantly, our screen was conducted *in vivo* on mutant animals, allowing us to interrogate organism-level phenotypes. We used this approach to screen for phenotypic targets of the essential *mir-35-42* family. By generating 1130 novel 3’UTR alleles across all predicted targets, we identified *egl-1* as a phenotypic target whose derepression partially phenocopies the *mir-35-42* mutant phenotype by inducing embryonic lethality and low fecundity. This study demonstrates that the application of *in vivo* whole organismal CRISPR screening has great potential to accelerate the discovery of phenotypic negative regulatory elements in the noncoding genome.

662B Understanding cluster assistance of microRNA biogenesis in *C. elegans*

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MicroRNAs are a class of genome-encoded small non-coding RNAs with an average size of 22 nucleotides. MicroRNA biogenesis employs a two-step mechanism. The first step is the cleavage of primary microRNA transcripts (pri-miRNA) into precursor microRNAs (pre-miRNA) by the DROSHA/DGCR8 Microprocessor complex. The second step involves the Dicer nuclease, which processes the pre-miRNA into mature microRNA products. Mature microRNAs that originate from a polycistronic transcript often vary widely in their expression levels, partially due to their different optimality in secondary structure for the Microprocessor complex. In mammalian cells, a phenomenon describing the indispensable role of one helper pre-miRNA in assisting the Microprocessor-mediated cleavage of another recipient pre-miRNA in the same cluster was recently described (cluster assistance effect). ERH and SAFB2 were identified as co-factors of the Microprocessor complex in this pathway. In *C. elegans,* about 35% of the well-annotated microRNAs are encoded in clusters, and we aim to understand cluster assistance in regulating microRNA biogenesis in *C. elegans.* Therefore, we generated mutants of the orthologous genes in *C. elegans* (*erh-1*, *erh-2* and *safb-2*) using CRISPR, followed by deep sequencing of miRNAs from each of these mutants in embryo and young adult stages to determine whether these factors play a role in cluster assistance *in C. elegans*.

663C Distinct pathways for exporting dsRNA in systemic RNAi

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In *C. elegans*, ingestion of double stranded RNA (dsRNA) can induce systemic RNAi. Despite widely using this phenomenon as a gene knockdown method, it has not been well understood how ingested dsRNA is transported within and exported from the intestine where dsRNA is taken up from the environment. We screened for mutants that show deficiency in the feeding RNAi and identified two novel genes in which lesions affect the intercellular transport of dsRNA. One is *Y39A3CL.1* encoding a novel endomembrane-associated protein that has no homologs outside of nematodes. Then, we refer to this gene as *rexd-1* (RNAi EXporting Defective). The other is *tbc-3*, which encodes a TBC domain-containing Rab GTPase activating protein. We found that REXD-1 and TBC-3 can act in a cell non-autonomous manner, suggesting that they promote export of dsRNA during systemic RNAi. Next, we examined genetic interactions between *rexd-1*, *tbc-3* and *sid-5*, which encodes an endosome-associated protein involved in transport of dsRNA across the intestine. The *tbc-3* mutation enhanced the RNAi resistance phenotype of both *rexd-1* and *sid-5* mutations, whereas the interaction between *rexd-1* and *sid-5* mutations showed relatively weak enhancement of the phenotype. These results indicate that REXD-1, TBC-3 and SID-5 act in redundant pathways in systemic RNAi. Furthermore, we found that *rexd-1*, *tbc-3* and *sid-5* triple mutants have strong resistance to RNAi triggered by ingested dsRNA except for genes expressed in the intestine. In contrast, RNAi was efficiently induced in the triple mutants when dsRNA was injected into the pseudocoelom. These results suggest that in *rexd-1*, *tbc-3* and *sid-5* triple mutants, neither uptake of dsRNA nor intracellular RNAi machinery but export of dsRNA from the intestine is strongly impaired. Despite inability to export dsRNA, transport of a yolk protein from the intestine to germ cells as well as dynamics of secretion signal-fused GFP was not affected in the *rexd-1*, *tbc-3* and *sid-5* triple mutants. Therefore, it is possible that transport of dsRNA is separately regulated from general secretion pathways. Our results revealed that REXD-1 and TBC-3, together with SID-5, constitute essential pathways for export of dsRNA from the intestine in systemic RNAi.

664A The RNA helicase CGH-1 regulates the liquid condensates of piRNA pathway factors to promote piRNA silencing in *C. elegans*

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PIWI-interacting RNAs (piRNAs) are small RNAs that defend genomes against transposons and regulate endogenous gene expression in animal reproductive systems[1]. Many of the piRNA pathway factors are enriched in various perinuclear foci, including P/Z granules and Mutator granules[2-4]. How these liquid condensates interact and coordinate for piRNA mediated gene silencing is not well understood. Using a sensitized piRNA reporter strain, we performed a candidate RNAi screen and identified CGH-1 as a novel factor required for piRNA silencing.

CGH-1, a conserved DEAD box helicase, is a key component of P bodies. CGH-1 localizes to cytoplasmic storage bodies (germline P bodies) and perinuclear P granules in the *C. elegans* germline. Our imaging analyses suggest that CGH-1 regulates the localization of piRNA pathway factors. In both *cgh-1* RNAi and knockout mutants, PIWI Argonaute PRG-1 is delocalized from perinuclear P granules; CSR-1, an Argonaute that counteracts piRNA silencing, is also not properly localized to P granules, while accumulation of two constitutive P granule components, PGL-1 and GLH-1, are largely normal. Perinuclear foci of Mutator protein MUT-16 and Z granule protein WAGO-4 are also partially dispersed in *cgh-1* mutants. Interestingly, CGH-1 foci accumulate at the cytoplasmic side of P granules, suggesting CGH-1-containing bodies represent a separate but closely related granule to P granules, or that these bodies represent a subdomain of P granules. In addition, crosslinking and IP-MS identified P granule factors, including PRG-1, GLH-1 and CSR-1, together with P body components, as CGH-1 interactors, suggesting a role of CGH-1 in mediating the interactions between these two granules.

As *cgh-1* is expressed broadly and *cgh-1* null mutants are lethal, we developed an auxin-inducible degron (AID) tagged CGH-1::degron::GFP strain to perform the germline specific depletion of GGH-1. Small RNA-sequencing suggests that piRNA levels are reduced slightly, while CSR-1, but not WAGO, bound 22G-RNAs have a 2-fold increase in *cgh-1* depleted animals.

In summary, our results support a model that the RNA helicase CGH-1 promotes piRNA silencing through regulating the liquid condensation of piRNA pathway factors at perinuclear foci. Our research further highlights the critical role of an RNA helicase in mediating the interaction between distinct phase-separated bodies.

1. Ozata et al., Nat Rev Genet (2019); 2. Lee et al., eLife (2020); 3. Placentino et al., EMBO J (2020); 4. Spichal et al., Nat Commun (2021)

665B Neuronal circuits and molecular pathways involved in olfactory imprinting in Caenorhabditis elegans

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Olfactory imprinting is a type of behavioural alteration produced during the early developmental stages of an animal. Like imprinting, associative learning can also alter the behavioural pattern. *Caenorhabditis elegans* is an excellent model to study alterations in neuronal connectomes because of their precise mapping and well-defined neurotransmitter pathways. *C. elegans* has been found to adapt to several learning paradigms, including olfactory, temperature, and salt chemotaxis, as well as olfactory imprinting. This study tried to elucidate the connectomes and neuro-modulators involved in olfactory imprinting and associative learning and memory. Expression of sra-11 receptors is critical for imprinted memory in worms. The difference in associative learning and memory after imprinting also suggests that AIY interneurons play a critical role in both these memory formations. We used a set of mutants with connectome defects to elucidate the neuronal circuitries involved in memory formation. Our results show a significant overlap between imprinting and associative learning; both sharing common neuronal circuits such as AWC, AIY, and RIA neurons in *C. elegans*. These results suggest that animals with imprinted olfactory memory have a better ability to learn and form both long-term and short-term memory.

Key words – Olfactory imprinting, connectome, neuromodulator, associative learning

666C Gravitaxis in *C. elegans* requires touch receptor neuron tubulins and TRPA-1

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The ability to sense Earth's gravitational pull is essential for orientation, navigation, and/or proprioception in many organisms, including plants, animals, fungi, and even protists. We have found that *C. elegans* dauers, and to a lesser extent, adults, negatively gravitax when migrating on agar in 50 cm vertically oriented test chambers. We hypothesize that this behavior is necessary for dauer larvae to migrate away from poor environmental conditions in a natural setting and that negative gravitactic behavior in dauers may be modulated by other sensory inputs to halt upward movement as the animals approach the surface of the ground. Supporting this hypothesis, we observed that upward migration is attenuated when dauers are exposed to constant light and/or background EM radiation present in the lab. To assess possible molecular mechanisms for gravity sensation, we investigated the requirement for mechanosensory components in the behavior. MEC-4 and MEC-10 are components of the DEG/ENaC channel involved in touch sensation and have been shown to be necessary for the stress response induced by hypergravity. Although we found that gravitaxis is only slightly defective in *mec-10* mutants and *mec-4* mutants show no impairment in the assay, *mec-7* and *mec-12* mutants are entirely defective for the behavior. MEC-7 and MEC-12 are tubulin subunits comprising the 15 protofilament microtubule structures unique to touch receptor neurons (TRNs). Significantly, touch-insensitive *mec-12(e1605)* mutants, in which the 15 protofilament structures are not disrupted, are also defective for gravitaxis, emphasizing the importance for the varied roles that TRN microtubules play in transducing mechanical force. Finally, we found that another, previously unrelated mechanoreceptor, TRPA-1, is also essential for negative gravitaxis in worms. Studies in *D. melanogaster* have shown that *trpa-1* homologs *pyx* and *pain* are required for negative gravitaxis in the absence of light. Although TRPA-1 has been identified as a putative mechanoreceptor in *C. elegans*, it is primarily known for sensing noxious cold in PVD neurons. A homolog of *trpa-1* is expressed in mammalian inner ear hair cells; hence, *C. elegans* may provide a potential model for vestibular and auditory systems in humans. Our findings provide evidence that two mechanosensory pathways function in parallel to mediate gravity-sensing in worms.

667A Discerning the Role of Neuropeptide(s) in *C. elegans* Thermotaxis Behavior

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The *C. elegans* genome encodes over a hundred and fifty neuropeptide genes, which consist of three classes and partake in various functions such as synaptic transmission, cellular signaling and development. Despite their ubiquitous nature, the role(s) played by neuropeptides in several behaviors in *C. elegans* remains elusive. One such behavioral paradigm is thermotaxis, a robust behavior wherein well-fed worms have been shown to migrate towards the region of past cultivation temperature when placed on a thermal gradient. Studies have suggested the possible involvement of neuropeptides in communication within the neural circuits required for thermotaxis, however, their exact role in thermotaxis regulation is unknown (Narayan, A., Laurent, G. and Sternberg, P. W. (2011) and Nakano, S. *et al.* (2020)). In order to confirm and further uncover how neuropeptide(s) might be partaking in thermotaxis, we must first identify which neuropeptide(s) are involved for this behavior. To this end, we are performing a genome-wide neuropeptide screen to look for neuropeptide candidates that

may be involved in thermotaxis behavior, along with cell-specific knockout for genes crucial for neuropeptide processing in *C. elegans*. Thus far, we have tested over a hundred mutants, and none have shown strongly altered thermotaxis behavior. In addition, we have tested the four known pro-protein convertases in *C. elegans* and found that *egl-3*, the *C. elegans* ortholog of human proprotein convertase 2 (PCSK 2), displayed abnormal thermotaxis behavior. However, an AFD-specific knockout of *egl-3* displayed thermotaxis behavior akin to wildtype, suggesting that *egl-3* mediated regulation of thermotaxis most likely takes place through an alternative pathway, prompting further questions pertaining to site of action and the role of the neuropeptides processed by *egl-3* in thermotaxis behavior. Altogether, through the genome-wide neuropeptide screen along with the study of the neuropeptide processing enzymes, we hope to gain a holistic insight into peptidergic modulation that controls thermotaxis behavior.

668B Detecting signatures of evidence accumulation in the feeding circuit

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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. Worms adapt their pumping rate to the available food in the environment.

It has been suggested that worms sample their surroundings by pumping and adjust their food intake accordingly, possibly using a decision process. We are interested in identifying the neural circuits, signals, and transfer functions in the worm that take place during this decision-making process. To study brain activity, we make use of SCAPE (Swept Confocally-Aligned Planar Excitation) microscopy. SCAPE is a novel light-sheet microscopy method that allows for whole-brain imaging in 3D at high volume rates. Furthermore, this technique allows imaging for long periods due to its low phototoxicity. We will show our advances in using this technique to image neural activity in C. elegans while feeding. We will discuss challenges in analyzing and interpreting these data, and show how the fast scanning speed of SCAPE improves the tracking of neurons.

669C Behavioral and Ca^{2+} imaging analysis of odor and temperature sensory integration in *C. elegans*

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Most of living organisms are exposed to multiple environmental stimuli at the same time in nature. When animals sense external stimuli of heterogeneous sources, they weigh and integrate the information in their nervous system to respond appropriately. It is essential for animals to make behavior choice for survival, however, the neural and molecular mechanism of multisensory integration is still largely unknown.

C. elegans exhibits behavioral responses against various stimuli such as temperature and odors. Previous studies have revealed detail neural networks and signaling pathways that are required to produce behavior in response to each stimulus. To understand how the nervous system process multiple information, we are investigating the behavior and neural response of worms that are exposed to both temperature and odor stimuli at the same time.

To examine the behavior choice of worms, we evaluated the direction of worm migration on the agar plate with thermal gradient and an odor. We also set up a microscope with auto-tracking stage that enable us to observe neural responses and locomotion of free-moving worms simultaneously with stimuli of temperature and an odor. We are currently analyzing the neural activities of sensory neurons and inter neurons in order to identify the neurons and the mechanisms that drive worm behavior. These results are expected to allow us to understand the detail neural mechanism of multi-information integration in a complicated environment.

670A Diacylglycerol content controls proper memory utilization through switching between forgetting and retrieving

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Utilizing memories properly through forgetting and retrieving is essential for animals to be adapted to changing environment. Yet, its regulatory mechanisms are not fully understood. We previously reported that TIR-1/JNK-1 pathway acts in AWC sensory neurons accelerates forgetting of the olfactory memory in another sensory neurons, AWA. By the Ca^{2+} imaging analyses, the sensory responses to diacetyl in AWA alter in accordance with the behavioral changes during memory acquisition to forgetting and hence the Ca^{2+} response can be considered as the sensory memory trace. In this study, by using unbiased genetic screening, we found that mutations result in increased diacylglycerol (DAG) content, such as *dgk-1*, *goa-1*, or *egl-30*

(gf), suppressed the forgetting defect in *tir-1* loss-of-function mutant. However, even though *goa-1;tir-1* double mutant shows forgetting in chemotaxis like wild-type animals, their Ca^{2+} responses to diacetyl in AWA did not recover similarly to that in *tir-1* mutant defective in forgetting, suggesting that the sensory memory trace is retained in AWA even though the double mutant exhibits behavioral forgetting. On the contrary, *AWC promoter::goa-1 (gf)* expressing mutant showed a forgetting defect in behavior, while it showed recovery in Ca^{2+} responses. Taken together, proper DAG content in olfactory circuit is important to regulate the switching between forgetting and retrieving of olfactory memories without modifying the sensory memory trace. These results raise two possibilities that inadequate DAG content interferes memory recall, and that, in addition to the sensory memory trace in AWA, memory traces in other neurons are required for the memory recall. We suppose that these two possibilities are not mutually exclusive, and that these are consistent with the mechanisms of the memory retention and recall in mice.

671B Insulin signaling underlies a heavy-tailed temporal organization in *C. elegans* episodic swimming

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Episodic bouts of animal behaviors are not periodic, but temporally organized in a scale-free manner with long memory in many animal species including human. It is known that depression in human is associated with defects in the scale-free temporal organization of behaviors and impacts on social fitness, and accordingly exacerbates disease state. *C. elegans* crawls in on agar plate and swim in a solution while switching between actively-moving state and inactive state, which is referred to as episodic behavior. We have shown that the residence time in each state in episodic swimming has a scale-free or heavy-tailed property with long memory. Here, we studied how the residence times between two states are cross-correlated by using detrending moving-average cross-correlation analysis (DMCA) to activity time series obtained from a long-term culture of *C. elegans*. We found that residence times in the active and inactive states are correlated with each other over the time scale from minutes to hours, or at the ultradian time scale. This temporal cross-correlation disappeared when cultured in M9 buffer without food bacteria, and restored when cultured M9 buffer only with glucose. Additionally, the temporal cross-correlation disappeared in the mutant animals of *daf-2* gene, which encodes the membrane receptor for insulin growth factor signal and in the mutant of *daf-16*, which encodes a downstream transcriptional repressor FOXO. Therefore, we conclude that the temporal cross-correlation is achieved by the insulin signaling. Glucose metabolism disorders and behavioral disorders are known to be closely related in human depression, raising the possibility that the temporal organization by insulin signaling found in *C. elegans* is conserved in humans.

672C Exploring the Role of Neuropeptide receptor 14 (NPR-14) in *Caenorhabditis elegans* Sleep Behaviour

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Studies in human and animal models have revealed a causative link between instances of narcolepsy, a sleep disorder which presents with fatigue and episodes of cataplexy, and disruptions in the orexin signalling pathway. In *C. elegans,* a G-coupled protein receptor called neuropeptide receptor 14 (NPR-14) has been identified as a potential ortholog to the human orexin receptor-2 (OX_2R). Previous phenotypic analyses of *npr-14* knockout strains revealed a marked reduction in adult locomotion and mechanosensory stimulation compared to wildtype. These findings, along with the proposed orthologous relationship to OX_2R, suggests that *npr-14* is involved in the regulation of sleep in *C. elegans.* The Raizen lab has shown that *C. elegans* displays two distinct sleep pathways: the developmentally timed sleep (DTS) pathway, and the stress induced sleep (SIS) pathway. The objective of our research is to elucidate whether *npr-14* plays a role in the DTS and/or SIS pathways. This will be achieved by observing DTS and SIS behaviour in *npr-14* knockout worms alone and in combination with knockout and overexpression mutations of known components of either pathway. Results of this research may uncover epistatic interactions between NPR-14 and components of DTS/SIS, therefore suggesting its potential position within either pathway. Successful characterization of *npr-14*'s role in *C. elegans* sleep may provide a model system in which the pathology of narcolepsy and other fatigue-presenting conditions may be explored.

This work is supported by a Discovery grant from the Natural Science and Engineering Research Council (NSERC) of Canada.

673A Sex differences in behavioral, cellular, and physiological responses to nutrient restriction in *C. elegans*

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Nutrient restriction is a universal challenge that requires animal nervous systems to coordinate cues about nutrient availability with current metabolic demands. Often, this requires adjustments to foraging behavior after nutrient restriction, and changes in physiology during prolonged food deprivation. Despite the ubiquitous requirement for nutrient intake, many animals show sex-specific responses to nutrient restriction. Sex differences in immediate responses to lack of food could emerge from sex-specific functions of shared neuronal circuits, while long term nutrient deprivation might bring about sex-specific changes to metabolism and usage of lipid stores. However, the mechanisms by which biological sex acts to regulate neuronal and physiological function and produce sex-specific responses to nutrient restriction is poorly understood. Past work from our lab indicates that *C. elegans* exhibits sexually dimorphic behavioral and neuronal responses to food deprivation. Here we investigate how biological sex regulates differences in *C. elegans* behavior and physiology due to nutrient restriction.

Our goal is to determine to what extent *C. elegans* exhibits sexually dimorphic behavioral and physiological responses to nutrient restriction and the mechanisms by which these sex-differences arise. We used behavioral recordings to determine sex-specific foraging strategies following immediate and sustained nutrient restriction and found that males show distinct foraging behaviors in which they appear to maintain local search patterns longer than hermaphrodites. After immediate and sustained nutrient restriction we found males show diminished slowing and satiety quiescence following re-feeding. Preliminary analysis of genetically sex-reversed animals suggests that dimorphic foraging relies on sex-specific interactions between the nervous system and peripheral tissues. To determine sex differences in physiological responses over extended periods of nutrient restriction, we quantified differences in stress response markers and stains for lipid storage and found sex-specific changes to some physiological responses. In the longer term, our approach provides a unique opportunity to explore potentially conserved mechanisms by which biological sex can regulate encoding of internal states and animal behavior.

674B Wandering versus waiting worms: Loss of nrx-1 decreases hyperactivity induced by food deprivation and octopamine

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Behavior requires the precise coordination of structural and functional properties of neuronal circuits, made up of neurons connected by synapses. Many neuronal and synaptic genes are associated with Autism Spectrum (AS) and schizophrenia (SCZ), suggesting that dysregulation of circuits may underlie characteristic behavioral phenotypes. Here, we ask if and how synaptic genes associated with AS/SCZ control behavior. Specifically, we are testing the role of conserved AS/SCZ genes in the *C. elegans* behavioral response to a simple external stressor: food deprivation. Using a PDMS fabricated "WorMotel" and computer vision image analysis, we record worm activity over 8 hours in fed or food-deprived conditions and find that food deprivation results in a sustained increase in activity in control worms that is easily quantifiable and reproducible. Ongoing high-throughput analysis of many conserved AS/SCZ genes using this paradigm, identified that *nrx*-1 is required for the behavioral response to food deprivation. Multiple loss of function alleles of *nrx-1*, the orthologue of the human synaptic adhesion molecule, *NRXN1,* resulted in reduced activity in response to food deprivation and octopamine, a candidate neuromodulator for this behavior. We find that expression of NRX-1 in all neurons rescues the mutant phenotype response to food deprivation conditions. Interestingly, mutation of the canonical binding partner of *nrx-1, nlg-1 (NLGN3),* does not alter behavioral response to food deprivation. However, *nrx-1; nlg-1* double mutants have a normal food deprivation response, demonstrating that the *nrx-1* phenotype is dependent on *nlg-1*. We are currently analyzing relevant neurons and synapses for changes that may underlie the altered response to food deprivation, in both wildtype and *nrx-1* mutants. Our results suggest *nrx-1* is involved in the response to the crucial external stimuli of food through a potentially novel function in octopamine signaling. Our work will elucidate the circuit and molecular mechanisms underlying the role of *nrx-1* on this behavior. Together with ongoing work on additional genes, we will identify overlap and interactions for AS/SCZ genes in a simple circuit and behavior and uncover novel molecular mechanisms for these genes in behavior generation.

676A Vibrations inhibit feeding behavior through a neural bottleneck in *C. elegans*

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A neural bottleneck is characterized by a convergence of multiple inputs onto a smaller number of neurons. This configuration implies that the network compresses information encoded in the incoming signals. However, it is unclear how biological neuronal networks may perform such a compression and how the information content may change. The pair of RIP neurons in *C.elegans* represent a simple implementation of a neural bottleneck. The RIPs receive converging sensory inputs and provide

the only connections to the network controlling pharyngeal pumping through a pair of gap junctions with the pharyngeal I1 neurons. To investigate the role of such a bottleneck motif, we supply controlled touch stimuli to a population of worms while observing changes in pumping behavior. Estimating information compression requires large amounts of measurements of both the input and output signals. We therefore implemented a high-throughput assay taylored to supplying vibrations as touch stimuli while observing feeding behavior in large worm populations. We present a custom image analysis tool termed 'PharaGlow' that can detect pumping events in several animals moving on standard cultivation plates. As expected, we find that pumping is inhibited by vibrations and this inhibition is abolished in mechanosensory defective mutants. Genetically disrupting the RIP-I1 gap junctions also abolished this inhibition. These results show that this connection is necessary for pumping inhibition by vibrations and provide us with the necessary tools to study sensory information encoding and compression in the RIPs neural bottleneck.

677B Alternative *mec-2* isoforms exhibit neuron type-specific expression and function

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Alternative splicing can contribute to functional diversity by creating multiple isoforms of a single gene. Alternative splicing has been widely observed but it is often unknown whether specific isoforms are expressed in unique cell types, and whether they are functionally relevant. Neuron-specific RNA sequencing (RNA-seq) of *C. elegans* in our lab showed that the conserved membrane protein *mec-2* exhibits unique splicing patterns in specific neurons. We found that the canonical long *mec-2A* isoform is expressed in mechanosensory neurons and was regulated by the *mec-8* RNA binding protein, but the non-canonical short *mec-2B* isoform was detected in neurons other than mechanosensory neurons. We have recently validated our RNA-Seq results using isoform-specific endogenous fluorescent tags, and determined that the *mec-2B* isoform is present in chemosensory neurons as well as mechanosensory neurons. We then tested whether there was a functional role for the *mec-2B* isoform in chemosensory neurons. Chemotaxis assays show that *mec-2* mutants have a significant deficiency in chemotaxis to various organic volatile odorants. Using the CRISPR-Cas9 system we forced the expression of one or the other isoform of *mec-2* to see whether there is a difference in behaviors. We found worms expressing only the short *mec-2B* isoform can sense odors but not soft touch, while worms expressing long *mec-2* can sense both odors and soft touch. When we re-introduced the long *mec-2* isoform into *mec-8* mutant, the *mec-8* touch insensitivity phenotype was rescued. These experiments therefore demonstrate regulation of *mec-2* alternative splicing resulting in isoforms with differential expression and function in different neuron types.

678C LITE-1 mediates behavioral responses to X-rays in *C. elegans*

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While prompt sensory detection of X-rays has been documented across a wide variety of species, relatively few studies have explored the underlying molecular mechanisms of these acute sensory responses. Here we report the discovery of an acute behavioral avoidance response in wild type *C. elegans* to X-ray stimulation. The endogenous *C. elegans* photoreceptor protein LITE-1, which mediates UV-avoidance behavior, was found to also underlie a similar behavioral response to X-radiation.

We observed that wild type nematodes exhibited a dramatic avoidance response to 1 Gy/s focused X-ray stimulation. The response consisted of a marked increase in activity, involving increased forward locomotion, omega bends, and reversals, observed within 2 s of stimulation onset and subsiding once the worm escaped the path of the focused X-ray beam. Moderate dose rates of 0.5 and 0.7 Gy/s evoked similar, but somewhat slower and less vigorous responses, while 0.2 Gy/s and sham (0 Gy/s) stimulation did not elicit any significant response. Nematodes with the *lite-1(ce314)* mutation, which renders LITE-1 dysfunctional, failed to exhibit the X-ray avoidance behavioral phenotype seen in wild type and *lite-1* intact mutant strains, suggesting that functional *lite-1* is critical for the X-ray avoidance response.

To verify LITE-1's sensitivity to X-rays and determine whether ectopic expression of *lite-1* can confer X-ray sensitivity to otherwise X-ray insensitive cells, we administered unfocused X-ray stimulation to swimming *pmyo-3::lite-1* worms, which transgenically express *lite-1* in myocytes. Transgenic, but not wild type, nematodes exhibited a strong paralysis response, quantified as a dramatic decrease in body bend frequency and sometimes accompanied by egg ejection, in response to the stimulation. High dose rates of 0.56 and 0.74 Gy/s produced the most extreme effects, however, paralysis responses were seen at X-ray intensities as low as 0.19 Gy/s.

Together, these results suggest that LITE-1 can function as an X-ray sensitive receptor, playing a critical role in the transduction of X-ray signals into neural activity to produce behavioral responses. This is the first demonstration of X-ray based optogenetic (X-genetic) manipulation of cellular electrical activity in intact behaving animals. As such, LITE-1 shows strong potential for use in this novel method of neuromodulation to transduce transcranial X-ray signals for precise, but minimally invasive manipulation of neural activity in mammals.

679A Discovery of a Highly-conserved Behavioral Role for an Interneuron Neuropeptide Receptor

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Throughout the animal kingdom, neuropeptide signaling mediates many survival-promoting behaviors such as foraging and the escape response. In *C. elegans*, members of two of the largest neuropeptide gene families, *flp* and *nlp*, bind to their cognate *frpr* and *npr*-encoding receptors to regulate many aspects of nematode behavior and physiology. However, the expression and function of several neuropeptide G-protein coupled receptors (GPCRs) in *C. elegans* still remain unknown. Here, we characterize 21 *frpr* and *npr* neuropeptide GPCRs with no pre-existing reagents and discover a conserved behavioral role for the interneuron neuropeptide receptor FRPR-14. We systematically screened 21 CRISPR-generated GPCR null mutants using two behavioral paradigms, the posterior light touch-induced response and freely-moving locomotion tracking assays. Although half of the receptor mutants screened had at least one phenotype, we found that only the *frpr-14* mutant was defective in both. A *frpr-14* GFP reporter is expressed in the AVJ and AIB interneurons, both of which are presynaptic to several command interneurons. However, only AVJ-specific *frpr-14* cDNA expression was sufficient to rescue the full repertoire of mutant phenotypes suggesting a broader behavioral role for AVJ compared to AIB. To identify FRPR-14's ligand in these behavioral contexts, we first screened all available *flp* mutants for visible motility defects. Following up with genetic epistasis analysis, we find FLP-3 to be a potential ligand for FRPR-14. We next applied a comparative approach by studying the orthologous *Cbr-frpr-14* in *Caenorhabditis briggsae*. A *Cbr-frpr-14* GFP reporter is expressed in a single pair of C. brigggsae interneurons with strikingly similar morphology and relative anatomical position as the *C. elegans* AVJ neurons. While cross-species *C. elegans* AVJ-specific expression of *Cbr-frpr-14* cDNA rescued all mutant phenotypes, a *Cbr-frpr-14* null mutant was only defective in the posterior light touch-induced response. Although FRPR-14's function at the cellular level is evolutionarily-conserved, the less evident conservation at the organismal level might be due to differing ligand expression patterns and/or neural architecture between species. Our results provide a framework for further studies on the evolution and function of neuropeptide signaling networks between two closely-related, genetically tractable species.

680B A microbial metabolite synergizes with endogenous serotonin to trigger *C. elegans* reproductive behavior

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Natural products form the basis of many therapeutics, including small molecules used to treat neurological and psychiatric disorders. We performed a behavior-based screen for natural products that affect serotonin signaling by using a simple serotonin-dependent behavior of *C. elegans*, egg laying. Our screen yielded agonists of G protein-coupled serotonin receptors, protein kinase C agonists, and a microbial metabolite not previously known to interact with serotonin signaling pathways: the disulfide-bridged 2,5-diketopiperazine gliotoxin. Effects of gliotoxin on egg-laying behavior required the G protein-coupled serotonin receptors SER-1 and SER-7, and the G_q ortholog EGL-30. Furthermore, mutants lacking serotonergic neurons and mutants that cannot synthesize serotonin were profoundly resistant to gliotoxin. Exogenous serotonin restored their sensitivity to gliotoxin, indicating that this compound synergizes with endogenous serotonin to elicit behavior. *In vivo* calcium imaging showed that gliotoxin stimulates egg-laying muscles and that its effect on muscles, like its effect on behavior, synergizes with serotonin. These data show that a microbial metabolite with no structural similarity to known serotonergic agonists potentiates an endogenous serotonin signal to affect behavior. Based on this study, we suggest that microbial metabolites might be a rich source of functionally novel neuroactive molecules. We will describe new screens we are performing to mine the microbial metabolome for small molecules that affect animal behavior.

681C Distinct neural circuits drive bimodal ethanol chemotaxis in *C. elegans*

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Environmental factors can modulate animals' perception of a stimulus and thus change the elicited downstream behavior. It has been shown that ethanol can cause intoxication in *C. elegans*, therefore ethanol may be a cue worms would like to avoid. However, ethanol may also represent information about food availability worms would like to seek since fruit fermentation often produces ethanol. In the process of looking for ethanol-elicited behavior, we find that ethanol elicits distinct chemotaxis behavior in *C. elegans* depending on the concentration of sodium chloride. Worms are attracted toward ethanol in the low sodium environment (positive chemotaxis), while they exhibit strong repulsive behavior to ethanol in the high sodium environment (negative chemotaxis). In sharp contrast, *C. elegans* exhibits unimodal behavior in response to other odorants such as isoamyl alcohol, diacetyl, or 2-nonanone, regardless of the sodium concentration. Interestingly, we have discovered only sodium but not potassium or osmolality as the determining factor for worm's bimodal ethanol chemotaxis behaviors. In searching for the signaling molecules involved, we have discovered that the positive chemotaxis depends on DAF-11, while the negative chemotaxis depends on CHE-1, GPA-3 and OSM-3. Ethanol chemotaxis in both directions depends on TAX-4 and EAT-4, while ODR-1 or ODR-3 is involved in neither. Analysis of the related neural circuit reveals that the negative chemotaxis to ethanol is likely mediated by the ASE chemosensory neurons, while the positive chemotaxis involves the AWC neurons. Our study provides novel insight into how environmental factors can bias chemotaxis behavior by engaging distinct chemotactic neural circuits. And it may provide a model to dissect how environmental factors modulate behavior.

682A ascr#3 imprinting is mediated by chromatin remodeling

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Imprinting is a form of long-lasting and robust memory which has been described in many animals including human. *C. elegans*, early experience of ascaroside#3 pheromone (ascr#3, C9, asc-ΔC9) enhances ascr#3 avoidance of adult hermaphrodites via the functional modification of the ascr#3 avoidance circuit. Upregulation of *odr-2* Ly-6-related GPI-linked signaling gene expression in the SMB motor neuron is required for ascr#3 imprinting (Hong et al., 2017). However, circuit and molecular mechanisms underlying imprinting need to be further investigated. Here we show that neuronal chromatin remodeling mechanism may play a critical role for ascr#3 imprinting. We first performed candidate gene search including *set-2* sSET1/MLL histone H3K4 methyltransferase gene. We found that ascr#3 imprinting is abolished in *set-2* mutants. We also found that, whereas expression of *set-2* under the control of own promoter did not rescue imprinting defect of set-2 mutants, these defects were partially restored upon expression of *set-2* cDNA under the control of pan-neuronal promoter. We are currently performing additional rescue experiment. We are also observing *odr-2* expression level and performing SMB Ca^{2+} imaging in *set-2* mutant background. This study will allow us to define a common logic for long-lasting memory formation and other forms of behavioral plasticity.

683B Identify the function of mechanosensitive channel PEZO-1 in *C. elegans* males

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The conversion of mechanical force to biological signals is crucial for the survival of animals. Mechanosensitive ion channels play a direct role in sensing physical stimuli and are evolutionarily conserved from bacteria to mammals. In mammals, PIEZO channels have been identified as ion channels that directly detect mechanical stimuli (Coste et al., 2010, Gnanasambandam et al., 2015, Syeda et al., 2015). *C. elegans* genome has a single PIEZO gene, *pezo-1*, which encodes 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 function, we first grouped 14 isoforms into short or long isoforms depending on the mRNA length and observed their expression patterns. We found that the promoter region of short isoforms is expressed in several tail neurons, including the 6th ray neurons of *C. elegans* males. Nine pairs of male ray neurons appear to have functional specialization and have been implicated to be involved in mating behavior (Zhang H, Yue X, Cheng H, et al., 2018). Interestingly, sensory endings of ray 6 neurons, but not other ray neurons, are not exposed to the external environment (Sulston and Horvitz, 1977), suggesting a distinct role in mechanotransduction during *C. elegans* mating. We are currently examining the contribution of PEZO-1 to mating behavior in males.

684C A chemosensory GPCR is required for a concentration-dependent behavioral switch in *C. elegans*

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C. elegans detects a large number of odorants and elicits a multitude of olfactory behaviors (Bargmann, 1993, Cell). Previous genetic and behavioral experiments have identified a set of signaling genes, including olfactory receptors, but the knowledge is still limited. Specifically, the mechanisms of how the same odorants can elicit either attractive or aversive responses depending on the chemical concentration have not been known in detail. Dimethyl trisulfide (DMTS) is an organic chemical, which smells like garlic and is derived from bacterial decomposition. Here, we show that *C. elegans* attracts to a low concentration of DMTS via the AWC neurons and avoids a high concentration of DMTS via the ASH neurons. We then find that chemosensory GPCR *sri-14* is required for both attraction to a low concentration of DMTS and aversion to a high concentration of DMTS. The defects of DMTS chemotaxis in *sri-14* mutants are restored when we expressed the wild-type *sri-14* gene to the AWC and ASH neurons for attraction and avoidance, respectively. Ca^{2+} responses of AWC and ASH upon acute exposure to DMTS are decreased in *sri-14* mutants. Moreover, ectopic expression of *sri-14* in the ADL neurons is sufficient to elicit Ca^{2+} responses to DMTS in the ADL neurons. Furthermore, we heterologously expressed the SRI-14 receptor in the mammalian cell and found that these cells confer dose-dependent response upon DMTS exposure, suggesting that SRI-14 is indeed a *bona fide* DMTS receptor. Next, we investigated how downstream interneurons translate and process DMTS signals from the ASH or AWC neurons to generate alternative behaviors. We found that the AIB interneurons, receiving signals from AWC and ASH neurons, exhibit distinct Ca^{2+} responses depending upon the concentration of DMTS; the AIB interneurons are activated upon removal of low concentration of DMTS or addition of high concentration of DMTS. Moreover, the *sri-14* mutation fully suppresses the AIB Ca^{2+} response to DMTS odor. Furthermore, we identified that the *glr-1* and *glr-5* glutamate receptors expressed in AIB are required for different concentration signals from low and high DMTS. Thus, our results demonstrate molecular and circuit mechanisms by which an animal connects chemosensory neurons detecting the same ligand to alternate downstream circuitry to efficiently trigger precise behavioral programs.

685A Role of MAPK/ERK signaling in neurons

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The MAPK/ERK pathway plays a critical role in regulating neuronal activity in *C. elegans.* The regulation of MAP3K Raf and its targets have been well-studied in growth and development, as unregulated ERK signaling causes cancer.However, the regulation of Raf activity in neurons and how ERK signaling affects neuronal activity are not understood.Overexpression of activated LIN-45/Raf (Raf*) in neurons results in loopy locomotion. While performing a forward genetic screen for suppressors of Raf*, we identified components that (1) affect the activity of Raf and (2) are downstream targets that directly affect neuronal activity. Loss of Kinase Suppressor of Ras 1 (KSR-1) suppresses the loopy locomotion of Raf* expressing worms. KSR-1 is known to heterodimerize with Raf, and this is critical for Raf function. Our experiments demonstrate that even Raf* requires KSR-1 in neurons to promote signaling. We also identified auxiliary subunits of the voltage sensitive cation channel NCA/NALCN as suppressors of Raf*. We found Raf* and the NCA mutant *nlf-1* were mutually suppressing, as *nlf-1* mutants exhibit a distinct “fainting” behavior that is suppressed by Raf*. We explored by what mechanism Raf* suppresses fainting, either by enhancing impaired NCA function or via upregulation of neuronal activity via a separate pathway. As certain tumors are electrically active tissues that hijack neuronal signaling pathways to promote cancerous cell growth, these studies will relate to both neurobiology and cancer biology.

686B Lipidomic analysis of the effects of exposure to ethanol on worms.

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The use of alcohol causes important physiological changes that contribute to the development of alcohol use disorder (AUD). These ethanol-induced changes are multifaceted and are incompletely characterized. In particular, the effects of ethanol on the composition and quantity of lipid species is under studied. We are particularly interested in lipidomic effects because several lines of evidence in our laboratory have implicated lipid metabolism in acute ethanol responses in worms. We therefore performed an unbiased lipidomic analysis of wild-type animals exposed for 16 hours to an intoxicating dose of ethanol. We found that several different lipid classes responded to the ethanol exposure, and that prominent among these was an increase in several different lipids containing odd-chain fatty acids (OCFA). *C. elegans* derive most OCFAs from their bacterial diet. In our ethanol exposure paradigm, the animals were exposed while feeding on OP50, so we examined if the bacteria were responding to the ethanol treatment. We found that the bacteria did not change their OCFA levels in response to

ethanol, suggesting that the ethanol-induced increase in OCFA was due to effects on the worms themselves. We also observed that the long chain polyunsaturated omega-3 fatty acid eicosapentaenoic acid (EPA) appeared to be moved from its storage form in triacylglycerides to other phospholipid species, suggesting that it was mobilized in response to ethanol exposure. We have previously shown that EPA is required for normal acute behavioral responses to ethanol (Raabe *et al*., 2014), and that dietary EPA modulates ethanol response behaviors in mice (Wolstenholme *et al*., 2018). Both OCFA and EPA have been implicated in modulating membrane fluidity; this suggests a model in which animals modify their lipidomes in response to ethanol, perhaps to regulate the function of membrane proteins that are targets of ethanol. Together, these results lend new insight into the role of lipids in the response to ethanol exposure and provide a rich dataset for future studies.

687C *Pseudomonas aeruginosa* Associated Volatiles Drive Chemotactic Behaviour and Immune Response In *C. elegans*

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Olfaction plays an important role in threat perception in higher organisms. To understand the role of olfaction in host survival, we studied interaction of *C. elegans* with *Pseudomonas aeruginosa*, a bacterium commonly found in the same ecological niche as this nematode. *P. aeruginosa* acts as a pathogen against *C. elegans*, colonizing its gut and reducing its survival. It is known that *C. elegans* actively detects and avoids P. aeruginosa (Zhang et al. Nature 2005). We have identified two volatiles produced by P. aeruginosa that elicit a behavioural response in *C. elegans*. One of the chemicals is an attractant and the other is a repellent for *C. elegans*. The amphid neurons AWA, AWB and AWC are mainly responsible for detecting volatile odorants. While AWA and AWC detect attractants, AWB is responsible for detecting repellents. By studying calcium dynamics in olfactory neurons, we have identified specific neurons which detect volatiles from *P. aeruginosa*.

Through qPCR analysis, we have observed induction of *P. aeruginosa* specific immune response genes upon exposure to the volatile repellent. The induction of immune response genes is dependent on functional olfactory neurons. Further, pre-exposure of worms to the identified repellent also increases their survival on *Pseudomonas aeruginosa* lawn.

Keywords: Host-microbe interactions, Olfaction, Chemotaxis, Immune response, *Pseudomonas aeruginosa*, Volatiles

688A Neuropeptidergic modulation of *C. elegans* learning behavior

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Neuropeptides are an evolutionarily conserved group of neuromodulators that regulate a wide range of adaptive behaviors, such as learning. Yet, unravelling the molecular and circuit mechanisms underlying this neuropeptidergic modulation is challenging due to the diversity of neuropeptide signaling pathways, and their 'wireless' extrasynaptic mode of action. Using reverse pharmacology, we have constructed a molecular map of the *C. elegans* neuropeptide-receptor network. Phylogenetic reconstruction of the evolutionary history of nematode neuropeptide systems across bilaterian animals revealed several nematode-specific diversifications of neuropeptide signaling in addition to evolutionarily ancient neuropeptide pathways. One of these ancient, conserved pathways is a neuropeptide Y/F (NPY/F)-like signaling system that is an important regulator of learning behavior both in Proto- and Deuterostomia. We found that NPY/F-like FLP-34 neuropeptides are required in serotonergic neurons for aversive olfactory associative learning, which is functionally similar to the role of NPY in vertebrate learning as well as to the role of NPF in invertebrate learning. NPY/F-like neuropeptides are released from serotonergic neurons and signal through the G protein-coupled receptor NPR-11 in the excitatory AIA interneurons to facilitate olfactory aversive learning. In addition, signaling through NPY/F-like receptor NPR-11 also affects learning in salt gustatory plasticity, a gustatory associative learning paradigm. NPY/F-like signaling is not the only neuropeptidergic signaling system affecting learning behavior; we discovered additional neuropeptides that appear to be important to the learning process as well, including peptides that are expressed in non-neuronal cells. Our current research focuses on unravelling the functions of such non-neuronal neuropeptide messengers in learning and other types of behavioral plasticity.

689B Transcriptional response to UVC irradiation in sleep deficient animals

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Sleep is an important and conserved behavioral phenomenon in animals. In *C. elegans*, there are two distinct sleep states, one during lethargus called developmentally timed sleep and stress-induced sleep. The function of sleep is still largely unknown.

Many hypothesize that it is a restorative state that allows resources to be allocated to cell processes, such as DNA damage repair. Stress-induced sleep is largely dependent on the ALA neuron and genetic mutants that lack the ALA neuron do not sleep following stress. Recently, UVC irradiation has been established as a strong and consistent inducer of sleep, also dependent on the ALA neuron. There is a strong transcriptional response following UVC irradiation. We asked if this transcriptional response supports the restorative function of sleep. We irradiated wild-type and ALA/sleep deficient mutants and did whole genome microarrays to look for differentially regulated pathways that tracked with the sleep phenotype. We also measured the transcriptional response to UVC in animals deficient in a germline. This allowed us to see the somatic cells' response to UVC irradiation, with and without the sleep behavior. Using gene module analysis we discovered differential regulation of several gene modules across groups, notably in the oxidative damage pathway. We also used Kegg pathway analysis and saw differences in regulation of longevity and axon regeneration pathways. These changes could lead to a basis for understanding the function of sleep and what pathways are important for repair and restoration during stress-induced sleep.

690C Using high-throughput behavioural assays to identify heritable natural genetic variants in three *Caenorhabiditis* species

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The most standard behavioural assay involves imaging worms crawling on NGM agar. Recent developments in hardware and software increase the throughput of these experiments by running multiple experimental replicates in parallel and by automating post-acquisition worm tracking and feature extraction. We imaged and analysed the crawling behaviour of 197 *C. elegans* wild isolates in the presence of the OP50 food source, and used genome-wide association (GWA) analyses to identify several quantitative trait loci that are linked to specific behavioural features.

Additionally, we explored multiple behavioural assays involving more "challenging" environments, and found that these assays reveal more pronounced behavioural differences at the species level (between *C. elegans, C. briggsae*, and *C. tropicalis*) compared to the standard crawling assay. We plan to apply these behavioural assays to hundreds of wild isolates from the three species, in order to identify natural genetic variants that underlie heritable behavioural traits across the *Caenorhabiditis* genus.

691A Acetylcholine Signaling Genes are Required for Cocaine-Stimulated Egg Laying in *Caenorhabditis elegans*

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Despite the toxicity and addictive liability associated with cocaine abuse, its mode of action is not completely understood, and effective pharmacotherapeutic interventions remain elusive. The cholinergic effects of cocaine on acetylcholine receptors, synthetic enzymes, and degradative enzymes have been the focus of relatively little empirical investigation. Due to its genetic tractability and anatomical simplicity, the egg laying circuit of the hermaphroditic nematode, *caenorhabditis elegans*, is a powerful model system to precisely examine the genetic and molecular targets of cocaine *in vivo*. Here, we report a novel cocaine-induced behavioral phenotype in *caenorhabditis elegans*, cocaine-stimulated egg laying. In addition, we present the results of an *in vivo* candidate suppression screen of synthetic enzymes, receptors, degradative enzymes, and downstream components of the intracellular signaling cascades of the main neurotransmitter systems that control *caenorhabditis elegans* egg laying. Our results show that cocaine-stimulated egg laying is dependent on acetylcholine synthesis and synaptic release, functional nicotinic acetylcholine receptors, and the *caenorhabditis elegans* acetylcholinesterases.

692B *C. elegans* as tool to study chronic stress implications

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Chronic stress is one of the main risk factors for depression and other neuropsychiatric diseases. Long exposure to chronic stress results in changes in brain gene expression that are deleterious for organisms. Due its relevance to human health, our goal is to investigate the molecular pathways disrupted by chronic stress to understand how this leads to such diseases. Using rodent models of chronic stress, we found that stress alters neuronal protein GPM6A. GPM6A participates in neuronal differentiation and morphology establishment and human *GPM6A* has been linked to schizophrenia, bipolar disorder, claustrophobia and suicide patients. This links GPM6A to the stress phenomenon and depression. Nevertheless, there is a

gap between the cellular GPM6A functions and its role in systemic stress response. To fill this gap, we use the nematode *Caenorhabditis elegans* as model due to shared features between nematode and mammal nervous system and because of the genetic tools available. *C. elegans* exhibits a GPM6A ortholog, the neuronal membrane glycoprotein 1 (NMGP-1), thus, here we used *C. elegans* as a simpler model to study NMGP-1 participation in stress response. First, worms expressing GFP under the *nmgp-1* promoter indicated us that *nmgp-1* expresses in sensory amphid and phasmid neurons and in the egg-laying apparatus. Second, we have characterized NMGP-1 functions using RNAi knockdown and two non-null, hypomorphic mutant alleles. Analysis of dsRNA (*nmgp-1)*-treated or mutant alleles showed an increased recovering time from the stress-resistant dauer stage and a reduced egg-laying rate with respect to control worms. In addition, defects in egg-laying induced egg retention (bag of worms) in *nmgp-1*-deficient worms. Also, worms lacking NMGP-1 showed a normal response to the attractant diacetyl, but an altered repulsive response to SDS. Moreover, morphologically, *nmgp-1*(RNAi) worms showed alterations on ASJ chemosensory neurons located at the nerve ring, responsible of *dauer* exit. Altogether these results suggest that NMGP-1 is involved in the stress response in *C. elegans*. To move forward, we will present and discuss a battery of experiments to score stress response: Temperature acute and chronic exposure, oxidative and osmotic stress. The characterization of stress response in worms lacking *nmgp-1* will allow us to deepen on the stress molecular bases and mental diseases.

693C A glial Cl^- channel is the master regulator of ASH neurons' response to touch

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In mammalian touch receptors, accessory cells, including glia, play a key role in mechanosensation. However, the mechanisms underlying such regulation are not fully understood. We show here that knock-out or amphid glia specific knock-down of Cl^- channel *clh-1* causes nose touch insensitivity in *C. elegans*. Analysis of ASH nose touch neurons' dendrites in *clh-1* mutants show no structural abnormality. However, Ca^{2+} transients in ASH neurons upon consecutive touch stimulations are altered in *clh-1* knock-out. More specifically, while in wild type ASH neurons the second touch elicits smaller transients, this adaptation is absent in *clh-1* knock-out animals. We further show that Ca^{2+} transients' adaptation is also lost in animals in which GABA transmission from amphid glia is inhibited or in which GABAA channel lgc-38 is knocked down in ASH neurons. Importantly, this phenotype is rescued in *clh-1* mutants by expression of Cl^- channels *clh-3* and rat ClC-2 in amphid glia, as well as by growth in high Cl^- plates. These results support that Cl^- excreted by glia via CLH-1 permeates through LGC-38 in ASH to mediate inactivation in these neurons upon consecutive touches. We also demonstrate for the first time that cAMP in ASH neurons favors Ca^{2+} transients' adaptation upon touch stimulation. Indeed, using both pharmacological and genetic approaches, we show that the lack of adaptation in *clh-1* is rescued by hyperactivation of adenylyl cyclase (*acy-1*) or by inactivation of phosphodiesterase (*pde-4*). Parallel behavioral experiments demonstrate that mutations and conditions in which adaptation of Ca^{2+} transients is lost, cause nose touch insensitivity, while mutations and conditions that restore Ca^{2+} transients' adaptation restore nose touch sensitivity. Taken together our data support that reduction in Ca^{2+} transients in ASH upon consecutive touches is essential for maintaining nose touch sensitivity and that both neuronal cAMP and glial Cl^- channel CLH-1 are required for this molecular mechanism. We suggest that this Cl^- mediated mechanism of functional interaction between glia and neurons might be conserved across species in touch receptors and elsewhere in the nervous system.

694A TRPM channels mediate learned pathogen avoidance following intestinal distention

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Upon exposure to harmful microorganisms, hosts engage in protective molecular and behavioral immune responses, both of which are ultimately regulated by the nervous system. Using the nematode *Caenorhabditis elegans*, we show that ingestion of *E. faecalis* leads to a fast pathogen avoidance behavior that results in aversive learning. We have identified multiple sensory mechanisms involved in the regulation of avoidance of *E. faecalis,* including the GPCR NPR-1-dependent oxygen-sensing pathway, an ASE neuron-dependent pathway, and an AWB and AWC neuron-dependent pathway. Colonization of the anterior part of the intestine by *E. faecalis* leads to AWB and AWC mediated olfactory aversive learning. Finally, two transient receptor potential melastatin (TRPM) channels, GON-2 and GTL-2, mediate this newly described rapid pathogen avoidance. These results suggest a mechanism by which TRPM channels may sense the intestinal distension caused by bacterial colonization to elicit pathogen avoidance and aversive learning by detecting changes in host physiology.

695B HACD-1 functioning in neural cells regulates cold acclimation in *C.elegans.*

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Temperature is important environmental information for biological reaction. Animals have acclimation mechanism to environmental temperature changes. We are studying cold acclimation and cold tolerance of nematode *C. elegans* to reveal how animals respond and acclimate to temperature (Ohta et al., *Nature commun*, 2014; Okahata et al., *Scienceadvances*, 2019; Takagaki et al, *EMBO rep*, 2020). Cold tolerance is a phenomenon that 25 degrees-cultivated wild-type animals can not survive at 2 degrees, whereas 15 degrees-cultivated wild-type can survive at 2 degrees. Additionally, 25 degree-cultivated wild-type is transferred to 15 degrees and stayed for 3 hours, they can survive at 2 degrees. This phenomenon is defined as cold acclimation (Ohta et al., *Nature commun*, 2014). We show here that HACD-1 has a role to regulate cold acclimation. HACD-1 protein is homologous to human 3-hydroxyacyl-CoenzymeA dehydrogenase (HADH) that catalyzes a beta oxidation reaction of the fatty acid metabolism in mitochondrial matrix. When 15 degree-cultivated *hacd-1* mutant animals are transferred to 25 degrees and stayed for 3 hours, *hacd-1* mutant showed abnormality of increasing survival rate of cold acclimation relative to wild-type.. HACD-1::GFP driven by *hacd-1* promoter were observed in several head neurons and intestine. We expressed *hacd-1* cDNA in intestine and/or almost all neurons of *hacd-1* mutant. The abnormal cold acclimation of *hacd-1* mutant was rescued by expressing *hacd-1* cDNA in almost all neurons, but not in intestine. HACD-1 expression in approximately 70 neuron pairs including essential neurons for cold acclimation such as thermosensory neurons (ASJ, ADL and ASG) and their interneurons (AIN and AVJ) driven by multiple neuron promoter also rescued abnormal cold acclimation of the *hacd-1*mutants. To determine the neuron type required for *hacd-1*-dependent cold acclimation, we are introducing a series of cell-specific rescue experiments. Since human HADH acts in fatty acid metabolism of mitochondrial matrix, neuronal HACD-1 could play a similar role in the metabolism of mitochondria, which maybe essential for cold acclimation. As ongoing progress, we are trying to measure a temperature of mitochondria in *hacd-1* mutant and wild-type by using genetically encoded temperature indicator tsGFP, to determine a relationship between mitochondria temperature and animal cold acclimation.

696C K2P channel TWK-40 Regulates Rhythmic Motor Program in C. elegans

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Background potassium (K^+) leak currents, conducted by two-pore domain K^+ channels (K2P), are critical for the stabilization of the resting membrane potential and facilitate repolarization. Although K2Ps are fundamental to widespread cellular actions, the K2Ps associated with motor behavior regulation remain enigmatic. Here we show that a K2P gene *twk-40* regulates the rhythmic defecation motor program (DMP) in *Caenorhabditis elegans*. Indeed, disrupting TWK-40 suppresses the expulsion defects of *nlp-40* and *aex-2* mutants. By contrast, a gain-of-function (*gf*) mutant of *twk-40* significantly reduces the expulsion frequency per DMP cycle. Consistently, heterogenous expression of TWK-40, together with in vivo whole-cell patch clamping, demonstrate that TWK-40 forms a voltage-insensitive K^+ selective channel, that regulates the resting membrane potential of the DVB neuron. In addition, we find that TWK-40 substantially contributes to the rhythmic activity of the DVB neuron. Specifically, DVB Ca^{2+} oscillations exhibit obvious defects in *twk-40* mutants. Expression of TWK-40(*gf*) in DVB recapitulates the DMP deficiency of *twk-40(gf)* mutants, and inhibits DVB Ca^{2+} oscillations in both wild-type and *twk-40(lf)* animals. Moreover, the intestinal muscles (IM), which are innervated by DVB to execute the expulsion action, also exhibit rhythm defects. IM-Ca^{2+} transient frequency, but not amplitude, was decreased and elevated in *twk-40(gf)* and *twk-40(lf)* mutants, respectively. Taken together, these results demonstrated that a K2P channel TWK-40 is essential for rhythmic motor regulation, which might provide a potential drug target for constipation.

697A Investigating the role of neuropeptide receptors npr-16 and npr-24 in Caenorhabditis elegans

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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. In mammals, the peptide hormone family somatostatins comprise a group of various secretory proteins that control neurotransmission, metabolism and

memory by acting antagonistically on their respective G-Protein Coupled Receptor (GPCR). In *C. elegans* over 50 GPCRs act as neuropeptide receptors (*npr*), many of which have mammalian orthologs including *npr*-16 and *npr*-24. 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. In *C. elegans*, the insulin/insulin-like growth factor signaling (IIS) pathway is an evolutionarily conserved and well-characterized pathway required for growth, metabolism and behaviour with a strong impact on longevity. Previous studies have indicated the increased longevity associated with *npr-16* and *-24* knockout strains. Whether the increased longevity of *npr-16* and *-24* knockout strains is achieved via the IIS pathway remains unknown. Overall, I hypothesize that *npr-16* and *npr-24* negatively regulate metabolism and development within *C. elegans* through the insulin signaling pathway. I will perform various phenotypic assays to determine the impact of the mutations on fat accumulation, dauer formation, locomotion, development and chemo-sensation. I will also create a construct with the promoter region of *npr-16* or *npr-24* fused to green fluorescent protein (GFP) using PCR fusion. The finalized amplicon with the GFP will be microinjected into *C. elegans* and used to test for localization. Finally, I will cross either *npr-16* or *npr-24* mutant strains with a *daf-16* knockout worm. DAF-16 is a key component of IIS pathway required for increased longevity. I will then test for rescue of wildtype behavior through phenotypic analyses. 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.

698B *C. elegans* learning strategy in T-mazes and aging-related interventions

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C. elegans' ability to exhibit associative, non-associative and imprinted memory in the context of chemical stimuli is well studied. We provide evidence for a new type of associative learning, in which nematodes learn to associate food with a combination of proprioceptive cues and information on the structure of their surroundings (maze), perceived through mechanosensation. By using our custom-made Worm-Maze platform, we demonstrate that *C. elegans* young adults locate food in T-shaped mazes and, following that experience, learn to reach a specific maze arm. The observed learning is a food-triggered multisensory behavior, which requires mechanosensory and proprioceptive input, and utilizes cues about the structural features of nematodes' environment and their body actions. Our findings suggest that *C. elegans* use a type of response learning strategy to achieve learning in the maze, and that structural features of the environment play a role in the acquired learning. In addition, we show that the observed aging-driven decline *C. elegans* learning in the maze can still be reversed at mid-age by starvation. Genetically induced dietary restriction (*eat-2*) and genetic alterations that extend lifespan (*eat-2, daf-2*) have an impact on nematodes' maze performance. Lastly, we share preliminary results of a computer vision-based, custom- made tracking algorithm, especially developed for use in the challenging, 3-dimensional maze environment. This is the first time that learning in a structured maze environment is methodically portrayed and extensively characterized in *C. elegans* nematodes.

699C 3-dimensional behavioral arenas for *C. elegans*

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In nature, *C. elegans* live in a rich 3-dimensional environment. However, their behavior has been assessed almost exclusively on the open, flat surface of NGM (Nematode Growth Medium) plates, the golden standard for *C. elegans* culture in the lab. We present a method to build 3-dimensional behavioral arenas for *C. elegans* by directly 3D-printing NGM hydrogel. This is achieved by using a highly customized fused deposition modeling (FDM) 3D-printer, extensively modified to employ NGM hydrogel as ink, i.e., the Parnon Printer. The result is the advancement of 3-dimensional complexity of behavioral assays. To demonstrate the potential of our method, we use the 3D-printed arenas to assess *C. elegans* physical barrier crossing ability, in the context of aging (young, middle-aged adults), feeding history (fully fed, starved animals) and prior experience (have been or not in the presence of a similar 3D structure before). We also explore the usage of 3D-printed structures to spatially confine

C. elegans egg laying behavior. *C. elegans* behavior in 3-dimensional environments is by definition not possible to explore on standard flat NGM plates. Therefore, the findings reported here would likely not have been brought to light if the Parnon Printer had not been developed. We consider these work a decisive step toward characterizing *C. elegans* 3-dimensional behavior, an area long overlooked due to technical constrains. We envision our method of 3D-printing NGM arenas as a powerful tool in behavioral neurogenetics and neuroethology.

701B Decoding locomotion from population neural activity in moving *C. elegans*

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The activity of an animal's brain contains information about that animal's actions and movements. We investigated the neural representation of locomotion in the nematode *C. elegans* by recording brain-wide neural dynamics in freely moving animals. We report that a population of neurons more accurately decodes the animal's locomotion than any single neuron. Neural signals are distributed across neurons in the population with a diversity of tuning to locomotion. Two distinct subpopulations are most informative for decoding velocity and body curvature, and different neurons' activities contribute features relevant for different instances of behavioral motifs within these subpopulations. We additionally labeled the AVA neurons within our population recordings. AVAL and AVAR exhibit activity that is highly correlated with one another, and they exhibit the expected responses to locomotion, although we find that AVA is not always the most informative neuron for decoding velocity. Finally, we compared brain-wide neural activity during movement and immobilization and observe that immobilization alters the correlation structure of neural activity and its dynamics. Some neurons that were previously correlated with AVA become anti-correlated and vice versa during immobilization. We conclude that neural population codes are important for understanding neural dynamics of behavior in moving animals.

702C Temperature-regulated gene expression changes driving plasticity in the AFD thermosensory neurons

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Animals must respond to changing external stimuli by altering their behavior and physiology in order to maintain fitness. Many of these responses rely on neuronal and neural circuit functional plasticity, which often persist long after cessation of the initial stimulus. Prolonged plasticity is typically achieved through changes in gene expression in the neurons that respond to stimuli and execute stimulus-specific behaviors. We sought to identify genes displaying expression changes in a stimulus- and cell type-specific manner, and determine both how these genes enable neuronal plasticity, and how they are engaged by external stimuli.

We used the *C. elegans* thermosensory neuron pair AFD as a model for stimulus-dependent neuronal plasticity. *C. elegans* exhibits robust thermotaxis towards a preferred temperature when placed on a temperature gradient, and this preference can be set and reset as a function of the animal's long-term temperature experience. The sensory and synaptic physiology of AFD is altered as a function of temperature experience, and this plasticity is necessary for correct thermotaxis. Temperature is a continuous, rather than discrete variable, and *C. elegans* is able to alter its behavior and AFD physiology precisely to match temperature changes over a large dynamic range. We previously showed that temperature experience alters gene expression in AFD. However, it is largely unknown what molecular logic might enable presumably analog changes in gene expression in response to the temperature stimulus. To identify temperature-dependent gene expression changes in AFD, we used translating ribosome affinity purification (TRAP) with GFP-tagged ribosomal subunits expressed specifically in AFD, followed by RNA-Seq. We have validated the temperature-dependent expression of a number of these genes with extrachromosomal and/or endogenous reporters. We are functionally characterizing these genes with behavior and calcium imaging assays, dissecting surrounding non-coding DNA to uncover *cis*-regulatory features, and placing them within previously described genetic pathways controlling AFD thermosensation.

Our long-term goal is to use AFD to derive general principles for how external stimuli can be transduced into gene-regulatory signals that modulate gene expression as a function of stimulus magnitude and dynamics, enabling neural plasticity that is precisely regulated as a function of variations in an animal's environment.

703A Uncovering the Molecular Mechanisms of Thermosensory Adaptation

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Thermotaxis, the movement of animals towards a preferred temperature on a thermal gradient, is a well-studied behavior in *C. elegans*. The precise navigation strategy exhibited by *C. elegans* on thermal gradients is dictated via sensation of the ambient temperature, and modulation of motor movement in a temperature experience-dependent manner. Thermosensation is mediated by thermosensitive guanylyl cyclases expressed in the AFD neuron pair, the primary thermosensory neurons in this nematode. Interestingly, AFD responds to temperature variations only above a temperature threshold that is determined by the animal's long-term temperature experience. We and others showed that in addition to long-term adaptation, the thermosensory response threshold of AFD also adapts rapidly to small temperature variations on a minutes-long timescale. These two AFD thermosensory adaptation mechanisms along with adaptation of AFD synaptic output threshold allow the animal to both adjust its thermosensory behaviors based on long-term temperature experience, and to retain sensitivity to small thermal fluctuations. We have described transcription-dependent mechanisms underlying long-term temperature adaptation of AFD thermosensory responses, but mechanisms of short-term adaptation are unknown. By using the fluorescent sensor FlincG3 to visualize changes in cGMP concentrations, we have determined that in addition to short-term adaptation of the calcium response threshold, cGMP dynamics in AFD also adapt rapidly. Rapid adaptation of the cGMP threshold is calcium-dependent. Ongoing experiments are aimed at identifying the signaling proteins and their targets in AFD that mediate rapid thermosensory adaptation.

704B SWI/SNF chromatin remodeling complexes regulate the expression of innate immunity genes and modulate acute responses to alcohol.

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Alcohol use disorder is a significant social problem. Despite intense study, we have only a limited understanding of the direct and indirect targets of ethanol, leading to difficulty in generating effective pharmacological interventions. We use *C. elegans* to model the acute effects of ethanol on neuronal function in order to identify molecular targets of ethanol and determine the mechanisms underlying the development of tolerance to those effects. We have found that SWI/SNF genes are associated with alcohol dependence in human populations and that SWI/SNF complexes are important for behavioral responses to alcohol in *C. elegans.*

SWI/SNF chromatin remodeling complexes consist of multiple subunits that can be combined to generate functionally and molecularly distinct chromatin remodelers. Two major subfamilies of SWI/SNF are BAF (Brm/Brg1-associated factors) and PBAF (Polybromo-BAF) and these complexes affect different aspects of the acute response to alcohol in worms. The initial response to alcohol has at least two components, initial sensitivity and the development of acute functional tolerance (AFT). We found that BAF complexes regulate initial sensitivity to ethanol, while PBAF complexes are required in neurons for the development of AFT (Mathies *et al*, 2015). We focus here on the role of PBAF in AFT. Using neuronal rescue of *swsn-9* (a PBAF subunit) and a temperature-sensitive allele of *swsn-1* (a core subunit of BAF and PBAF), we identified 603 genes whose expression is regulated by PBAF in neurons and in adults (Mathies *et al.,* 2020). Among the transcriptional targets of PBAF were genes involved in the innate immune response. Innate immunity is regulated by several conserved signaling pathways, including the p38 MAPK, ERK MAPK, TGFß, and insulin pathways, as well as the stress response. We asked if any of these pathways regulates acute behavioral responses to alcohol by testing representative genes in each pathway. We found strong evidence for the involvement of the p38 and ERK MAPK pathways in the development of AFT. We are currently examining genes that are regulated by both SWI/SNF and PMK-1 (p38 MAPK) to identify downstream mediators of AFT.

705C Genetic Mechanisms of Isothermal Tracking Behavior in *Caenorhabditis elegans*

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Maintaining goal-oriented behaviors in a dynamic environment could be a demanding task, which requires perpetual transformation between multisensory signals and motor commands to actuate actions in a coherent sequence. Nervous systems can process such complex computation beyond perception levels and execute complex behaviors in dynamic environments. How do the nervous systems achieve this efficient computation in animals performing complex tasks? To address the neural mechanisms required for this precise computation in sensorimotor systems, we focused on the regulation of isothermal tracking behavior, subjective to plasticity in their cultivation experience. On a thermal gradient under their physiological temperature range, *C. elegans* can migrate toward their previous cultivation temperature (thermotaxis) and then track isothermally along it at the precision of less than 0.1°C differences for up to several minutes. Previous works from our

lab and others have revealed the critical circuit involved in isothermal tracking behaviors, but detailed genetic mechanisms underlying this precise sensorimotor computation remain unexplored. From a candidate screen for mutants defective in isothermal tracking behaviors but normal in thermotaxis ability, we found that a mutant carrying a large deletion in the putative G-protein coupled receptor kinase gene *grk-1* displayed fragmented isothermal tracks at the cultivation temperature instead of long isothermal tracks observed in wild-type animals. Pan-neuronal *grk-1* expression is sufficient and required to maintain the isothermal tracking behavior. Although expressing *grk-1* in key thermosensory neurons and interneurons, including AFD, AWC, AIY, and RIA, failed to restore the isothermal tracking defect of the *grk-1* null mutant, simultaneous expression of *grk-1* in two premotor interneurons AVE and RIM fully rescued the isothermal tracking defect of the *grk-1* null mutant. While our previous analysis showed that the animals with genetic ablation in either AVE or RIM could perform isothermal tracking behavior, our current genetic analysis on *grk-1* identified regulatory roles of AVE and RIM interneurons that maintain the isothermal tracking behavior. The identification of AVE and RIM mediated *grk-1* signaling led us to propose the pathway for isothermal tracking behavior is distinguishable from that of thermotaxis. Our study provides insights into precise sensorimotor computation of goal-driven behaviors in a dynamic environment.

706A Long isoforms of mechanoreceptor pezo-1 control pharyngeal gland cell activity in the nematode Caenorhabditis elegans

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Two PIEZO mechanosensitive cation channels, PIEZO1 and PIEZO2, have been identified in mammals, where they are involved in numerous sensory processes. While structurally similar, PIEZO channels are expressed in distinct tissues and exhibit unique properties. How different PIEZOs transduce force, how their transduction mechanism varies, and how their unique properties match the functional needs of the distinct tissues where they are expressed remain all-important unanswered questions. The nematode Caenorhabditis elegans has a single PIEZO ortholog (pezo-1) predicted to have twelve isoforms. While all isoforms share many transmembrane domains, they differ in the number shared, particularly in those underlying the differences between PIEZO1 and PIEZO2 in mammals. Here we use translational and transcriptional reporters to show that long pezo-1 isoforms are selectively expressed in mesodermally derived tissues (such as muscle and glands). We show that pharyngeal muscles, glands, and valve all rely on long pezo-1 isoforms to respond appropriately to the presence of food. Specifically, we found that gland cell activation is modulated by food presence and density, but that in the absence of long isoforms of pezo-1 gland cells had reduced activity that did not significantly respond to food. The number of pezo-1 isoforms in C. elegans, their differential pattern of expression, and their role in experimentally tractable processes make this an attractive system to investigate the molecular basis for functional differences between members of the PIEZO family of mechanoreceptors.

707B Exoribonuclease ERI-1 regulates ascr#3 avoidance behavior in *C. elegans*

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Small RNAs are short, non-coding RNAs that can regulate gene expression. Recent studies suggested that small RNAs pathway modulates animal behavior (Juang et al., 2013; Posner et al., 2019; Picao-Osorio et al., 2015). However, the mechanisms by which small RNAs regulate animal behavior are not fully understood. *C. elegans* secretes a complex pheromone mixture called ascarosides. A pheromone component, ascr#3 (asc-ΔC9, C9) has been shown to elicit avoidance behavior in wild-type hermaphrodites (Jang et al., 2012). Here, we investigate the roles of small RNAs in ascr#3-mediated avoidance behavior. First, we screened candidate mutants including *rrf-3, ergo-1, drh-3, dcr-1, alg-4; alg-3,* and *rde-4* of which gene products are known to regulate small RNA pathways. We found that four alleles of *eri-1* mutants exhibit defects in ascr#3 avoidance. We then restored the phenotype by expressing *eri-1* cDNA under the control of its own promoter and pan-neuronal promoter. Next, we examined expression patterns of *eri-1* and found that *eri-1* is expressed in a few neurons in the head and tail. We identified that these neurons include either AVJ or AVH in the head and PVP in the tail. Besides, we found a correlation between *eri-1* expression level in AVJ/AVH but not in PVP and ascr#3 avoidance; animals showing high *eri-1* expression appear to exhibit increased ascr#3 avoidance and vice-versa. Moreover, we found that *eri-1* expression levels in AVJ/AVH and ascr#3 avoidance are increased with maternal age. Taken together, these results indicate that exoribonuclease *eri-1* regulates ascr#3 avoidance behavior, which provides an opportunity to study the roles of small RNAs in animal behavior.

708C Viral infection in *C. elegans* causes sleep, which is necessary for survival and energy maintenance

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Cellular stressors including heat shock and ultraviolet radiation (UV) cause sleep in the nematode *Caenorhabditis elegans*. This stress-induced sleep is characterized by locomotor quiescence, cessation from feeding, an increased arousal threshold, and rapid reversibility. We have identified a novel stressor which causes sleep in *C. elegans*: the Orsay virus. The Orsay virus is the only known virus to infect *C. elegans* and is related to the nodavirus, which infects other invertebrates as well as vertebrates including mammals. The Orsay virus exclusively infects intestinal cells. Despite infection of non-neuronal cells, Orsay caused sleep through a similar neuronal mechanism as sleep caused by other stressors. We hypothesized the same genetic and neural circuitry which controls sleep in response to UV and sleep would also control sleep in response to Orsay. Similar to UV and heat, mutations which affected development of the ALA neuron and processing of neuropeptides reduced sleep in infected animals. Conversely, mutations which affected development of the RIS neuron (which is required for UV/heat sleep) did not affect sleep in response to Orsay. ALA-defective animals, which had reduced sleep, had decreased survival compared to wild-type animals. This effect on survival was not observed in the RIS-defective animals, which slept normally. Transgenic overexpression of somnogenic FLP-13 neuropeptides increased the survival of infected ALA-defective animals. The survival benefit of sleeping animals was not explained by a difference in viral replication levels or in the transcriptional intracellular pathogen response. Infection with Orsay caused a decrease in global ATP levels, and this decrease was more severe in ALA-defective animals. The decreased survival of virally-infected animals was accompanied by the proliferation of OP50 bacteria inside the gut of the animal, and in some cases beyond the intestines. Lifespan was extended in virus-infected animals when they were grown on UV-killed OP50 bacteria, suggesting that bacterial superinfection is partially responsible for virus-induced lethality. These findings suggest that sleep is beneficial for recovery from infection due to its effect on energetics, and this model presents a novel opportunity to explore the protective role of sleep in host-pathogen interactions.

709A Klinotactic versus klinokinetic steering strategies implemented in neuroanatomical models for *C. elegans* thermotaxis

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Steering behavior is a prevalent strategy of navigation in which animals make gradual adjustments of their moving direction. The current view holds that steering behavior is a direct orientation to a destination based on temporal comparisons of sequential stimulus samples. This orientation strategy is categorized as klinotaxis. However, for a small-size animal, *C. elegans*, the difference of signal intensity sampled through its sinusoidal locomotion is subtle and potentially overwhelmed by noise, variability, and non-uniformity in environmental signal.

We herein constructed neuroanatomically-grounded models that reproduce *C. elegans* thermotactic steering behavior and found that model worms implemented klinokinetic steering strategy; persistent thermal input sensed not through sinusoidal but through forward locomotion of model worms controlled their curving rates, thereby steering indirectly to a destination temperature. An evolutionary algorithm was employed to find configurations of the model that reproduce empirical positive thermotaxis in *C. elegans* population. In all the evolved models, steering curvature were modulated by temporally persistent thermal signals sensed beyond the time scale of sinusoidal locomotion of *C. elegans*. Persistent rise in temperature decreased steering curvature resulting in straight movement of model worms, whereas fall in temperature increased curvature resulting in crooked movement. This relation between temperature change and steering curvature reproduced the empirical thermotactic migration up thermal gradients and steering bias toward higher temperature. Further, spectrum decomposition of neural activities in model worms showed that thermal signals were transmitted from a sensory neuron to motor neurons on the longer time scale than sinusoidal locomotion of *C. elegans*.

These results indicate a previously unrecognized mechanism of *C. elegans* steering behavior which does not rely on its sinusoidal locomotion. The klinokinetic steering strategy proposed in this work is potentially employed in real worms and enabling reliable orientation behavior overcoming environmental noise and variability.

710B Predatory feeding behavior is modulated via three serotonin receptors and other genetic factors in the nematode *Pristionchus pacificus*

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To intake proper food, animals have developed various feeding strategies. *Pristionchus pacificus* is a satellite model nematode for comparative studies with *Caenorhabditis elegans*. *P. pacificus* exhibits two types of feeding behavior: bacterial feeding and predatory feeding toward other nematodes. While bacterial feeding is observed in most species of nematodes in the same taxonomical clade, predatory feeding is only seen in the family Diplogastridae. Thus, predatory feeding is considered to be an evolutionally novel behavior. Previous studies revealed that the neuromodulator serotonin is a key factor to modulate predatory feeding behavior in *P. pacificus*. To elucidate downstream mechanisms regulating predation, we focused on serotonin receptor orthologs that have been functionally characterized in *C. elegans*. Using the CRISPR/Cas9 system, we produced flame-shift mutants of five serotonin receptors in *P. pacificus*. We examined predatory feeding behavior in those mutants and found that *Ppa-ser-5* mutant and *Ppa-ser-1; Ppa-ser-7* double mutant decreased predation toward *C. elegans* larvae. During predation, the *Ppa-ser-1; Ppa-ser-7* mutant decreased tooth movements that are assumed to be important for predation. *Ppa-ser-1* was expressed in the anterior pharyngeal muscles including the dorsal tooth muscle and *Ppa-ser-7* was expressed in pharyngeal neurons including the M1 neuron, which is innervating onto the dorsal tooth muscles. Interestingly, the expression patterns of those serotonin receptors are partially different between *C. elegans* and *P. pacificus*, implying that changes in the expression patterns may be associated with evolution of the predatory feeding behavior in *P. pacificus*. To find novel genetic factors that are critical for predatory feeding behavior, we performed a forward genetic screening. Among 5150 strains, we found five mutants that are almost completely unable to kill the prey worms. We are now searching for the responsible genes and examining a new genetic mechanism and evolution of predation using those mutants.

711C Computational Neuroethology to Bridge the Gap between Connectome, Neural Dynamics, and Behavior

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One of the current scientific challenges is to understand how the brain works. To address this challenge, we must bridge the gap between the information available about neural circuitry, activity, and behavior. With the growing recognition of the central roles that embodiment and situatedness play, the true challenge is even more difficult: to understand how behavior is grounded in the dynamics of an entire brain-body-environment system. It is also increasingly recognized that the largest challenge we face is not in collecting the data, but in developing the computational modeling tools that will allow us to understand the system. *C. elegans* is a uniquely qualified target for such an integrated modeling of a complete animal. I will provide an overview of the current challenges and state of the art on the effort to develop such a model. Despite the substantial behavioral and anatomic knowledge available, we still do not know the strengths and signs of the synaptic connections or the response characteristics of the majority of the neurons. However, it would be a mistake to wait until more data is gathered before we begin to develop the computational models. I have developed a unique computational approach using optimization techniques to explore the space of unknown electrophysiological parameters of the nervous system necessary to generate organism-like behavior. Because the models are necessarily underconstrained, each successful parameter search produces an ensemble of models that are consistent with the known anatomy, physiology, and behavior of the organism. We then analyze the properties of this entire ensemble using techniques ranging from model neuron recordings, neural and behavioral manipulation and lesion studies to parameter clustering, dynamical systems theory, and information theory. The focus of the analysis is to identify different possible classes of solutions and to thoroughly understand the operation of the highest-performing exemplars of each. This insight can then be used to suggest specific experiments that could decide between the different possibilities. In addition to accelerating the discovery and understanding of the neural mechanisms underlying specific behaviors of interest, I will show how this methodology allows us to begin to address key theoretical challenges in a situated, embodied, and dynamical understanding of adaptive behavior more generally.

712A Dopamine signaling mediates a homeostatic compensation of locomotor bending amplitude in *Caenorhabditis elegans*

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Locomotion in a natural context requires an animal to adapt its motor patterns to changes in the environment or unexpected external perturbations. The mechanisms by which *C. elegans* sets and maintains the amplitude of its dorsoventral bending wave during locomotion are largely unclear. We used spatially restricted optogenetic perturbations of body wall muscles to

increase or decrease the bending amplitude at mid-body and found that this resulted in a decrease or increase, respectively, of the bending amplitude of the head. We hypothesized that the modulation of the head amplitude was a compensatory response to a change in midbody amplitude, sensed via proprioceptive feedback. To test this idea, we designed a microfluidic device to partially immobilize a portion of the worm's body. We found that partial physical immobilization of the midbody resulted in an increased amplitude of the head, consistent with the result of optogenetic experiments. Next, we conducted a candidate screen for genes required for this posterior-to-anterior compensatory effect. We found that neither *dat-1* (dopamine transporter) nor *cat-2* (tyrosine hydroxylase) mutants display compensatory response to mid-body perturbation, suggesting that this locomotory modulation requires dopamine signaling. Furthermore, testing of dopamine receptor mutants *dop-1, dop-2, dop-3,* and *dop-4* showed that compensation depends only on the D2-like DOP-3 receptors. These results reveal a dopamine-mediated homeostatic modulation mechanism for setting the amplitude of *C. elegans* bending waves.

713B Human pain gene ortholog screen in *C. elegans*

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Chronic pain affects up to 30% of the population and can be caused by different factors such as injuries or genetic mutations. Chronic pain can take various forms like migraines, different musculoskeletal conditions (low back pain, fibromyalgia), neuropathic pain disorders triggered by cancer or diabetes, as well as visceral pain disorders. Available drugs are not always effective and could trigger many undesirable side effects. In the recent years, genes responsible for those pathologies have been screened in the population with methods, such as linkage analysis and genome wide association studies. Those approaches have revealed a plethora of new candidates but, for the majority of them, we know nothing about their function in the pain pathway. Because studies on pain in human are very limited, we propose *C. elegans* as a complementary model organism to study conserved nociception genes.

In the present study, we first determined a list of *C. elegans* orthologs for candidate human pain genes reported in the literature and recovered available mutants (109 mutants). We then screened these mutants for alterations in noxious heat-evoked reversals using as high-throughput, computer-assisted behavior analysis pipeline. Using different heat intensities and comparing naive animals with animals repeatedly stimulated with heat, we obtained a set of measures reflecting baseline sensitivity profiles, and the ability to adapt (desensitize) in response to repeated stimuli. Twenty-two mutants displayed significant alterations and could be clustered in different categories, including fast-adapting mutants, non-adapting mutants, and mutants with exacerbated naive sensitivity. As a whole, our study suggests that *C. elegans* represent a promising model to tackle the function of recently identified human pain-associated genes and set the bases for additional studies on specific candidates.

Topics: neurobiology, behavior

Keywords: noxious heat avoidance; sensory plasticity; pain modelling

714C Behavioral studies, responses and chemical synapses on EphR/ephrin deficient mutants

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In *C. elegans,* EphR/ephrin signaling pathways play pivotal roles in multiple biological functions, including epidermal morphogenesis, male tail development, cell migration, cell proliferation, cell morphology, neurite outgrowth and germ line apoptosis. Eph receptor, *vab-1(dx31)* mutants and ephrin ligands, *efn-1(e1) and efn-4(bx80)* mutant exhibit strong phenotypes like embryonic lethality, larval lethality, notched head, tail deformities and axon guidance cues. The amphid sensory neurons including the olfactory-sensing AWA and AWC neurons, temperature-sensing AFD neurons and gustatory-sensing ASE neurons, send chemical synapses primarily onto the paired AIY interneurons. The AIYs lie posterior to the pharynx and suppress turns and reversal behavior in wild-type nematodes. Ventral AIY neurite morphology is disrupted *in vab-1, efn-1 and efn-4* mutants, whereas dorsal extension is disrupted in *efn-4* mutant animals. How these neuronal defects affect sensory-to-interneuron physiology and behavior is not known. Preliminary studies on ASE neuron morphology revealed that *vab-1* and *efn-1* have only a minor role in ASE development, suggesting that distinct axon guidance programs are used for the ASE sensory neurons versus AIY interneurons. However, we find that food seeking behavior is significantly affected in *vab-1* and *efn-4* mutants when compared to wild-type. These data suggest that EphR/ephrin control of AIY interneuron morphology is required, in part, for correct sensory circuit function.

715A Two thermosensory neurons AFD and AWC regulate purity of frequency components in temperature-evoked sinusoidal crawling

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Migration behavior by the innate force such as walking, flapping and crawling is composed of periodic bending and stretching of the body parts. These behaviors consist of various frequency components. Walking includes marching consisting of a single frequency component and tottering consisting of multiple frequency components. Despite quantifying the purity of frequency components of the behavioral states and elucidating neural mechanisms controlling them are important for ethology and neuroscience, it is unclear how the nervous systems regulate the purity of frequency components. To address this question, we quantified the crawling behavior of *C. elegans* responding to thermal stimuli by using spectral entropy that represents the purity of frequency components of crawling behavior. Animals were subjected to two types of temperature stimuli: linear increments across the cultivation temperature (T_c) of animals with slow (0.02°C/s) and rapid (0.1°C/s) rate. Around T_c with slow temperature increment, animals showed pure crawling behavior consisting of small number of frequency components. To identify neurons required for the purity control of the crawling under temperature increment, we examined spectral entropy for the strains, in which thermosensory neurons AFD and AWC were genetically ablated. AFD-ablated animals showed more disordered crawling near T_c than that of wild type under slow temperature increment, indicating that AFD is required for pure sinusoidal crawling near T_c under slow temperature increment. By contrast, AWC-ablated animals showed purer crawling near T_c than that of wild type under both slow and rapid temperature increments, indicating that AWC disorders sinusoidal crawling near T_c. To clarify the relationship between AFD and AWC in the purity regulation of the crawling behavior, we performed behavioral test using a strain defective in temperature sensing in both AFD and AWC. This strain showed additive phenotype of those of AFD- and AWC-ablated strains under both slow and rapid temperature increments, suggesting that AFD and AWC regulate sinusoidal crawling in parallel. We also found that AFD and AWC affected each other's calcium responses to the thermal stimuli. Our results suggest that two different sensory neurons that communicate each other possess opposing functions in regulation of a temperature-responding behavior: one organizes and the other disorders sinusoidal crawling.

716B Characterization of *C. elegans* acid-sensing DEG/ENaCs and their role in rhythmic behavior

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While the roles of classical neurotransmitters and neuromodulators in neurotransmission are well-established, less attention has been paid to the effect of unconventional signaling molecules such as protons on neuronal signaling. In *C. elegans*, protons secreted from the intestine are able to generate motor contractions of the defecation motor program (DMP) independent of the nervous system (Beg et al., 2008). Proton-receptors from the DEG/ENaC family; called acid-sensing ion channels (ASICs) have been described in vertebrates, but their roles in behavior are mostly uncharacterized. We have been investigating *C. elegans* acid-sensing DEG/ENaCs and their role in modulating behavior via neuronal and non-neuronal tissues. In an initial screen using heterologous expression in *Xenopus* oocytes and two-electrode voltage recording, we identified two groups of *C. elegans* pH-sensitive DEG/ENaCs based on their response to neutral and low pH. One group, including ACD-5, DEL-4 and the previously characterized ACD-1, is open at neutral pH and blocked at low pH, while the other group, including ASIC-1, ACD-2 and DEL-9, becomes activated at low pH. The proton-inactivated channel ACD-5 is highly expressed at the apical lumen in the intestine where it senses proton-fluctuation and maintains the intestinal pH. Gain-of-function mutations show a more neutral intestinal pH and prolonged DMP intervals demonstrating the importance of protons in the generation of behavior. In contrast, the proton-activated DEL-9 is expressed in neurons and muscles. In the motoneuron AVL, DEL-9 drives the execution of the DMP enteric muscle contraction, while in the vulva muscles it is responsible for the timing of egg-laying. Mutants show prolonged inactive states during the rhythmic behavior of egg-laying and an egg-retention phenotype that can be rescued. This demonstrates that proton signaling is important for diverse physiological processes and behaviors.

717C cGMP phototransduction pathway is involved in light avoidance behavior in the nematode *Pristionchus pacificus*

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Sensing light is an important function for most organisms, which is mediated by photoreceptors such as opsins and cryptochrome. Although nematodes do not have eyes and known photoreceptors, worms can react to light and show avoidance behavior. Recently, a new photoreceptor LITE-1 has been identified in the nematode *Caenorhabditis elegans*.

However, LITE-1 is only conserved in the genus *Caenorhabditis*, and the mechanism of light-sensing in other nematodes is unknown. To elucidate the mechanism of light-sensing, we use 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. We established a light-avoidance assay in *P. pacificus*, based on *C. elegans* studies. Similar to *C. elegans*, illumination of the short-wavelength light induced avoidance behavior in *P. pacificus*. To reveal the mechanism of the light avoidance behavior, we investigated mutants of six neurotransmitter-related genes. GABA and glutamate mutants had a defect in light avoidance, indicating that GABA and glutamate are required for light avoidance in *P. pacificus*. To identify genes involved in light-sensing in *P. pacificus*, we performed a forward genetic screening of the light avoidance behavior and isolated three UV light-unresponsive mutants. By next-generation sequencing, we found that two mutants have mutations in *Ppa-tax-4* and *Ppa-daf-11*, respectively. TAX-4 (a cyclic GMP-gated channel) and DAF-11 (a guanylate cyclase) are components of phototransduction in *C. elegans*. To confirm these genes are responsible for the light-unresponsive phenotype, we knocked out *Ppa-tax-4* and *Ppa-daf-11* by the Co-CRISPR method we have established previously in *P. pacificus*. These mutants decreased the light avoidance, suggesting that *Ppa-tax-4* and *Ppa-daf-11* are required for the light avoidance behavior and a part of the light-sensing mechanism is conserved between *C. elegans* and *P. pacificus*. For the other mutant, we will narrow down a candidate region by SNP mapping to identify a responsible gene. Future analysis of these mutants may promote an understanding of the mechanism for light detection in nematodes.

718A

Mechanisms of context-dependent processing of odor valence in *C. elegans*

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The survival of all animals is critically dependent on their ability to detect and respond appropriately to environmental cues. It is particularly important for animals to integrate information such as internal state and contextual cues in order to generate flexible and adaptive behaviors. One of the most important sensory modalities is olfaction; animals rely on olfaction to locate food sources, avoid pathogens and predators, and communicate with each other. However, a given odorant can elicit attractive or repulsive responses depending on context, intensity, and experience. How odor valence is robustly but flexibly encoded in neural circuits remains to be fully explored.

Here we show that *C. elegans* is normally attracted to the straight-chain alcohol 1-hexanol (HEX). However, in the presence of saturating levels of isoamyl alcohol (IAA), animals are instead robustly repelled by this odorant. Two different sensory pathways converge to mediate the behavioral response of *C. elegans* to HEX. Under normal conditions, both the AWC and ASH sensory neurons respond to HEX as assessed via *in vivo* calcium imaging. Genetic ablation and mutant analyses indicate that AWC is required for attraction to HEX, and appears to override ASH-driven avoidance behavior. However, while AWC exhibits the expected “odor OFF” calcium response to HEX in the absence of IAA, HEX instead elicits a robust “odor ON” calcium response when saturating levels of IAA are present. This response switch occurs cell-autonomously and is specific to HEX. The HEX/IAA-dependent ON response requires the ODR-3 Ga protein, whereas the HEX alone OFF response is mediated by other Ga proteins. Preliminarily, it appears that the ON responses in AWC alone can drive repulsion to HEX under IAA saturation conditions, and in the absence of AWC, repulsion can be driven by ASH. This previously uncharacterized neural coding strategy may allow *C. elegans* to efficiently use a small number of chemosensory neurons to tune and correctly regulate context-dependent olfactory behavioral plasticity.

719B Identify the function of calcium-activated chloride channel Bestrophin in *C. elegans*

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Bestrophin is a calcium-activated chloride channel of which gene family is identified in many organisms. In humans, bestrophins are encoded by the four genes; *BEST1-4*. BEST channels are expressed in multiple tissues and appear to 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 largely unknown. To investigate the expression patterns of *best* genes in *C. elegans*,

we expressed a green fluorescent protein (GFP) under the control of the promoter of each best gene. We found that these genes are expressed in most, but not all, cell types, including intestine, muscle, hypodermal and neuronal cells. For example, *best-3* and *best-4* genes are expressed in an excretory cell, which has been implicated in osmoregulation. We are also characterizing nine mutants, including *best-3* and *best-4*. Furthermore, we plan to perform electrophysiology using the *best* cDNAs to examine physiological characteristics of the *C. elegans* BEST channels.

720C Construction of the map of odorants and olfactory neurons in *C. elegans*

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Animals must recognize and discriminate among thousands of chemicals to generate the correct behavioral response. Understanding basic design of a sensory system in simple animals gives the opportunity to elucidate detailed molecular and neural mechanisms underlying sensory responses in higher animals. The small nematode *Caenorhabditis elegans* detects many odorants via a few neuron pairs, including the AWC neurons, and elicit a multitude of olfactory behaviors (Bargmann, 1993, Cell). Due to the established connectome, the olfactory system of *C. elegans* provides an excellent model system in which to explore the circuit mechanisms underlying olfactory processing at the single-cell resolution. Previous genetic and behavioral experiments have identified a set of signaling genes, including olfactory receptors, but the knowledge is still limited.

Here we are trying to construct a comprehensive map of odorants and olfactory neurons in *C. elegans*. We first screened volatile chemicals that are not tested previously and found that animals respond to a set of volatile chemicals, including acetic acid, acetoin, formaldehyde, 1-propanol, or 2-propanol. We further identified that the AWC neurons are required for chemotactic responses to these chemicals. We then measured intracellular Ca^{2+} transients in response to 3,4-hexanedione, acetic acid, heptanoic acid, and (-)-pulegone in AWC-specific GCaMP3 transgenic worms and found that AWC alters Ca^{2+} activities in response to 3,4-hexanedione, heptanoic acid, and (-)-pulegone. Overall, this study indicates that *C. elegans* olfactory systems detect a large spectrum of volatile chemicals via the distinct chemosensory neuron-type.

721A Investigating the Genetic Interaction Between Ciliary *bbs-5* and *nphp-4*

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Primary cilia are critical sensing and signaling hubs that extend from nearly all mammalian cell types. Ciliopathies are a spectrum of human disorders associated with defects in cilia formation and function that result in a wide and variable range of clinical features, often with low genotype to phenotype correlation. This phenotypic variability may stem from the presence of multiple modifier alleles causing different degrees of primary cilia dysfunction. To identify modifier alleles, we conducted a mutagenesis screen in *C. elegans* with a primary mutation in the ciliary transition zone component, *nphp-4(tm925)*, and looked for secondary mutations that caused ciliary dysfunction. From this screen, we identified a mutation in the BBSome component, *bbs-5(yhw62)*, as a genetic modifier of *nphp-4(tm925)* mutants. By assessing a variety of behaviors associated with ciliated sensory neuron function, including chemotaxis and egg laying, we find that our novel *bbs-5(yhw62)* mutation causes enhanced ciliary defects when compared to an existing mutation, *bbs-5(gk537)*. In addition, we observe that *bbs-5; nphp-4* double mutants carrying either *bbs-5* mutation display dye filling defects in ciliated sensory neurons and defects in dauer formation not seen in single mutants alone. We have also investigated whether the genetic interaction between *bbs-5* and *nphp-4* is conserved in two vertebrate models, zebrafish and mouse. In adult *Bbs5; Nphp4* zebrafish, we observe scoliosis and disorganization of the outer nuclear layer of the retinal photoreceptor cells, although these phenotypes are also observed in adult *Bbs5* single mutant animals. In contrast to zebrafish, mice with congenital expression of both *Bbs5* and *Nphp4* do not survive to weaning age. Juvenile induction of *Bbs5* loss in an *Nphp4* mutant background is associated with seizure-like activity and early mortality. These results suggest that the genetic interaction between *bbs-5* and *nphp-4* identified in *C. elegans* that causes enhanced ciliary dysfunction may be conserved in mice, but not in zebrafish. The mammalian conservation of this genetic interaction provides excellent rationale to pursue further mechanistic studies in *C. elegans*.

722B C. elegans-based chemosensation strategy for the early detection of cancer metabolites in urine samples

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Olfaction is one of the primary mechanisms through which many animals adapt to environmental changes. Olfactory receptors, which in all animals belong to the G-protein coupled receptors (GPCRs) family, play a crucial role in distinguishing the wide range of volatile or soluble molecules by directly binding them with high accuracy. Chemosensation is particularly developed in organisms lacking long-range sensory mechanisms like hearing and vision. The genome of the nematode Caenorhabditis elegans possesses a remarkable number of genes encoding chemosensory receptors, making it able to detect a similar number of odorants as mammals, despite the extremely low number of chemosensory neurons available. Here, we show that C. elegans displays attraction towards urine samples collected from women with breast cancer but avoids those from healthy subjects. This behavior is strongly influenced by the female hormone cycle. Behavioral assays performed on animals in which the AWC sensory neurons were genetically ablated demonstrate an essential role of these neurons in sensing cancer odorants. Calcium imaging experiments on AWC neurons dramatically increase the accuracy in discriminating between positive and control samples (with an accuracy of 97.22%). Also, chemotaxis assays performed on mutant animals harboring individual deletion in genes encoding GPCRs expressed in AWC neurons allow us to identify candidate receptors that are likely to be involved in binding cancer metabolites. This finding suggests that a specific alteration of a restricted number of metabolites is sufficient for the highly accurate cancer discriminating behavior of C. elegans, which may allow in principle to identify the fundamental fingerprint of breast cancer.

723C Comparison of taste preferences between two divergent nematode species

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Many different genes and proteins are involved in the molecular pathway of chemosensation. Guanylyl cyclase genes (*gcys*) are known to help detect water-soluble salts and are located in the amphid sensory neuron in the model organism *Caenorhabditis elegans*. Our study focuses on whether the role of the *gcy* family and chemosensory neurons are conserved between free-living and host-associated nematode species. *Pristionchus pacificus* is an entomophilic nematode species that has a host association towards the Oriental beetle, but nothing is known about its gustatory sensitivity. We found that *C. elegans* and *P. pacificus* are equally attracted to bromide and chloride. In addition, we found that *P. pacificus* are repulsed by sodium whereas *C. elegans* are attracted to the salt. When tested with acetate discrimination chemotaxis assays, both species' abilities to sense salts are reduced significantly.

In *C. elegans*, one of the *gcys*, *gcy-22,* is exclusively expressed asymmetrically in the ASER taste neuron and is known to have a board effect on salt sensing. However, the ASE homolog in *P. pacificus* is likely not lateralized in function. In the *P. pacificus* genome, there are 5 *gcy-22* homologs, but it is unclear which if any of these *gcy-22* homologs have conserved gene functions in salt chemotaxis.

Using CRISPR/Cas9 editing system, we investigated the possible gustatory functions of a *gcy-22* homolog, *PPA34960*. We recovered 3 viable loss or reduction-of-function alleles in *PPA34960*: *csu79*, *csu80*, and *csu81*. When testing the mutants to salts, *csu80* have a stronger attraction to NH_4Br. *csu79* have a stronger attraction to NH_4Cl while *csu80* are attracted to NH_4Cl at a level comparable to wildtype. We are currently conducting more salt chemotaxis assays for different salts such as LiCl and NH_4I. The characterization of guanylyl cyclase would contribute to understanding the molecular pathway of chemosensation and host-seeking abilities in *P. pacificus* and other host-associated organisms.

724A *C. elegans* Can Learn To Associate a Temporally Precise Delivery of Paired Stimuli

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Associative learning allows animals to adapt to multiple environmental stimuli that occur proximally in space and time. How molecular and cellular interactions control the formation, maintenance and degradation of learned memories in precise spatiotemporal terms is not fully understood. *C. elegans* can be trained to associate multiple cues and exhibit learned locomotor responses. Short wavelength light is an aversive stimulus that triggers a *C. elegans* escape response (including reversals). We have developed a novel trial-by-trial associative conditioning paradigm for *C. elegans* that utilizes the pairing of a neutral odor stimulus and a noxious light stimulus. After training, worms learned to reverse to the once-neutral smell. This memory is short-term, with memory responses rapidly decreasing over minutes. The transient temporally precise nature of the stimuli delivery has allowed us to demonstrate that timed order and contiguity of stimuli presentation are critically required for the learning, both being features key to associative learning. In a standard classical-conditioning paradigm, the odor and light stimuli overlap in time. Notably, worms were even able to learn associations in a trace-conditioning procedure in which the presentations of the light and odor stimuli were separated in time. In

humans, trace but not standard classical-conditioning is thought to be associated with awareness of the stimulus contingencies.

Mutants defective in dopamine, glutamate, and octopamine exhibited defects in learning rates and efficiencies. Intriguingly, mutants defective in serotonin learned more rapidly and more consistently than wild-type worms. How the interplay of order of stimuli and neuromodulation produce an optimal adapted learning process that is sensitive to precision in stimulus timing is incompletely understood. By studying how these factors influence each other at a single-cell level resolution of analysis across a well-defined neural circuit, we hope to gain a detailed understanding of the molecular, cellular and circuit mechanisms that underlie learning processes.

725B The role of mitochondria calcium uniporter in *C. elegans* odor learning

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Calcium is essential for neuronal function – for activation, synaptic transmission, and also for modulating sensory adaptation and synaptic strength. Calcium concentration is carefully regulated in the cell by actively sequestering calcium into ER and mitochondria by transporters and calcium binding proteins. Mitochondrial calcium uniporter (MCU) is the transporter that permits calcium entry into the mitochondrial matrix, where, in addition to buffering cytosolic calcium, it regulates mitochondria-mediated cellular processes such as ATP synthesis and apoptosis. However, the role of MCU and mitochondrial calcium in neuronal function remains largely unknown. In this study, we sought to find the role of MCU and mitochondrial calcium in neurons in *C. elegans*, by using simple odor learning paradigms.

We found that, whereas wild type strain shows significantly decreased chemotaxis index in response to 60 min pre-exposure, *mcu-1* mutants show only a modest decrease. This was the case only for odors detected by the AWC sensory neuron, such as benzaldehyde and butanone. Restoring MCU-1 in all neurons and AWC resulted in normal adaptation, showing that MCU-1 in the sensory neuron is sufficient for adaptation. Using the heat shock inducible promoter, we found that mcu-1 is required during early larval development (egg or L1 stage), and restoring expression after L4 or adult stage did not improve odor learning. Surprisingly, we found that overexpression of mcu-1 in the AWC neuron resulted in delayed forgetting of the odor memory. This suggests that mcu-1 may regulate the plasticity of odor memory depending on its expression levels. We are currently investigating the downstream mechanism of our observations.

726C Neuropeptides regulate a novel *C. elegans* oviposition behavior displayed in a three-dimensional environment

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The *C. elegans* ecological niche is a complex and diverse three-dimensional environment found mostly in rotting fruit or soil compost. However, *C. elegans* behavior and physiology have mostly been studied in 2D culture in the lab. Previously, we designed a cultivation habitat which we term NGT-3D and NGB-3D in which growth, brood size, and lifespan in NGT-3D are comparable with 2D NGM plates. Using this 3D cultivation, we observed that the adult hermaphrodites spread the bacteria out from the center in a “nest” like pattern and remain at the edge of this nest. We then observed a novel stereotypical oviposition behavior in which the mother worms occasionally wander away from the bacterial nest, lay eggs, and then return to the bacteria. Using a candidate mutant analysis, we found that mutants defective in the neuropeptide PDF-1, as well as its cognate receptor PDFR-1 that regulates roaming behavior, were also defective in the novel 3D oviposition behavior laying eggs close to the bacteria. In addition, we identified mutants defective in the FMRF-like neuropeptide FLP-17 and its cognate receptor EGL-6 that together inhibit egg-laying were also defective in the novel 3D behavior. FLP-17 is expressed in the BAG sensory neuron, and the EGL-6 receptor expresses in the HSN neuron that regulates egg-laying. We showed that both *egl-46* mutants that lack the BAG neuron as well as *egl-1* mutants that lack the HSN neuron were also defective for the novel 3D oviposition behavior. To understand what cue may be causing mother worms to lay eggs away from OP50 in 3D, we found that 2D culture of *C. elegans* with a tiny “dot” colony of OP50 could elicit a similar egg-laying behavior in which the N2 mothers lay eggs far away from the bacteria. We found that low oxygen exacerbates the behavior, whereas high oxygen restores egg-laying in OP50. However, *flp-17* mutants displayed defects in the oxygen-dependent egg-laying behavior. Finally, we tested whether defects in 3D oviposition behavior could induce decreased reproductive fitness in 3D and showed that *flp-17* mutants have decreased brood sizes in 3D cultivation compared to 2D. Although low oxygen itself does not affect brood size, we found that cultivation

with a high density of OP50 may be toxic to the young. Overall, we speculate that the novel 3D oviposition behavior may be a maternal behavior that increases the mothers' reproductive fitness.

727A Stimulation of egg laying in *C. elegans* by *Salmonella* lipopolysaccharides (LPS) is dependent on a Gα protein expressed in chemosensory neurons

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Lipopolysaccharides (LPS) of *Salmonella enterica* serotype typhimurium stimulates egg laying in *C. elegans*N2 strain. Among the 23 G protein α subunits in *C. elegans*, we found that GPA-11 is a component in the signaling pathway, since the loss-of-function mutant did not respond to LPS stimulation while displaying wildtype stimulated egg-laying response to serotonin. We dissected the mutant hermaphrodites at the adult stage which are usually selected for egg-laying assays and found that they bore similar number of eggs to that of N2 worms. This confirms that the blunted response to LPS egg-laying stimulation in the mutants was not due to any limited number of eggs stored in the uterus, but was because of the absence of functional GPA-11 protein. The mutant was known to respond poorly to the repellent octanol but are wild-type for other traits studied. Therefore, our reported observation reveals another defect of the mutant. Besides, the defective egg-laying response to the bacterial toxin LPS is likely to be linked to innate immunity, so the involvement of the chemosensation-related GPA-11 that is expressed in the chemosensory ASHs and ADL neurons suggests that there is an interaction between chemosensation and innate immune responses. Moreover, GPA-11 is epistatic to TPH-1 involved in serotonin biosynthesis, but the G protein-coupled receptor with which GPA-11 interacts is yet to be identified. The egg-laying system responsive to LPS stimulation may provide insights into this complete signaling pathway. It is also noteworthy that GPA-11 in another *Caenorhabditis* species, *C. briggsae*, is dysfunctional because of an altered translation frame resulted from an insertion in the first exon. Future investigation is needed for understanding the possible dispensability of GPA-11 in innate immunity and/or chemosensation in the genus.

728B Mechanism for the munchies: endocannabinoid modulation of food preferences in *C. elegans.*

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The mammalian endocannabinoid system, comprised of the endocannabinoids AEA (N-arachidonoyl-ethanolamine) and 2-AG (2-Arachidonoylglycerol), their receptors, CB1 and CB2, and their metabolic enzymes, is thought to integrate internal energy state and sensory food cues to modulate feeding. For example, cannabinoids, acting on CB1, can increase preference for more palatable, calorically dense food: a response called *hedonic amplification*, colloquially known as "the munchies." In mammals, cannabinoids can increase sensitivity to odors and sweet tastes, which may underlie hedonic amplification. We are developing *C. elegans* as a model in which to investigate the neurogenetics of hedonic amplification. We have found that exposure to AEA, an endocannabinoid common to mammals and *C. elegans*, increases the worm's preference for strongly preferred (more palatable) bacteria over weakly preferred (less palatable) bacteria, mimicking hedonic amplification in mammals. Furthermore, AEA acts bidirectionally, increasing consumption of strongly preferred bacteria while decreasing consumption of weakly preferred bacteria. We also found that deletion of the CB1 ortholog, NPR-19, eliminates hedonic amplification, which can be rescued by expression of the human CB1 receptor, establishing a humanized worm for cannabinoid signaling studies.

Deletion of the olfactory neuron AWC, which directs chemotaxis to food, abolishes hedonic amplification. Consistent with this finding, calcium imaging revealed that AEA bidirectionally modulates AWC's activity, increasing its responses to strongly preferred food and decreasing its response for weakly preferred food. Furthermore, AEA's effect on AWC requires NPR-19. However, GFP expression analysis revealed that NPR-19 is expressed ~21 neuron classes but, surprisingly, not in AWC. Although AEA's effect could be mediated by NPR-19-expressing neurons presynaptic to AWC, nearly complete elimination of fast synaptic transmission, via a mutation in *unc-13*, has no effect on modulation. Instead, AEA's effect on AWC is mediated via *unc-31*-dependent dense-core vesicle release. We are now working to identify the NPR-19-expressing neurons that pass the cannabinoid signal down to AWC, thereby modulating chemosensation and leading to hedonic amplification.

729C Is activation of ASER neurons sufficient to generate state dependent learning?

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In state dependent learning (SDL), the internal state of an organism must be the same when performing a learned behavior as it was during acquisition of learning. An example of such a state is ethanol intoxication. We have demonstrated that

ethanol intoxication during learning can confer state-dependency to olfactory adaption (Bettinger & McIntire, 2004). Olfactory adaptation is when previous exposure to an odorant results in a diminished behavioral response to the odorant. Animals trained while sober can demonstrate that they recall olfactory adaptation in the presence of intoxicating doses of ethanol. However, animals trained while intoxicated only express adaptation if they are tested in the intoxicated state. These state dependent effects of ethanol require dopamine. Dopamine is not required for olfactory adaptation, indicating that there is a novel input to the adaptation circuit that confers state dependency. Our goal is to identify the neuronal circuitry and molecular mechanisms underlying SDL. We are using mutant analysis to identify other signals in SDL. HEN-1 is a secreted ligand that interacts with its receptor SCD-2; this pathway has previously been shown to be required for integration of sensory stimuli (Ishihara *et al*., 2002). We have found that *hen-1* and *scd-2* are required for SDL. We localized the requirement for *hen-1* in SDL to the ASER neuron; killing ASER eliminates SDL, and we could rescue state dependency in *hen-1* mutants by expressing *hen1* only in ASER. We rescued SDL in *scd-2* mutants by expressing *scd-2* only in AIA neurons, demonstrating that expression of *scd-2* in AIA neurons is sufficient for SDL. Together, these results suggest a model in which HEN-1 released from the ASER neuron signals the AIA neuron through SCD-2, and this signaling causes olfactory adaptation to become state dependent. We are now using an optogenetics approach to ask if simply activating the ASER neuron, in the absence of ethanol, can trigger state dependency. Our ultimate goal is to understand the circuit and the signals, which include dopamine and HEN-1, that produce state dependency for olfactory adaptation to better understand how alcohol affects the learning process.

730A Constructing a tool box for imaging and stimulating pharyngeal neurons to understand foraging behavior in *C. elegans*

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To understand the interplay between neural circuit structure, activity, and behavior requires the ability to observe the action of a set of neurons underlying that behavior while the animal is behaving in a well-known circuit. Work in other organisms, such as the lobster has already yielded insight into variability of neural activity, expression and yet remarkable stability in behavioral output. However, these studies often are limited to dissected preparations and behaviors inferred from muscle or motor-neuron activity.

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 calcium indicator GCaMP.

We will 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 (forming an AND gate), split cGAL, to achieve specific expression in targeted neuron(s). We then cross these drivers with their UAS effector strains, such as GFP, optogenetic activator/inhibitor and GCaMP, to achieve neuron-specific expression of fluorophores or other desired proteins.

To search for the promoters that drive unique expression in pharyngeal neurons, we have curated information from the literature and transcription databases. We will generate different “promoter::cGAL” driver lines and then verify the expression using the GFP effector lines and the neuroPAL multicolor Atlas. Successful candidates will then be crossed to other optogenetic and GCaMP effector lines for targeted neuronal manipulations and calcium imaging of foraging animals.

We will present our progress in creating such a toolbox. These collectively will pave the way to understand the neuronal basis of foraging behavior in *C. elegans*.

731B *C. elegans* chooses food exactly as if maximizing economic utility

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The well-functioning brain makes decisions that maintain and improve the animal's welfare. These decisions are based on subjective values assigned by the chooser to available goods and actions. However, the neural mechanisms of value assignments, and choices based on them, remain obscure. To investigate this problem, we used a classic measure of utility maximization, the Generalized Axiom of Revealed Preference, to quantify internal consistency of food preferences in *Caenorhabditis elegans*, a nematode worm with a nervous system of only 302 neurons. Using a novel combination of microfluidics and electrophysiology, we found that *C. elegans* food choices fulfill the necessary and sufficient conditions for utility maximization. Moreover, as in many other animals, preferences are learned, a process we found to require intact dopamine signaling. Preferences are expressed as a modulation of the worm›s normal foraging movements rather than feeding rate. Food-specific responses of identified chemosensory neurons known to direct foraging are strengthened by training, suggesting they may be part of the value-assignment system. The demonstration of utility maximization in an organism having a nervous system of only 302 neurons sets a new lower bound on the neuronal requirements for its execution, and offers the prospect of an essentially complete explanation of value-based decision making at single neuron resolution.

732C High-throughput EPG recordings reveal the food exploitation-exploration trade-off

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Whole-animal screens complement traditional screens based on cultured cells and single-celled organisms. In *C. elegans*, two of the most common whole-animals screens involve locomotion and pharyngeal pumping. Whereas high-throughput locomotion screens are in wide use, high-throughput pharyngeal screens are lacking. Electrophysiological measures of pharyngeal activity – *electropharyngeograms* (EPGs) – offer higher temporal resolution and signal to noise ratio than manual or optical methods. Recently, the ease and efficiency of EPGs was improved by placing individual worms in tight-fitting microchannels. Electrical resistance formed where the worm contacts the channel walls generates voltage differences sufficient to resolve individual pharyngeal action potentials. However, this approach is currently limited to only eight worms per recording.

To address this deficiency, we explored the utility of bulk EPG recordings made by increasing the length and width of the recording channel to accommodate hundreds or even thousands of worms. In bulk recordings, where worms are loosely arrayed in the channel and unrestrained, the voltage signal produced by each worm is insufficient to resolve individual action potentials. However, the composite voltage signal, which can be conceptualized as the sum of many individual, asynchronous EPGs, can be analyzed by computing its power spectrum. We found that EPG power spectra generally have just two peaks: a low frequency peak at 0-2 Hz, and a high frequency peak at 4-6 Hz. Using serotonin dose-response curves, optogenetic inhibition of pharyngeal muscles, and pharyngeal pumping mutants, we found that the high frequency peak reflects neurogenic pumping whereas the low frequency peak reflects myogenic pumping together with locomotion. As serotonin concentration is increased, the high and low frequency peaks are enlarged and diminished, respectively. We propose that the high frequency peak reflects the proportion of worms that are nearly stationary but feeding vigorously (*exploitation*), whereas the low frequency peak reflects the proportion of worms that are moving rapidly but feeding intermittently (*exploration*). Thus, the new method quantifies not only neurogenic pharyngeal pumping, but also the distribution of animals across these mutually exclusive behavioral states.

733A Male-specific Responses to State-dependent Hermaphrodite Signals Facilitate Mate Preference in *C. elegans*

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Natural habitats of free-living nematodes can be complex and changeable. For a mixed population to reproduce dynamically, *C. elegans* males must interpret complex mixtures of signals provided by animals with different sex states, developmental stages, fitness levels, or even dissimilar species. A preference for comparatively healthier mates likely provides fitness advantages. Chemical (e.g., sex pheromones) and physical cues (e.g., vulva-locating) have both been found to be important for mate recognition, but the relative importance of different signals is not well understood, nor is the way in which males integrate these signals to make corresponding decisions. To understand how males might assess and compare the sexual attractiveness of potential mates, we quantified mate preference using an assay that measures the relative attractiveness of distinct mates ("targets") to "tester" males.

Specifically, we found that males can distinguish hermaphrodite targets from male targets; they also recognize *daf-22* hermaphrodites as less favorable mates compared to wild type hermaphrodites. These findings confirmed the contribution of ascaroside pheromones in mate preference, for *daf-22* lacks ascarosides. We considered flexible nutritional states

altering fitness and attractiveness of mates and found that males prefer well-fed targets over nutrient-deprived targets in a *daf-22*-dependent manner. Using genetically sex-reversed animals, we found that the production of *daf-22*-dependent sex pheromones requires the hermaphrodite state of intestine. Moreover, non-ascaroside signals provided by adult hermaphrodites, likely germline-derived and/or contact-dependent, also contribute to mate preference, based on comparisons between hermaphrodite and male targets at different developmental stages in both wild type and *daf-22* backgrounds. In addition, cuticle O-glycans may mediate mate recognition, as males dislike *bus-2* hermaphrodites, in which O-glycan profiles are changed. Furthermore, mate preference behavior is regulated by the genetic sex of the nervous system, as pan-neural sex reversal alters preference patterns. These studies advance our understanding of male-specific neural mechanisms behind mate preference and the integration of multi-sensory signals.

735C Food Deprivation Induces Behavioral Changes that Require Metabolic Reprogramming and Non-Canonical Insulin Signaling

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Behavioral changes are easily attributable to external influences such as temperature, light exposure, and chemical stimuli. However, the effects of internal states such as infection status, stress, and hunger on animal behavior are often less obvious. We sought to determine how an internal state modifies animal behavior and define the pathways necessary to encode the behavioral change. Sensory integration is a conserved behavior in which an animal, or population of animals, must integrate attractive and repulsive signals simultaneously to decide whether to approach or avoid a cue. We conducted sensory integration assays in which populations of *C. elegans* are presented with an attractant, diacetyl, just beyond a repellant copper barrier. We show that acute food deprivation reversibly reduces copper sensitivity, allowing animals to engage in a "risky behavior": starved animals cross the toxic copper barrier to reach the attractant ~4 times more often than well-fed animals. Our results suggest that decreased copper sensitivity in food-deprived animals requires the transcription factors MondoA and HLH-30 within intestinal cells, which likely detect and respond to the lack of food. Others have shown HLH-30 translocation to intestinal nuclei is correlated with the expression of a few insulin-like peptides, many of which we show are required for the hunger-induced behavioral change. The insulin receptor DAF-2 is required to sense and respond to these insulin-like peptides. We demonstrate that expression of *daf-2* and downstream non-canonical insulin signaling molecules in the ASI chemosensory neurons sufficiently rescues this food deprivation-induced risk-taking behavior. Our work suggests that the internal state of hunger or food sensation links animal behavior to intestinal metabolism and neuronal function.

736A Vitamin B12 regulates chemosensory receptor gene expression via the MEF2 transcription factor

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Dynamic changes in gene expression is one strategy that animals can use to modify their behavior to dietary changes, but the molecular mechanisms underlying diet-dependent modulation of gene expression are unclear. We show here that the expression of the candidate *srh-234* chemoreceptor gene localized in a single ADL sensory neuron type of *C. elegans* is downregulated when animals are fed a *Comamonas* DA1877 bacterial diet relative to a low vitamin B12 diet of *E. coli* OP50 bacteria. This dietary effect of vitamin B12 on *srh-234* expression levels in ADL neurons is distinct from the starvation response we previously reported for *srh-234* regulation (Gruner, Grubbs et al, PLoS Genetics, 2014; and Gruner, Grubbs, McDonagh et al. PLoS Genetics, 2016). Remarkably, this diet-modulated effect on *srh-234* expression levels is dependent on vitamin B12 endogenously produced by *Comamonas*, because mutant strains of *Comamonas* bacteria that are deficient in vitamin B12 production fail to reduce expression levels of *srh-234* in ADL. Further analysis suggest that vitamin B12 likely does not act as an olfactory cue released by *Comamonas* bacteria, and does not depend on the modification or metabolism of *E. coli* bacteria to regulate *srh-234* expression. It is more likely that *E. coli* bacteria function as the vehicle for vitamin B12, because animals eating *E. coli* deficient in the *tonB* transporter shown to allow import of vitamin B12 from the extracellular environment suppress in part the vitamin B12-mediated reduction in *srh-234*. Mutations in the *C. elegans mrp-5*, a candidate vitamin B12 transporter, reduced but did not abolish *srh-234* expression in ADL in animals fed both *E. coli* and *Comamonas*, suggesting a yet unknown role for *mrp-5* in *srh-234* regulation. Propionate supplementation can bypass the repressing effects of vitamin B12 on *srh-234* regulation. Consistent with these findings, we found that mutations in *pcca-1* and *pccb-1* encoding for biosynthetic enzymes in the canonical propionate breakdown pathway suppressed, at least in part, the *Comamonas*-induced reduction in *srh-234* expression. We previously reported that the MEF-2 transcription factor acts together with bHLH factors to regulate the starvation-dependent modulation of *srh-234* expression. We found that mutations in *mef-2*, but not in *hlh-30*, are

necessary for regulation of *srh-234* mediated by vitamin B12. Together, our findings suggest a MEF-2-dependent mechanism by which diet-supplied vitamin B12 transcriptionally tunes individual chemoreceptor genes in a single sensory neuron type, which in turn may change an animals' responses to biologically relative metabolites in their diet.

737B C. elegans PEZO-1 is a Mechanosensitive Channel Involved in Food Sensation

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PIEZO channels are force sensors essential for physiological processes including baroreception and proprioception. The *Caenorhabditis elegans* genome encodes an ortholog gene of the *Piezo* family, *pezo-1*, expressed in several tissues including the pharynx. This myogenic pump is an essential component of the alimentary canal in most animals whose contraction and relaxation depends on mechanical stimulation elicited by food content. Whether *pezo-1* encodes a mechanosensitive channel and contributes to pharyngeal function remains unknown. Here, we leverage genetics, microfluidics, and electropharyngeogram recordings to establish that *pezo-1* is expressed in the pharynx, including a proprioceptive-like neuron, and regulates pharyngeal function. Knockout and gain-of-function mutants reveal that *pezo-1* is involved in fine-tuning pharyngeal pumping frequency, sensing osmolarity and food quality. Patch-clamp electrophysiology analyses in primary *C. elegans* embryo cultures demonstrate that *pezo-1* encodes a mechanosensitive channel. Our findings reveal a novel role for *pezo-1* in regulating food sensation in worms.

738C Reversal behavior upon encountering a cliff involves mechanosensation

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The precipice response is a little-understood phenomenon in which *C. elegans* move rapidly away from edges or gaps in an agar plate. First described by Chalfie et al. (2014), this behavior has yet to be characterized by the research community, and up until now potential underlying mechanisms remained unexplored. We hypothesized that mechanosensation underlies the precipice response and that mutant worms with deficient mechanosensation would exhibit the precipice response less frequently than N2 wild-type worms. Our experiments sought to 1) characterize the precipice response and craft a sufficient biological definition, and 2) test our hypothesis about mechanosensation's role in the precipice response.

N2 wild-type worms were used to define the precipice response. Using a wire or dog whisker pick, worms were transferred onto small chunks of agar with smooth, 90° edges. We observed that, indeed, worms had a strong tendency to quickly reverse when their head went over the edge of the agar. We defined the precipice response as a reversal initiated within two seconds of the head going over a 90° edge. This reversal must extend for a full sine wave within two seconds. In an initial dataset of 32 N2 worms, 26 of them (81%) exhibited the precipice response.

To test whether mechanosensation plays an important role in the precipice response, we tested three strains of worms with varying degrees of mechanosensory deficiency: *mec-3(e1338)*, *mec-10(e1515)*, and *trp-4(sy595)*. *mec-3* mutants do not respond to light or harsh touch, while *mec-10* and *trp-4* mutants exhibit partial loss of response to mechanical stimuli. In blinded trials, we found that *mec-3* mutants exhibited the precipice response less frequently than N2, *mec-10,* or *trp-4* worms. Neither *mec-10* nor *trp-4* exhibited the precipice response at a frequency that was significantly different from the N2 strain. These results show that mechanosensation underlies the precipice response, and imply that near total loss of mechanosensation may be necessary to have a profound impact on the precipice response. This study is an illuminating first step in discerning the mechanisms of the precipice response in *C. elegans*, and may have broader implications in animal behavior.

739A Study on molecular mechanisms of gait switching in *C. elegans*

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Animals exhibit multiple locomotion modes referred to as gaits and switch gaits depending upon external or internal conditions. However, molecular mechanisms underlying gait switching are not fully understood. The nematode *Caenorhabditis elegans* is a good model system that allows us to study gait switching because animals exhibit well-defined and flexible locomotive behaviors. For example, animals crawl on solid surfaces with low frequency and short wavelength (Karbowski et al., 2006) and swim under liquid with high frequency and long-wavelength (Korta et al., 2007; Pierce-Shimomura et al., 2008). To identify the molecular mechanisms of gait switching, we first performed a candidate mutant search. We found that

lim-4(ky403) mutants showed normal body posture during swimming but delayed crawl-to-swim onset time and reduced swimming frequency. *lim-4* encodes a LIM homeodomain protein and is expressed in several neurons, including SMB and SAA (Sagasti et al., 1999; Kim et al., 2015). To investigate the functional role of *lim-4*-expressed neurons in gait transition, we genetically ablated the SMB and SAA neurons and found that SMB but not SAA ablated worms showed defects in onset time and swimming frequency, suggesting that the SMB neurons play a role in gait transition. To identify additional genes that mediate gait switching, we performed EMS mutagenesis and isolated seven mutant alleles (*lsk56*, *57*, *58*, *59*, *66*, *67*, *68*) defective in onset time and/or swimming frequency. Specifically, *lsk58* and *lsk66* mutants showed normal onset time but slow swimming frequency. Moreover, *lsk57*, *lsk58*, and *lsk68* mutants showed defective body posture during swimming, whereas *lsk58* and *lsk59* showed decreased swimming speed. We are currently identifying molecular lesions of these mutants.

740B Tyramine influences associative learning outcomes and is linked to a novel learning phenotype in a purine biosynthesis mutant

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Adenylosuccinate lyase deficiency (ASLD; OMIM #103050) is a rare inborn error of purine metabolism disorder. Patients with ASLD have decreased activity of adenylosuccinate lyase (ADSL), which catalyzes two non-sequential steps in the biosynthesis of purines. ASLD is associated with degenerative motor deficits, seizures, mild-to-moderate cognitive impairment, autistic-like behavior, and seizures. We have revealed a novel learning phenotype in *C. elegans* animals with reduced adenylosuccinate lyase (ADSL-1) activity and are using the model to probe etiology of the neurobehavioral manifestations of ASLD.

Naïve control animals have a positive chemotactic response to NaCl but change their behavior after starvation (aversive cue) in the presence NaCl (positive cue). After cue-pairing, control animals become indifferent to NaCl in a chemotaxis assay (an associative learning phenotype). *adsl-1(RNAi)* animals retain the naïve chemotactic behavior of control animals, suggesting normal sensory function. However, *adsl-1(RNAi)* results in an altered learning behavior relative to control animals in response to the cue-pairing. Instead of indifference to NaCl, the *adsl-1(RNAi)* animals are robustly repelled by NaCl in response to the cue-pairing.

We have examined the role of product depletion in this behavioral phenotype via supplementation of cultures with purines and the role of enzyme substrate accumulation using small molecule drugs that inhibit early steps in purine biosynthesis. Both supplementation with adenosine and treatment with lomotrexol effectively revert the learning phenotype in *adsl-1(RNAi),* suggesting that both effects of reduced ADSL-1 activity are required to manifest the learning phenotype.

We used LC-MS to look for clues in the metabolome that might point to how behavior is altered and found that tyrosine levels are elevated in *adsl-1(RNAi)* animals. Because tyramine and octopamine are produced from tyrosine, we used genetic and pharmacological approaches to probe the role of these neurotransmitter/neuromodulators in associative learning. *tdc-1* mutants, which cannot synthesis tyramine, phenocopy *adsl-1(RNAi)*, but *tbh-1* mutants, which produce tyramine but not octopamine, do not. Additionally, supplementation of *adsl-1(RNAi)* animals with tyramine reverts the learning phenotype, suggesting that the behavioral phenotype of *adsl-1(RNAi)* is caused by a lack of tyramine production. Tyramine is produced by only a few cell types in *C. elegans*, including the RIM neuron that coordinates reversals during locomotion, providing a link to the behavioral output. We have revealed a novel learning phenotype associated with a purine biosynthesis mutant and are elucidating the metabolic changes in response to *adsl-1* depletion that will impact on the downstream effectors identified as important in mediating the behavioral deficiencies.

741C Decision-making in *C. elegans: Neuronal mechanisms underlying behavioral choice*

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Animals constantly weigh opposing sensory cues to decide and execute appropriate behavioral responses as they navigate their environment. These decisions and their subsequent behaviors demonstrate the nervous system's ability to integrate and process heterogenous sensory information (i.e. risk or reward) into proper decision making neural correlates. We test a value-based decision-making model using responses to pheromones. We discovered that the pheromone, osas#9, which incorporates the neurotransmitter octopamine is released exclusively by starved first larval stage (L1) animals to elicit a strong avoidance response in conspecifics (Chute et al., 2019). This avoidance response is strongly dependent on the physiological state of the animal and is attenuated by the concurrent detection of food odor(s). This suggests that the possibility of nearby food sources can override an innate avoidance to the starvation cue, osas#9. The interplay between opposing olfactory sensory signals provides a strong paradigm for studying decision-making in the context of multisensory integration. Additionally, we

have observed disrupted decision-making in a neurodegenerative model of worms that expresses the $A\beta_{42}$ peptide. We will present data that elucidate the signaling networks necessary for mediating attenuation to osas#9 allowing us to deconstruct the neural circuits controlling decisions in both healthy and disease states of the worm.

742A Electrophysiological properties of amphid sensory neurons in *C. elegans*

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Since the lack of voltage-gated sodium channels in *C. elegans* neurons, it has long been believed that *C. elegans* neurons transmit electrical signals by passive propagation. However, we have recently reported regenerative depolarization in a major gustatory sensory neuron, ASEL, upon stimulation with an increase of environmental NaCl concentration. This all-or-none membrane depolarization may set the threshold for the sensory neuron to respond significant environmental changes. To test this possibility, therefore, we examined electrophysiological properties of other amphid sensory neurons. Membrane depolarization of amphid sensory neurons were recorded upon current injection by whole-cell patch-clamp techniques. All 14 types of amphid neurons, which consist of 10 pairs of symmetrical neurons and two pairs of asymmetrical neurons, AWCon/AWCoff and ASEL/ASER, showed supralinear depolarization upon current injection. In this meeting, we will report membrane depolarization of several amphid neurons upon puff stimulation of natural substances.

743B UNC-7/Innexin Regulates Transmission of Temperature Information during *C. elegans* Thermotaxis

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Innexins are transmembrane proteins, and form gap junction channels and non-junctional hemichannel. Innexins are known to play fundamental roles in the information processing in the nervous system. We previously showed that INX-4, a member of innexin family, is required for thermotaxis and functions in the AFD thermosensory neuron. Since the thermotaxis abnormality of *inx-4* mutants was very mild, we hypothesized that other innexins might also function to regulate thermotaxis. To test this hypothesis, we examined the thermotaxis behaviors of most of the innexin gene mutants present in the *C. elegans* genome and also assessed whether overexpression of the innexin genes in AFD affects thermotaxis. Our genome-wide survey revealed that UNC-7/Innexin is important for regulation of thermotaxis; overexpression of UNC-7 in AFD caused a thermophilic defect. We also generated strains lacking *unc-7* only in AFD using Cre/*loxP* system and found that *unc-7(AFD KO)* animals displayed a cryophilic defect. These results indicate that UNC-7 functions in AFD to regulate thermotaxis.

To identify the critical period of *unc-7*, we utilized the auxin-inducible degron system and found that the activity of *unc-7* in AFD is required later than the larval stage, suggesting that *unc-7* acts at the stage after the development of AFD is completed. Calcium imaging revealed that *unc-7* mutations did not affect temperature-evoked calcium response in AFD, indicating that UNC-7 functions in the downstream of calcium influx. We also investigated whether UNC-7 functioned as gap junctions or hemichannels. Previous study indicated that the UNC-7(Cysless), in which four cysteines in the extracellular loops are substituted to alanine, cannot form gap junction while maintaining the hemichannel activity. We therefore asked whether UNC-7(Cysless) can rescue the thermotaxis defect of *unc-7(AFD KO)* animals. While the expression of wild-type UNC-7 only in AFD rescued thermotaxis defect of *unc-7(AFD KO)* animals, UNC-7(Cysless) did not. This result suggests that UNC-7 regulates thermotaxis by functioning as gap junctions.

Our results suggest that UNC-7 functions as gap junctions to transmit temperature information from AFD to another neuron. We are currently identifying the gap junction partner innexin and neuron of UNC-7 in AFD. Through our research, we hope to understand the role of UNC-7-mediated gap junction in thermotaxis and reveal the dynamics of the gating of gap junctions during animal behavior.

744C Solute Carrier family 46 and aquarius intron-binding spliceosomal factor mediates temperature tolerance

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We are studying mechanisms for animal temperature response by utilizing temperature tolerance of nematode *C. elegans*. *C. elegans* can survive at 2℃ after grown at 15℃, while animals cultivated at 20℃ can not survive at 2℃. This cold tolerance is regulated by tissue networks containing neurons, intestine and sperm (Ohta et al., *Nat commun*, 2014; Okahata et al., *Science Advances*, 2019), in which temperature is received by DEG/ENaC, TRP and unidentified G protein-coupled receptor

in sensoryneurons (Takagaki et al., *EMBO rep.*, 2020; Ohnishi et al., *Scientific rep.*, 2020). To identify new molecules of cold tolerance, we introduced three approaches as follows.

(1) We decoded genome of a new cold tolerance mutant KHR018 by NGS, and found the responsible gene *slcr-46.1* that encodes a homologue of a human amino acid transporter on the lysosomal membrane called human SLC46A3 (SoLute Carrier family 46 member 3). *slcr-46.1* gene was expressed in neuron, intestine, cuticle and muscle cells. *slcr-46.1* knock out mutant showed defective cold tolerance, which was rescued by expressing wild-type *slcr-46.1cDNA* in neuron and muscles. Human SLC46A3 is known to be involved in catabolic metabolite transport that excretes the antitumor agent maytansine from lysosomes, but other functions remain unclear. Our future study will provide novel insights into SLC46-mediated biological reactions.

(2) We isolated two novel mutants that were isolated as background mutations of two known mutants. By deep DNA sequencing, one of their responsible mutations is narrowed down to 17 candidate genes. We are trying to measure cold tolerance of these gene' mutants.

(3) Temperature tolerance is essential for not only animal but also plant. By studying natural variations of Arabidopsis, a variation of splicing factor is found to be involved in heat tolerance. We found that the mutant animal of its *C. elegans* homologue gene AQR (aquarius intron-binding spliceosomal factor) exhibited defective heat tolerance. Interestingly, *C. elegans* AQR mutant also showed supra-normal cold tolerance. These suggest that AQR plays an opposite positive and negative regulatory role in heat and cold tolerance respectively. We are trying to identify downstream molecules of AQR by using transcriptome analysis.

745A acute exposure to thallium acetate results in behavioral changes, activation of the stress response and accumulation of metals in the model *Caenorhabditis elegans*

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thallium is a rare metal used in the electronics industry. its toxic effects have already been observed in humans and the nematode c*aenorhabditis elegans* (c*. elegans*). this study evaluated the effects of thallium acetate on the behavior of c*. elegans* and antioxidant response. adult worms wee synchronized and remain in m9 buffer for approximately 24 h, to obtain worms only in l1 stage larval. the worms l1 were exposed to thallium acetate in concentrations of 50, 100, 150, 200, 250, 500, and 1000 µm for 1 h. after that, worms were transferred to new ngm plates with bacteria and allowed until the l4 larval stage. animals were tested for survival, 24 h next to the exposure, and the pharyngeal pumping and the defecation cycles, in l4 stage. the larval development was monitored from l1 larval stage until adulthood and body bend assays were performed in individual worms, during 20 s, into a plate without bacteria. after one min of adaptation, worms were scored for the number of body bends performed in 20 s. the reproductive capacity of the animals was also evaluated, by counting eggs from individual worms. the subcellular localization of daf-16 and expression of sod in stress response were evaluated in a fluorescence microscope. the amount of Tl^+ was determined in l1 stage worms using icp-ms. the worms were survival affected after 100 µm to 1000 µm exposure and absence or reduction of contractions in the pharyngeal muscles was observed in worms treated at 1000 µm. acute exposure to thallium acetate altered the peristaltic movement in the intestinal tract of worms of 500 to 1000 µm. the *c. elegans* exposed remained paralyzed for a few minutes after the treatment, which could positively impact the developmental delay. the results showed a significant increase in body movement. there was a significant reduction in egg-production at concentrations above 100 µm, suggesting that thallium acetate also affects the worm's reproductive system. in the l1 larval stage worms, there was a significant activation of the antioxidant pathway, evidenced by the translocation of daf-16 from the cytosol to the nuclear region, but in the l4 larval stage, there was no translocation. sod-3 expression was only evidenced in the exposed worms l4 stage, indicates an activation of the antioxidant response to thallium acetate. the Tl^+ body load increased in a dose-dependent manner, after the development of the worm, part of the Tl^+ was not excreted, bioaccumulating in the body at lower levels. in this study, we verified new information concerning the toxicity induced by thallium acetate, using c*. elegans*. we recognize that additional studies are needed to clarify the mechanisms underlying the adverse effects of thallium, and the possible neural damage caused.

746B Identification of Neuropeptides Accelerating Forgetting in *C. elegans* with A Reverse Genetic Approach

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Animals acquire and store information as memories that are required for 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 *Caenorhabditis elegans* (*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. Previously, our studies showed that TIR-1/JNK-1 pathway in AWC sensory neurons accelerates forgetting of olfactory memory through releasing of "forgetting signals". However, the molecular basis of "forgetting signals" remains elusive.

In this study, we identified a substance of "forgetting signals" through a reverse genetic approach. Firstly, to check the possibility that neuropeptides work as "forgetting signals", we analyzed forgetting phenotypes in mutants of neuropeptide processing enzymes, and found that mutants of various neuropeptide-processing enzymes showed forgetting defect, suggested that neuropeptides might be responsible for "forgetting signals".

Furthermore, to identify the neuropeptides that serve as "forgetting signals" secreted from AWC sensory neurons, we searched for candidate genes by using a database of single-cell RNA sequencing, CeNGEN (*C. elegans* Neuronal Gene Expression Network), and identified 12 candidate neuropeptide genes. By genome editing using CRISPR-Cas9, we created these mutants and analyzed their forgetting phenotype. Among these candidates, mutants of one neuropeptide gene showed forgetting defect. Moreover, injection of the genomic fragments derived from wild type could recover the forgetting phenotype in these mutants. These results suggest that this neuropeptide is responsible for accelerating forgetting. Further analyses of its function will reveal how memory forgetting are regulated through signaling pathways including "forgetting signals" and its receptors.

747C Individual behavioral differences in *C. elegans*

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C. elegans is attracted or repelled by many odorants. The strength of the response varies depending on the odorant and its concentration. However, the response is not 100% even for a strong odorant. For example, isoamyl alcohol, which is one of the strongest attractants for *C. elegans*, attracts more than 90% of the worms but does not about the remaining 10% in the typical experimental settings. Besides, individual worms behave differently even when they have the same genetic background. It remains unknown in detail how such individual differences in behaviors are created. This study aims to elucidate the unknown mechanisms that make individual behavioral differences in a "homogeneous" group.

To quantitatively analyze individual worms' behavior, we developed a system that records worms' behaviors in a 6 cm plate every 0.04 s by using a digital 4K single-lens reflex camera and quantitates the behaviors by using DeepLabCut, a deep learning-based animal tracking software tool. As a first trial, we examined chemotaxis behaviors for isoamyl alcohol by using this system. Our preliminary result showed that the majority of worms (~95%) migrated to the attractant, but a few of them (<5%) were not attracted and continued to move over a wide area in the plate.

We plan to investigate the mechanisms that make such individual behavioral differences by combining data-driven and experimental analyses.

748A Characterizing the Role of the Mechanosensitive Ion Channel TACAN in *C. elegans* Osmosensation

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Mechanotransduction occurs via ion channels whose gating is controlled by mechanical stimuli. Recently, the transmembrane protein TACAN was identified as a mechanosensitive ion channel crucial for sensing mechanical pain in mice, and TACAN homologs were shown to be highly conserved across other species such as humans and nematodes. The nematode *C. elegans* is an ideal model organism to study the molecular properties of TACAN, given its mapped connectome, simple behavior, and capacity for genetic manipulations. Our preliminary data suggest that the uncharacterized TACAN homolog in *C. elegans* is involved in worm mechanosensation, specifically contributing to the detection of osmotic stimuli.

749B Mechanosensory behaviors associated with host seeking and host infectivity in the skin-penetrating nematode *Strongyloides ratti*

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Skin-penetrating parasitic nematodes, such as the threadworm *Strongyloides stercoralis*, infect approximately 600 million people worldwide. During the infective life stage, *Strongyloides* infective larvae (iL3s) engage in behaviors that increase the likelihood of contact with a host. Upon contact, iL3s penetrate the skin of the host and develop into parasitic adults that colonize the gastrointestinal tract. How *Strongyloides* iL3s sense mechanical cues and the extent to which mechanosensation enables both location and infection of a host are not yet understood.

To characterize the role of mechanosensation in location of a host, we first studied the response of the parasitic nematode *S. ratti* to mechanical cues such as vibration and touch. In preliminary experiments, *S. ratti* iL3s moved toward applied 50 Hz vibrations. In contrast, *S. ratti* free-living adults moved away from vibrations of the same frequency. Additionally, *S. ratti* iL3s reacted less frequently to gentle touch along the body and harsh touch at the nose relative to free-living adults.

To examine how mechanosensation enables infection of a host, we developed an assay to study skin penetration *in vivo*. We observed that iL3s penetrate into 1% low-melt agarose media, whereas free-living adults do not. We are now characterizing the behaviors of iL3s that are penetrating rat skin.

We have also identified *S. ratti* homologs of several *C. elegans* genes that encode mechanoreceptors. We are now generating reporter constructs to examine the expression patterns of these genes, and to identify and label putative mechanosensory neurons in *S. ratti*. To study how the neurons sense and respond to mechanical stimuli, we will use a combination of calcium imaging and neuronal silencing. Additionally, we will disrupt candidate genes using CRISPR/Cas9-mediated targeted mutagenesis, and then compare the behaviors of mutant and wild-type iL3s to identify genes and signaling pathways required for host-seeking and host-invasion behaviors. Together, these experiments will provide insight into the neural and molecular mechanisms that drive mechanosensory behaviors in skin-penetrating nematodes.

750C Integration of neuronal connectivity, activity and synaptic plasticity drives sexually dimorphic learning in *C. elegans*

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Sexually dimorphic traits stemming from distinct processing of environmental cues bring about the holistic survival of the species and are mostly driven by sexual selection. However, the underlying mechanisms driving sex-dependent complex decisions remain largely unknown.

Previous studies have shown that *C. elegans* hermaphrodites are able to shift their preference from attraction to aversiveness to a specific pathogenic bacterium *Pseudomonas aeruginosa* (PA14), after certain hours of exposure (Zhang et al, 2005). Using a modified version of this learned avoidance behavior, we show that *C. elegans* makes sexually dimorphic choices based on short past experiences. The hermaphrodites learn efficiently, as was previously shown. However, males do not learn to avoid PA14, even after training, thus retaining their initial preference. Using a blind testing paradigm, we show that larval stages of both sexes are unable to shift the preference, suggesting that maturation of nervous system is necessary for short term experience-based plasticity. By performing fast killing assays, we show that the lethality kinetics toward PA14 toxins is similar in the sexes, thus ruling out dimorphic susceptibility. To understand tissue specificity in developing a learned response, tissue specific sex-reversal experiments were performed and the importance of the nervous system was unraveled. In the same vein, we show that short term exposure does not involve signaling from the gut. Previous work has shown the importance of the interneuron AIY within several learning circuits (Ha et al, 2010; Jin et al, 2016). By analyzing AIY synaptic activity using fluorescently labelled RAB-3, we show that, upon short-term exposure, there is a decrease in synaptic activity, specifically, in an axonal region that forms connections with the interneuron RIA (Colón-Ramos et al, 2007). While this trend was prevalent in both the sexes, close examination of Ca^{2+} fluxes in the same region, using calcium indicators, reveal dimorphic responses to the odor of PA14. These results suggest that downstream encoding of short-term learning is sex-dependent and thus, its neuronal connectivity.

To summarize, we present a model in which dimorphic processing of environmental cues, in a sex-specific manner, evokes diverse behavioral phenotypes that help shape the fitness of the species.

751A Investigating DNA damage during associative learning in the nematode C. elegans

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Learning allows an organism to modify its future behavior as a result of past experience in ways that promote survival and reproduction. For learning to occur, a past experience must be encoded by changes at the molecular, cellular, or circuit levels. Over years of work in multiple organisms a variety of mechanisms for storing experiential information have been elucidated. These include shorter term transcriptional or translational responses as well as longer term synaptic remodeling and alterations to neuronal connectivity. Interestingly, recent evidence suggests that neuronal activity can also lead to changes in DNA through the formation of double strand breaks (DSBs) (Madabhushi et al., 2015). In some cases, these DSBs occur in targeted locations that facilitate the expression of neuronal genes involved in learning and memory (Madabhushi et al., 2015). However, the induction of DNA damage during a learning paradigm in a freely behaving animal has not been investigated, nor the consequences of damage on future neuronal activity. We propose to monitor the occurrence of DNA damage during an associative learning paradigm in the nematode C. elegans whose compact, fully mapped nervous system makes it ideal for the dissection of molecular and cellular components of learning. In this inquiry we will make use of an NRDE-2 mutant strain which we previously found shows defects in associative learning as well as an accumulation of DSBs under stressful growth conditions. We will ask whether the role of NRDE-2 in preventing or repairing DNA damage is related to its requirement for optimal associative learning or whether DNA damage is increased in neurons after learning in the absence of functional NRDE-2. Through these studies we hope to determine whether modifications to DNA can serve a role in encoding learned behavior.

752B Long-term behavioural imaging for characterizing the dauer exit decision

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Upon unfavourable conditions during its early development, the nematode *C. elegans* can develop into a dauer diapause lava, an alternative developmental stage geared for survival, characterized by distinct morphology and behaviour. Once conditions sufficiently improve, larvae actively reverse dauer-specific behavioural and morphological traits and resume reproductive development. Therefore, the decision to exit the dauer stage is an important moment in the worm's life history that has to be tightly controlled and robustly encoded. However, the temporal dynamics of behavioural adaption during dauer exit as well as corresponding changes in neuronal activity remain unknown. Furthermore, how different environmental factors influence the decision's temporal dynamics is unclear. To fill this gap, we aimed at characterizing the dauer exit decision, focusing on the behavioural change for quantitatively describing the decision process. We designed and implemented the WormObserver, a new end-to-end solution for long-term behavioural tracking of thousands of *C. elegans* larvae over several hours that allows long term observation of dauer but also other larvae stages and is compatible with a standard stereoscope. We were able to identify the timing of key events during dauer exit, such as the onset of growth and, more specifically, how the timing of these events changes under different environmental conditions (e.g. presence vs. absence of bacteria). Moreover, using automated image analysis and machine learning, we have established dauer specific behavioural motifs as well as how and when these motifs change during the course of dauer exit. By performing our behavioural analysis in the context of different exit triggers, we could show that ingestion of food is required for inducing a behavioural motif change during dauer exit. Ultimately, precise characterization of the dauer exit decision process will improve our understanding of how robust integration of environmental information can encode a developmental decision in a multicellular organism.

753C Principles for coding associative memories in a compact neural network

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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 the four types of olfactory associative memories (short- and long-term memories, each as positive and negative associations) are encoded within the compact neural network of *C. elegans* worms. By combining these robust training paradigms with fast confocal calcium imaging using multi-neuron and whole-brain imaging approaches, we systematically traced experience-dependent activity changes down to the level of individual neurites. Interestingly, short-term, but not long-term, memory broadly altered memory-evoked responses of chemosensory neurons. Modulated activity in three neurons, namely AWA, AWC, and ASE, sufficed to discriminate between the different memory states. This economy in memory-coding neurons increases memory capacity and limits non-innate behavioral responses. In contrast, long-term

memory was relegated to deeper layers of the network. Primary interneurons, AIY and AIA, exhibit memory-state-dependent activation signatures, allowing the sensory system to resume innate functionality. In conclusion, olfactory associative memory appears to follow a hierarchical and temporally structured encoding logic.

754A Exploring natural genetic variation influencing ethanol response behaviors.

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The propensity to develop alcohol use disorder is strongly influenced by genetics, but it has been difficult to identify allelic variation in humans underlying abuse liability. Natural allelic variation in the human population is likely to largely consist of mild change-of-function alleles of important genes that contribute to the physiological effects of ethanol. Only certain genes and pathways can be modified to affect alcohol response phenotypes, and only certain types of alleles are likely to be maintained in a natural population. Studies of laboratory-induced mutations in animal models have yielded a good understanding of the neuropharmacology of ethanol, but much remains to be learned about relevant natural allelic variation that impacts alcohol use phenotypes in humans.

We aim to advance the understanding of the natural allelic variation that exists and the pathways that can be modulated in wild populations to modify the neurobiological response to alcohol in wild isolates of *C. elegans*. We examined four French wild strains (JU1511, JU1931, JU1941, JU1926), of which two were isolated from a fruit (apple) and two from a plant (hogweed), for three acute ethanol response behaviors. We tested low dose locomotor activation, high dose initial sensitivity, and high dose induction of acute functional tolerance. We found that there is substantial phenotypic variation between the four strains for these phenotypes, strongly suggesting that there is natural allelic variation that modulates acute ethanol responses. To identify the causal variants, we are performing quantitative trait locus mapping using a panel of approximately 200 recombinant inbred lines (RILs) derived from these four wild parent strains. The phenotypes vary continuously among the RILs tested. We have found transgressive RILs for each of the three behavioral phenotypes, indicating that there are multiple alleles in the population that can assort independently. Finally, at least some of the alleles influence specific aspects of the behavioral response to ethanol, because there is low correlation between the phenotypes in the RILs.

Together, our observations indicate that there is extensive natural genetic variation underlying independent aspects of the ethanol response behaviors in *C. elegans*. We will map these to loci and aim to confirm causal alleles using genome editing.

755B Male Locomotor Responses to Ascaroside Sex Pheromones

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In order to adapt to an ever-changing environment, animals must constantly monitor their surroundings in order to adjust their behavior accordingly. One such stimulus that has been shown to play a large role in influencing many animal behaviors is pheromones. Pheromones can be defined as chemicals secreted by an animal that trigger some sort of a response from other members of the same species. In *C. elegans,* the ascaroside pheromones, particularly ascaroside #3 (ascr#3), have sex-specific ways of influencing behavior; males exhibit attraction to ascr#3 while hermaphrodites exhibit a slight repulsion to ascr#3. Furthermore, males have been found to exhibit both a greater number of reversals on ascr#3 as well as spend a greater amount of time on areas treated with ascr#3. However, the specific locomotor behavior that males display when detecting ascr#3 remains poorly characterized. More generally, patterns of locomotor behavior in males have not been well characterized. On a patch of food, hermaphrodites stochastically switch between two locomotor states, roaming and dwelling. Male locomotor states, on the other hand, appear to differ from hermaphrodites; previous work has suggested that males may have a distinct framework for these states. To investigate these issues, we are examining the locomotor behavior of males with or without ascr#3 present. By recording behavior and analyzing patterns over multiple timescales, we will compare the architecture of male motor behavior to that of hermaphrodites and understand how it is influenced by pheromone cues. Our results will further elucidate how genetic sex is able to influence behavior and thus give further insight into the role genetics plays in the development and function of neural circuits.

756C Identifying the GPCRs involved in detecting valproic acid, an anticonvulsant and mood-stabilizing drug, by using *C. elegans* as a chemosensor

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Valproic acid (VPA) is a short-chain fatty acid derived from the medicinal plant *Valeriana officinalis*. 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 mechanism of action is not known. Furthermore, prenatal exposure to VPA is associated with birth defects, cognitive deficits, and an increased risk of autism. Thus, a better understanding as to how VPA exerts its therapeutic effects may provide insights needed to develop better therapeutics to promote mental health. To identify the molecular targets of VPA, we are using *C. elegans* as a chemosensor because of its defined nervous system, well-characterized chemosensation behaviors, and conserved signaling pathways. In chemotaxis assays, we found that *C. elegans* are attracted to VPA and this behavioral response is eliminated in animals lacking the AWC chemosensory neurons. Given that chemosensory transduction in these cells commonly depends on G protein-coupled receptors (GPCRs), we determined how VPA attraction is affected in G protein mutants. In this way, we discovered that attraction to VPA is likely to be mediated by a GPCR(s), since *egl-30* Gα protein mutants are indifferent to VPA. Indifference towards VPA, in this case, suggests that in the absence of functional *egl-30*, upon activation, the GPCR(s) is unable to transduce an intracellular response that eventually leads to attraction. To determine the identity of the GPCR(s) involved, we used the *C. elegans* Neuronal Gene Expression Map and Network (CeNGEN), a single-cell RNA seq database, to generate a list of 85 candidate GPCRs expressed in the AWC neurons. By conducting chemotaxis assays with global null mutants of candidate GPCR genes, we found that *frpr-2* null mutants, but not those affecting other GPCRs we tested, are indifferent to VPA. Thus, FRPR-2 is a putative receptor for VPA in *C. elegans*. To test this hypothesis, we will knock out and rescue FRPR-2 specifically in the AWC neurons and express FRPR-2 in heterologous cells.

757A The conserved transcription factor *mef-2* regulates sickness induced sleep

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Sleep disruption is a common feature of neurodevelopmental disorders. As such, gaining a detailed mechanistic understanding of sleep circuit development will help elucidate causative factors in neurodevelopmental sleep disorders. Previous work has illuminated aspects of the development of sleep controlling neurons ALA (van Buskirk and Sternberg ‘10) and RIS (Turek et al ‘13), but much is yet to be discovered regarding the larger sleep circuit and how sleep signals are interpreted by the body. *C. elegans* experience stress or sickness induced sleep (SIS) in response to infection, radiation treatment, heat shock, or osmotic stress. During SIS, the ALA neuron is activated by Epidermal Growth Factor (EGF) to release the sleep-inducing neuropeptides FLP-13, FLP-24, and NLP-8. Differential effects of these peptides on feeding and movement quiescence suggest that parallel pathways regulate different aspects of quiescence during sleep (Nath et al, ‘16). Myocyte Enhancer Factor 2 (MEF-2) is a transcription factor that regulates the expression of genes involved in development of both muscles and neurons. In *C. elegans*, *mef-2* regulates muscle to neuron signaling at the neuromuscular junction (Simon et al, ’08). We find that animals lacking *mef-2* function are defective in movement but not feeding quiescence during SIS. *Mef-2* mutants are resistant to EGF but not FLP-13 overexpression induced movement quiescence, suggesting that *mef-2* functions in the development of the ALA neuron. Surprisingly, we find that muscle (*myo-3* promoter) but not neural (*rab-3* promoter) *mef-2* expression rescued the SIS defects in these animals. Our working hypothesis is that *mef-2* regulates the development of neuronal sleep circuitry by acting in muscle cells.

758B Identifying genes that contribute to social defects in autism using wild-isolate *C. elegans*

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder often characterized by significant problems with social skills. Human genetic studies have associated ASD with mutations in over 200 genes; however, it is unknown which ASD candidate genes are causal versus coincidentally associated with social deficits. Testing for causality of ASD-related phenotypes in such a large list of genes is challenging, especially when one considers that expressivity of ASD phenotypes may depend on mutations across multiple genes. We sought to leverage the convenient behavioral genetics of *Caenorhabditis elegans* to test for social deficits in orthologs of ASD-associated genes. *C. elegans* displays social behaviors through social feeding and clumping. The common wild-type lab strain N2 displays weak social behavior due to a background mutation in the neuropeptide-Y-like receptor, *npr-1*, but most natural wild strains are strongly social. We evaluated levels of social behaviors of 24 wild strains that harbor unique, naturally occurring variants predicted to alter function of conserved ASD candidate genes. We discovered that strains with predicted change-of-function mutations in ASD- implicated genes demonstrated significantly lower levels of social behavior compared to control social strains without these mutations. These genes include orthologs of human genes *BCL11A*, *PDCD2*, *SHANK3*, *STX1A*, *NLGN1*, *IL-17*, *IL17R*, *CHD2*, and *SETD5.* In addition to finding strains with dual

defects in bordering and clumping, intriguingly, we identified strains that exhibited selective defects in one of the two social behaviors suggesting that they are genetically separable. We also analyzed the evolutionary conservation of ASD-implicated genes to characterize critical regions for naturally occurring variations. Identification of genes and their critical regions that may predict ASD phenotypes when mutated could help medical professionals and genetic counselors identify at-risk families, while also providing useful insight to explain causality in the biological foundations of ASD-related phenotypes.

759C Identification of calcium/calmodulin-dependent protein kinase I (CMK-1) phospho-targets relevant for nociceptive plasticity in *C. elegans*

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Nociception is a conserved process serving as a self-protection system alerting animals of potential damage and underpinning different forms of pain in humans. Some chronic pain conditions may arise from maladaptive modulation in the nociceptive pathway, including within nociceptors, the primary nociceptive sensory neurons. 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 adapt to repeated stimuli causing a desensitized, analgesia-like state. The worm ortholog of mammalian CaMKI/IV, CMK-1 (calcium/calmodulin-dependent kinase-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.

Here, we performed *in vitro* CMK-1 kinase assays on both peptide and protein from total worm isolates in order to identify direct kinase target candidates via shotgun phosphoproteomics. For *in vivo* direct/indirect target determination, we carried out stable isotope labeling by amino acids (SILAC) using high-throughput quantitative phosphoproteomics. We used duplex SILAC, where ''light'' 12-carbon and ''heavy'' 13-carbon amino acids were incorporated into nematode proteins for measuring the amounts of phosphorylated proteins in wild type and *cmk-1* null animals. By combining results obtained from these different studies, we were able to ascertain CMK-1 phosphorylation consensus and develop a list of potential CMK-1 targets. Mutants for these candidates were then tested for heat avoidance behavior to determine changes in naive sensitivity to noxious heat and/or adaptation. We used a computer-assisted high-throughput analysis pipeline to quantify heat-evoked reversals in naive animals and animals exposed to repeated stimuli. While wild type animal sensitivity decreased in response to the repeated heat stimuli, some mutant animals failed to adapt. In conclusion, our study reveals several potential CMK-1 targets that may have an important role in behavioral plasticity.

760A Glucose impacts HSN morphology and induces an egg-laying defective phenotype dependent of the serotonin-signaling pathway

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Glucose impacts HSN morphology and induces an egg-laying defective phenotype dependent of the serotonin-signaling pathway

Manuel Axel Ruiz and Pamela Padilla

Peripheral neuropathy is a common and often painful complication that results from a chronic state of hyperglycemia. In recent years, research has shown that in humans altered levels of serotonin is highly associated to a chronic state of hyperglycemia. Additionally, altered levels of serotonin are associated with various neurological disorders. However, the mechanism by which hyperglycemia impacts serotonin processes is not understood. Here we are using the genetic model system *Caenorhabditis elegans* as a hyperglycemia model to further understand the impact of a glucose-supplemented diet on the animal's egg-laying behavior. *C. elegans* possess a nervous system that is composed of 302 neurons. In *C. elegans* the Hermaphrodite-Specific serotonergic motor Neurons (HSN) play an essential role in regulating egg-laying. Using this model organism, we conducted genetic and cell biological analysis to examine the role a glucose-supplemented diet has on the serotonergic HSN motor neurons. Our preliminary data shows that a glucose-supplemented diet impacts egg-laying and HSN morphology. A significant proportion of animals raised in a glucose-supplemented diet have a higher incidence of intra-uterine egg-hatching, HSN axonal degeneration, and abnormal HSN morphology. We used genetic mutants to dissect out the role the serotonin pathway has on the response to a glucose diet. Furthermore, through RNA-sequencing, we examined how a glucose-supplemented diet alters the expression of genes responsible of serotonergic functions. This work will lead to the further understanding of the molecular mechanisms that are involved on altered serotonergic neuronal functions as a result of a chronic hyperglycemic state.

761B Olfactory memory consolidation requires the TRPV channel OSM-9 in sensory neurons of the circuit.

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Memory is one of the most important abilities of the brain. It is defined as an alteration in behavior as a consequence of an experience. For example, the C. elegans nematode will downregulate its chemotactic response to the innately attractive odor, butanone, if the odor is not paired with food. Through repeated, spaced training with this odor in the absence of food, C. elegans will maintain this memory for a prolonged period of time. Although transient receptor potential (TRP) channels are classically thought of as primary sensory receptors, it was reported that the OSM-9/TRPV5/TRPV6 (TRP vanilloid 5/6) channel is required for single exposure learning. Here we describe a new role for osm-9 in consolidation of memory that is induced by repeated, spaced training. In this paradigm, osm-9 mutant animals learn as well as wild-types, but are unable to consolidate the memory. Though sleep is required for memory consolidation, loss of the TRPV channel OSM-9 does not affect sleep. This indicates that the TRP channel promotes memory in a process that acts outside the sleep pathway. We investigate the endogenous expression pattern of OSM-9 and show that it is not expressed in the butanone-responsive AWC olfactory sensory neuron. Instead, it is expressed in the paired AWA olfactory neuron, the ASH nociceptive neurons, the OLQ and two other unidentified sensory neurons which are most likely ADF and ADL as they express osm-9 mRNA. Because OSM-9 acts in sensory neurons that do not participate in butanone sensation, this indicates that the circuit participates in olfactory memory consolidation.

762C Systematic Behavioral Screen of 21st Chromosome Gene Overexpression in *C. elegans*

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Down syndrome (DS) is caused by overexpression of 21st chromosome (Hsa21) genes due to the presence of an extra copy of Hsa21. A major goal in DS research is to understand the individual contribution of each Hsa21 gene to the numerous phenotypes that accompany DS. Progress is being made using mouse models to understand the role of certain Hsa21 genes. However, most Hsa21 genes have not been studied in any detail. To circumvent the time and cost of mouse models for single-gene studies, we are leveraging the speed and affordability of the nematode *C. elegans*. Our lab found that *C. elegans* has 51 highly-conserved orthologs of Hsa21 genes. By studying loss-of-function mutants and performing RNAi for these orthologs, we found that 14 are essential and 10 are required for neural or muscular function in *C. elegans* -- 3 of which had not previously been studied. Through epistasis analysis, we found that the novel molecule, MTQ-2, appears to modify G-protein signaling to regulate rates of cholinergic synaptic transmission. Additionally, to probe how overexpression (OE) of individual Hsa21 genes contributes to neuronal, muscular, and developmental phenotypes related to DS, we are generating a complementary set of 51 *C. elegans* transgenic strains that each overexpress a different Hsa21 ortholog. We are performing quantitative behavioral analyses with the OE strains to deduce which genes cause neural or muscular dysfunction when overexpressed. Thus far, we discovered a specific behavioral defect for six OE strains including EVA-1, a novel slit receptor involved in axon guidance recently discovered in *C. elegans*. By identifying Hsa21 genes that cause phenotypes when overexpressed in *C. elegans*, this study will highlight individual Hsa21 genes and pathways to prioritize for further study in other models and to consider as potential therapeutic targets for improving health in those with DS.

763A Transducing touch by a titin-related protein in the worm

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Of the five traditional senses, touch is the first one available to us when we are born. Despite being a relatively little investigated sensory modality, touch governs our daily lives, from walking and feeding to kissing and cuddling. How mechanical forces reach the mechanoelectrical transduction channels remains an open question. To address this knowledge gap, we study the mechanotransduction pathway of *C. elegans'* gentle touch, which relies on the six touch receptor neurons that tile

the body into two receptive fields. We showed that a protein-protein interaction motif at the MEC-2 C-terminus is critical for mechanosensation, without interfering with its localization. To understand the function of MEC-2 C-terminus in touch, we conditionally coupled it to different components of the cytoskeleton. This artificial coupling was able to recover nearly wildtype functions of MEC-2, implying a role in force transfer. We then combined genetic engineering, microfluidics, and FRET-based molecular tension microscopy and found that MEC-2 is under tension when a force is applied to the body wall of the worm. To uncover an endogenous binding partner of MEC-2, we performed a neuronal RNAi screening. Surprisingly, interference of *unc-89*/*obscn*, which encodes for a protein related to the giant human titin, led to decreased touch response and colocalizes with wt but not mutant MEC-2 in TRNs. In summary, our study sheds light into how mechanical forces propagate to the mechanosensitive channels and show the first example for titin in neuronal mechanotransduction.

764B Role of Insulin and Insulin-like pathway in Learning and Memory of *Caenorhabditis elegans*

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Insulin is implicated in various neurodegenerative diseases including Alzheimer's Disease that primarily result in memory dysfunctions. Hence, it has been a topic of growing interest among scientists across the globe. We used the model organism Caenorhabditis elegans to elucidate the role of insulin pathway in the learning and memory processes. In C. elegans, the insulin pathway is mediated by a number of insulin peptides and a receptor, daf-2.

Our approach included an appetitive memory paradigm associated with an odor to analyse the learning and memory alterations in this model system. Our study involving both short- and long-term trainings showed that insulin affects learning and memory in a differential manner. Post- dauers with a developmental history of downregulated insulin pathway showed a defective memory recall. However, some mutants in the initial pathway of insulin, like the ligand ins-1 and the receptor daf-2, show better retention of memory. We were also able to show that this altered memory is not through the known Akt-1 pathway. In our study, we also found that excess insulin antagonises the pathway and shows extended memory. Our studies also point towards the implications of dopamine in the process. The calcium imaging data also correlates with the role of dopamine neurons in this memory. This work establishes the role of the insulin pathway in learning and memory and that insulin pathway modifies the learning and memory of the worms.

Key Words: insulin, Learning and memory, ins-1, daf-2, dauers

765C Discriminating between sleep and exercise-induced fatigue using computer vision and behavioral genetics

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Fatigue is poorly understood, beyond muscle energetics. For *C. elegans*, swimming is a more energetically costly behavior than crawling and can be classified as a form of exercise (PMID 28395669). After prolonged swimming, *C. elegans* cycle between active and inactive bouts (PMID 19011210); inactive bouts may be sleep or quiescent rest. Sleep is the most well characterized period where *C. elegans* show locomotion quiescence; however, we have shown that the quiescent bouts observed after prolonged swimming exercise do not fully fit the behavioral criteria required to be classified as sleep (PMID 32811254). It is likely that this locomotion quiescence is a fatigue-induced state of quiescent rest. Using a computer vision program and other approaches we also tested the role of several genes previously implicated in other behavioral quiescence to determine whether they also affect quiescent bouts induced by prolonged swimming. We have found that while there is some overlap between genes involved in regulating behavioral quiescence during *C. elegans* sleep and exercise-induced quiescence, distinct pathways underlie these behaviors. Ultimately, we hope to better understand the pathways involved in regulating fatigue and quiescent bout cycling.

766A Temperature-stressed *C. elegans* males prioritize food over mating resulting in sterility

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Sexual reproduction has one ancient and deeply conserved weakness: It is temperature sensitive. As climate-change associated infertility increases across species, understanding how temperature stress affects fertility has become an essential endeavor. We are interested in understanding the behavioral components of male fertility that are temperature sensitive in *C. elegans*

using two wild isolate strains (JU1171 and LKC34) and the canonical lab wild type strain (N2). We have previously shown that *C. elegans* males have a reduced response to hermaphrodites under moderate temperature stress (27°), with lesser effects seen in germline-associated processes. However, what behavioral or physiological somatic processes are impaired in temperature stressed males were unknown. We hypothesized that diminished male interest in mating temperature stressed males could be attributed to: (1) diminished pheromone response, (2) impaired touch response, (3) increased food drive, (4) lowered movement velocity and physical endurance, and/or (5) decreased mating drive. Surprisingly, we found that temperature stress increases pheromone response, a potentially maladaptive change that does not result in mating success. We also show that temperature stress perturbs the balance in male food and reproductive drives: Temperature stressed males appear to prioritize food over mating. In addition, we provide evidence that temperature-stressed males have diminished movement velocity and physical endurance, which may also contribute to their sterility especially in strains like N2 where hermaphrodites exercise reproductive choice and avoid males until they have depleted their compliment of sperm. We also report that LKC34 is a previously unrecognized wild isolate where hermaphrodites employ reproductive choice. Collectively our results paint a picture where a temperature-stressed male's fertility is reduced by a preoccupation with pheromones and food, lowered mating drive and weakened physical capacity. In essence, modest changes in temperature have profound effects on behaviors vital for reproductive success. Changes in behavior caused by temperature stress may prove to be conserved components of fertility that have been previously under-studied in comparison to facets like sperm production, and thus merit additional scrutiny to more fully understand why males experience catastrophic loss of fertility under moderate temperature stress.

767B Defining the Gap Junction Circuit that Modulates Aversive Chemosensory Behavior in *Caenorhabditis elegans*

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An organism's behavioral response to any sensory cue is reflective of an integration of both internal and external signals within the nervous system, making behavior a complex and context-dependent phenomenon. The sensory system mediates these detections through networks of neurons, which utilize both chemical and electrical synapses to communicate information.

In *C. elegans*, aversive stimuli are primarily detected by the ASH polymodal nociceptive neurons. These neurons form electrical synapses (gap junctions) with other amphid neurons that modulate avoidance behavior. We previously found that the gap junction innexin proteins INX-4 and INX-20 are critical for modulating the sensitivity of animals to select noxious stimuli, including bitter tastants such as quinine. Animals lacking either innexin are hypersensitive to dilute concentrations of these stimuli. Our data suggested that the role of gap junctions in this context is to regulate cGMP dynamics in the ASHs. We will provide an update on our efforts to identify additional innexins that function in the neural circuit that regulates ASH-mediated nociceptive sensitivity.

768C Therapeutic Ultrasound's Effects on the Developing Nervous System of *C. elegans*

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Ultrasound is widely used in diagnostic and therapeutic medical procedures, and it is becoming an important tool in biomedical research. As an ultrasound beam interacts with tissues in its path, changes known as "bioeffects" can result. In previous work, we showed that adult *C. elegans* exposed to therapeutic ultrasound exhibited a slow, irregular movement that we termed "writhing." The posterior region of the body was sometimes more severely affected than the anterior region was, which suggested that nervous system damage had occurred. Further characterization of the structural and functional changes is underway in adults and in larvae. We have hypothesized that ultrasound exposure during early development may lead to nervous system defects that persist into adulthood. To test this hypothesis, L1 animals were exposed to a half-lethal dose of 1-MHz therapeutic ultrasound. After they reached adulthood, their movement was normal. Chemotaxis assays suggested, however, that their sensory nervous system was impaired. Because many genes are conserved between *C. elegans* and humans, future work may help us understand the proteins and biochemical pathways that mediate ultrasound-induced damage and repair.

769A Behavior of *C. elegans* on lifespan-promoting bacterial diets

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C. elegans is a powerful model to study host-microbe interactions and address experimentally how microbes influence nervous system integration of sensory information. *C. elegans* respond to changes in food availability and environmental conditions through coordinated behavioral, physiological and metabolic responses controlled by signaling in olfactory sensory neurons. While examining the diet-dependent phenotypes of *C. elegans* on bacteria found in *C. elegans'* natural and laboratory environments, we noticed that despite the Red bacteria providing a longer lifespan to the worms residing on it, the worms are found less often on this food source when another food is present. When worms are given the option of another bacteria, they will leave the Red bacteria and move to the other diet. Avoidance and attraction behavior have been observed in previous studies, linking neuropathways to dietary response and physiological changes. Among these physiological responses influenced by the sensory neurons is both lipid metabolism and lifespan. Previous studies have demonstrated that loss-of-function and gain-of-function olfaction mutants of Ga proteins affect lipid content and lifespan of *C. elegans*. In light of these behaviors and changes in physiology, it will be beneficial to investigate if particular neuropathways are responsible for alterations in fat content and lifespan due to differential dietary exposure. The expansion of the bacterial food options to use in the laboratory will provide a critical tool to better understand the complexities of bacterial diets and subsequent changes in physiology, behavior and gene expression.

770B *C. elegans* regulates its behavior via serotonergic signaling to find food and hydrogen peroxide protection.

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Hydrogen peroxide is a pervasive chemical weapon used by many species to damage their prey or to protect themselves from their pathogens. Cells rely on conserved defense mechanisms, including catalases, to avoid the damage that hydrogen peroxide inflicts on their macromolecules We recently discovered that *C. elegans* represses those defenses in response to sensory perception of *E. coli*, the nematode's food source in the lab, because *E. coli* can deplete hydrogen peroxide from the local environment and thereby protect the nematodes[1]. Here, we investigated the extent to which *C. elegans* can tell the difference between bacteria that provide hydrogen peroxide protection and bacteria that do not.

To address that question, we determined the extent to which hydrogen peroxide modulates the behavior of *C. elegans* in food lawns of wildtype *E. coli* and mutant *E. coli* unable to degrade hydrogen peroxide. In the absence of hydrogen peroxide in the environment, *C. elegans* did not exhibit a preference between these two bacteria stains. However, in the presence of hydrogen peroxide, *C. elegans* remained on the wildtype bacteria lawn but was much more likely to leave the lawn of the bacteria that do not degrade hydrogen peroxide. This nematode behavior was conserved when we used catalase-positive and catalase-negative bacterial strains from the natural *C. elegans* microbiome, suggesting that this decision-making occurs in the natural environment. To determine how *C. elegans* senses and responds to bacteria and environmental hydrogen peroxide, we measured calcium dynamics in ciliated sensory neurons. We identified several neuronal classes that respond to hydrogen peroxide in a bacteria-dependent manner. In addition, genetic analysis showed that serotonin signaling regulates this nematode behavior. Our findings demonstrate that the cross-kingdom interactions between *C. elegans* and bacteria in their microbiome determine the nematode food-leaving behavior via serotonergic signaling, enabling nematode populations to find safety from hydrogen peroxide.

1. Schiffer JA et al. (2020). *Caenorhabditis elegans* processes sensory information to choose between freeloading and self-defense strategies. eLife.

771C Rpamide neuropeptides NLP-22 and NLP-23 mediate egg-laying quiescence during stress induced sleep

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Sleep is a reversible state in animals, characterized by the inhibition of behaviors that occur periodically during wakefulness. The transitions from wake to sleep states are regulated by signaling pathways that are not fully understood. *Caenorhabditis elegans* demonstrates a reversible sleep-state following acute exposure to environmental stressors, termed as stress induced sleep (SIS). In adult animals, SIS is characterized by the inhibition of movement, feeding and defecation, and each of these behaviors is largely regulated by neuropeptidergic signaling from the ALA and RIS interneurons. We find that egg-laying behavior, a fundamental process, is also inhibited during SIS. Additionally, we show that egg-laying quiescence (ELQ) is a function of mechanisms that are partially independent of the ALA and RIS. Specifically, RPamide neuropeptides encoded by the genes *nlp-22* and *nlp-23* signal directly to the terminal structure of the egg-laying circuit, the vulval muscles, to induce ELQ.

Our work suggests that signaling from multiple neuropeptidergic neurons is required to inhibit essential behaviors during SIS in *C. elegans*.

772A Sperm regulates behavioral states in hermaphrodites

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Sex differences in behavior allow animals to effectively mate and reproduce. Sex differences in behavioral states underlie the sex difference in the regulation of sex-shared behaviors such as locomotion. *C. elegans* hermaphrodites switch between two distinct behavioral states, roaming and dwelling, in which animals have high and low locomotor activity, respectively. Hermaphrodites spend most of the time in the dwelling state and various neuromodulators are involved in this regulation. We previously analyzed sex differences in the behavioral states and found that males spend less time in the low activity dwelling state than hermaphrodites on food. This behavioral difference allows males to explore a larger area of the bacteria lawn than hermaphrodites, which is beneficial for males to find mates and for hermaphrodites that can self-fertilize to stay with food. Dopamine generates the sex differences in the behavior by having the opposite effects in males and hermaphrodites, as the dwelling state was increased in males and decreased in hermaphrodites in dopamine-deficient cat-2 mutants, compared to the wildtype counterparts. Hermaphrodites produce both sperm and eggs, but exhaust sperm before eggs and effectively become females later in their life. It was previously shown that loss of sperm in hermaphrodites changes their behavior. In this study, I examined the behavioral states of sperm-defective mutants and found that sperm-defective hermaphrodites exhibit increased roaming compared to wildtype hermaphrodites. This may be adaptive in that this allows hermaphrodites lacking sperm to explore a larger area for mates as they require males to reproduce. Furthermore, the effect of losing sperm was decreased in cat-2 mutants, suggesting that dopamine plays a role in the regulation of behavioral states by sperm.

773B *Neuronal SKN-1B Modulates Nutritional Signalling Pathways and Mitochondrial Networks to Control Satiety*

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The feeling of hunger or satiety results from integration of the sensory nervous system with other physiological and metabolic cues. This regulates food intake, maintains homeostasis and prevents disease. In *C. elegans,* chemosensory neurons sense food and relay information to the rest of the animal via hormones to control food-related behaviour and physiology. Here we identify a new component of this system, SKN-1B which acts as a central food-responsive node, ultimately controlling satiety and metabolic homeostasis. SKN-1B*,* an ortholog of mammalian NF-E2 related transcription factors (Nrfs), has previously been implicated with metabolism, respiration and the increased lifespan incurred by dietary restriction. Here we show that SKN-1B acts in two hypothalamus-like ASI neurons to sense food, communicate nutritional status to the organism, and control satiety and exploratory behaviours. This is achieved by SKN-1B modulating endocrine signalling pathways (IIS and TGF-β), and by promoting a robust mitochondrial network. Our data suggest a food-sensing and satiety role for mammalian Nrf proteins.

774C Analysis of AIA interneuron in forgetting of olfactory memory in *Caenorhabditis elegans*

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Forgetting is an important process for the survival of animals towards changing environments. However, the mechanism for forgetting remains unclear. *Caenorhabditis elegans* (*C. elegans*), which are attracted to volatile odorants (Naïve), display weak chemoattraction after prolonged exposure to odorant without food (Adaptation). These adapted wild-type animals are able to recover their chemoattraction toward the conditioned odorant after 4 hours of normal cultivation on food (Recovery). This behavioral recovery is regarded as forgetting. Our previous study showed that TIR-1/JNK-1 pathway in AWC neurons regulates forgetting of the olfactory adaptation memory which is retained in AWA neurons. Yet, the forgetting mechanism between AWC and AWA are not fully revealed as no direct connection is reported between AWC and AWA. To investigate this, we examined the neurons which located downstream of AWC and AWA in olfactory circuit. Here, we discovered that animals without functional AIA interneurons displayed a defect in forgetting of olfactory adaptation for both diacetyl and isoamylalcohol, suggests that AIA interneurons are required for the regulation of two distinct olfactory adaptation memory. Acute silencing of AIA during recovery by expressing *Drosophila* histamine-gated chloride channel (HisCl1) cell specifically was sufficient to cause forgetting defect, indicates the activity of AIA during recovery is required to actively regulate forgetting.

To elucidate the relationship of AIA and AWC during forgetting, the behavior of animals was examined when AIA is ablated in *tir-1* gain-of-function mutant which showed adaptation phenotype due to excessive forgetting. Lack of functional AIA neurons not only suppressed the excessive forgetting behavior in *tir-1(gf)* mutant, but also cause a defect in forgetting behavior. This result revealed that AIA work downstream of TIR-1 pathway in AWC to regulate forgetting. Overall, we hypothesize that AIA is indispensable for the attraction to odorant in animals that have just recovered from adaptation. We are currently examining the relationship between AWA and AIA in active forgetting of olfactory adaptation by checking the Ca^{2+} transient in AWA at different phases (naïve, adaptation, and recovery).

775A Decoding temperature-dependent behavioral states in *C. elegans*

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Animal behavior is controlled by genetic components, environmental cues, past experience, and internal state. How these factors interact to determine behavior across timescales is poorly understood. Here we use high-content recording and quantitative analysis of *C. elegans* behavior to systematically dissect the impact of sensory input, sensory history, and internal state in the modulation of temperature-dependent behavioral states. Using clustering and principal component analysis (PCA), we show behavioral states under different conditions can be represented as a unique code of worm postural and functional behavioral parameters. Under constant environmental condition, worms show 'steady-state' behavior, which remains unchanged over long timescales. In response to a change in internal state or external environment, 'steady state' behavior undergoes a transition and reaches a new 'steady state'. During the transition, some behavioral parameters remain unchanged while others show a temporary or long-term change. We found that long-term thermosensory history, thermosensory input from the environment, and change in internal state interact to modulate 'steady-state' behavior. These results provide insights about plasticity in worm behavioral code across timescales. Future investigations are directed to take a deeper dive and understand the mechanisms modulating 'steady state' and transition in the temperature-dependent behavioral states.

776B Neuronal transcription elongation factor TCEB-3 positively regulates cold tolerance in C elegans

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The cold tolerance of C. elegans that is regulated dependent on a cultivation- temperature, in which the nervous system, intestine, sperm and muscle coordinately function (Ohta et al., Nature commun, 2014; Ujisawa et al., PNAS, 2018; Okahata et al., Science advances, 2019). We reported DEG/ENaC DEG-1 and xanthine dehydrogenase XDH-1 as a positive regulator for cold tolerance (Takagaki et al., EMBO rep,2020), yet the mechanism for the positive regulation in cold tolerance remains largely unknown. We now investigate new positive regulator TCEB-3 and its site of action in cold tolerance.

Wild-type worms cultivated at 15°C can survive at 2°C, however tceb-3 mutant cultivated at 15°C cannot survive. TCEB-3 is the elonginA known as a conserved transcription elongation factor in a stress-responsive manner. In C. elegans, TCEB-3::GFP fusion protein was localized in the nucleus of many head and tail neurons, intestine and muscle cells. Decreased cold tolerance in tceb-3 mutants was rescued by expression of wild-type TCEB-3 in neurons, but not by expression of TCEB-3 in muscle cells or the intestine. These results suggest that the neural function of TCEB-3 confers cold tolerance. We are performing cell-specific rescue experiments of TCEB-3 to identify functional neurons that TCEB-3 involves in cold tolerance. A transcription elongation factor TCEB-3 probably affects a part of gene expression, which is a reason why tceb-3 mutant showed low cold tolerant phenotype. Here we conducted transcriptome (RNA-seq) analysis to investigate downstream factors of TCEB-3. Comparison of transcripts in tceb-3 mutant and wild-type worms revealed that 325 downregulated-genes and 452 upregulated- genes were identified in tceb-3 animals. The gene ontology categories significantly enriched in the downregulated transcripts including stress response, extracellular material, proteolysis and sperm-related proteins. Interestingly, transcription levels of one GPCR gene that is expressed in thermosensory neuron was downregulated in tceb-3, and the transcription level of two genes that is known to be involved in cold tolerance were upregulated in tceb-3. We speculated based on these results that a transcription elongation factor TCEB-3 promotes to survive at 2°C after 15°C- cultivation in neuronal cells.

777C GRK-2 signaling in sensory neurons regulates the ability of *C. elegans* to travel long distances

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G protein-coupled receptor kinases (GRKs) play key roles in terminating neuromodulator signaling by phosphorylating and deactivating G protein-coupled receptors (GPCRs). Although GRKs are critical for brain function, their particular role in

regulating neuronal activity is largely unexplored. *C. elegans* GRK-2 is required for normal chemosensation, egg-laying, and locomotion. Our previous work suggests that GRK-2 acts in premotor interneurons to promote locomotion and swimming by negatively regulating the D2-like dopamine receptor DOP-3. Here we show that GRK-2 also regulates the ability of animals to travel long distances (exploration or roaming) in a manner independent of DOP-3. Using neuron-specific rescuing experiments we show that GRK-2 acts in multiple sensory neurons to mediate this behavior. Further analysis using mutants with defects in ciliated sensory neurons indicates that *grk-2* and the cilium-structure mutants act in the same pathway to regulate exploration. We then tested candidate mutants and also used mutagenesis to identify suppressors of the exploration defect of *grk-2* mutants. We identified a neuropeptide signaling pathway involving the neuropeptide FLP-1 and a neurohormonal signaling pathway involving the sulfotransferase SSU-1 that contribute to GRK-2- mediated exploration. Our studies of GRK-2 revealed neurons and molecular pathways that modulate exploration behavior.

778A Investigating the overlap between on-food exploration behavior and off-food behavioral responses to alcohol

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Variation in genes that modulate the acute level of response to alcohol are predicted to play a significant role in the predisposition of individuals to develop alcohol use disorder (AUD) over their lifetime. We are using *C. elegans* to identify genes that are important in mediating behavioral responses to ethanol with the goal of highlighting candidate genes that are likely to play similar roles in humans. In a forward genetic screen for mutations that have reduced sensitivity to the effects of ethanol on the speed of locomotion in the absence of food, we identified a nonsense mutation in the *nep-2* gene, an ortholog of the mammalian metalloprotease *neprilysin*. In *C. elegans*, *nep-2* has been shown to be important in regulating the impact of population density on olfactory adaptation behavior (Yamada *et al*. 2010). Our working hypothesis is that NEP-2 cleaves a peptide that acts to suppress an effect of ethanol on locomotion speed, and that in the absence of NEP-2 function, that peptide accumulates and counters the effect of ethanol to a greater extent, leading to reduced ethanol sensitivity.

While investigating neuropeptide receptors (NRs) for a role in mediating this *nep-2* effect on ethanol sensitivity, we examined a potential link between ethanol sensitivity and roaming and dwelling behavioral states. In an exploration assay, which serves as a proxy measure for the degree of roaming, we found that *nep-2* mutants have increased exploration measures relative to wild-type controls, while mutants for two NRs (*pdfr-1* and *npr-25*) each have reduced exploration on food and are hypersensitive to ethanol off food. This suggests that regulation of exploration-related behavioral states may also impact sensitivity to ethanol. We are now testing the degree of correlation between exploration and sensitivity to ethanol by performing exploration assays on mutants that alter behavioral responses to ethanol, and by examining the ethanol sensitivity of mutants that are known to alter the frequencies of roaming and dwelling. These experiments will enable us to classify genes and pathways that are important in mediating behavioral responses to ethanol.

779B Expressing human epithelial Na channel subunits in *C. elegans* to model human salt taste

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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 the salivary proteome is different in salt sensitive and salt insensitive tasters. We hypothesize that salivary proteases regulate ENaC open-probability and salt taste.

We use *C. elegans* as 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 will generate a humanized NaCl-taste worm model that expresses all three human ENaC subunits in the ASH cells. We use 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. In these animals we see weak GFP expression in the cell bodies of the ASH neurons. We are currently in the process of integrating *sra-6::mScarlet* on chromosome II, where we will subsequently introduce the human *SCNN1B* gene, and *sra-6::tagBFP2* on chromosome IV, for subsequent introduction of the *SCNN1C* gene. We expect that the ENaC expressing animals will be less attracted or even

repelled by salt. To reduce interference of the *C. elegans* NaCl-taste machinery, we will use *che-1* mutant animals, that lack functional ASE neurons. This 'humanized' model system will subsequently be used to study the possible role that proteolytic processing of ENaC may have in salt detection.

780C CAMTA tunes neural excitability and behavior my modulating Calmodulin expression

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Calcium signaling (Ca^{2+}) regulates a wide range of essential biological processes in multiple tissues. In particular, in the nervous system, it mediates signal transduction, synaptic release and activity-dependent transcription important for learning and memory. The Ca^{2+} sensor Calmodulin (CaM) plays a key role in Ca^{2+} signaling, however, what mechanism regulates neuronal CaM levels is unclear. The CaM-binding transcription activators (CAMTAs) are expressed broadly and selectively in the nervous system, and CAMTA mutations confer multiple behavioral defects in different species including mouse and human. Using single-neuronal-type RNA-seq and ChIP-seq, we show that the sole *C. elegans* CAMTA, *camt-1,* is a master regulator of neuronal CaM expression. The pleiotropic behavioral and neuronal Ca^{2+} signaling defects in *camt-1* mutants can be rescued by supplementing neuronal CaM. CAMT-1 binds multiple sites in the calmodulin promoter and deleting these sites phenocopies *camt-1*. CAMT-1 not only stimulates CaM expression, but can also inhibit it when CaM levels are high, by a feedback mechanism requiring CaM binding sites in CAMT-1. We also show that *Drosophila* CAMTA regulates CaM levels suggesting that CAMTAs mediate an evolutionally conserved mechanism that controls neuronal CaM levels, thereby regulating Ca^{2+} signaling, physiology and behavior.

781A The *C. elegans* Shugoshin (SGO-1) is a cilia resident protein that interacts with TAC-1/TACC

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In organisms with monocentric chromosomes, the Shugoshin family of cell cycle regulators have conserved functions in spindle attachment and sister chromatid cohesion during mitosis and meiosis. In worms, the sole Shugoshin homolog (*sgo-1*) has early roles in meiosis but is otherwise dispensable for gamete and embryo viability. We have identified a novel function of SGO-1 in sensory neurons. *sgo-1* is expressed in a series of adult amphid and phasmid neurons where the protein localizes to the base of cilia. *sgo-1* mutants display behavioural and dauer entry defects consistent with dysfunctional cilia. These phenotypes are in turn rescued by a *sgo-1(+)* construct specifically expressed in cilia-producing neurons. In a screen for SGO-1-interacting proteins, we identified TAC-1, a regulator of spindle microtubule dynamics. TAC-1 co-localizes with SGO-1 in the base of cilia and a *tac-1* mutation suppresses *sgo-1* sensory and dauer entry defects. We are exploring the hypothesis that SGO-1 influences microtubule maintenance in the ciliary axoneme through the recruitment of TAC-1 to the basal body. These results add to a growing body of evidence suggesting that kinetochore and centrosomal proteins are transiently targeted to cilia and represent the first description of a non-nuclear role of Shugoshin in a post-differentiated cell type.

782B Glial mediators of K^+ and Cl^- transport shape *C. elegans* olfaction and taste

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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 channels 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 NaCl. Given, the recent report that Amsh glia function as odorant receptor cells, Ca^{2+} imaging experiments are underway to determine whether these

channels and transporters alter the response of Amsh glia or sensory neurons (or both) to these sensory cues. 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.

783C Untying the Gordian Knot: unravelling spatiotemporal activity in the *C. elegans* neuropeptide-receptor network

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Neuropeptides are important modulators of organismal physiology and behavior. Yet, the rules and constraints by which these modulatory substances achieve both local and widespread physiological effects remain poorly understood. Currently lacking is a comprehensive analysis of the neuropeptide signaling network at an organismal systems level. The *C. elegans* genome shows a broad diversity of neuropeptide pathways, harboring around 150 genes encoding neuropeptides and a similar number of peptide GPCRs. Using reverse pharmacology, we have systematically mapped the biochemical network of neuropeptide-receptor interactions in the *C. elegans* nervous system. By screening for neuropeptide-GPCR couples, we identified receptors for all *C. elegans* RFamide-like peptides (FLPs) and many neuropeptide-like proteins (NLPs). These peptidergic pathways are organized into a dense signaling network including promiscuous neuropeptides and receptors. To further understand the functional organization of peptidergic circuits, we have adopted genetically-encoded sensors for neuropeptide-receptor activation that allow characterizing the spatiotemporal activity patterns of neuromodulatory signaling axes in the network. Using optogenetics, we 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*. CAPA-1 signaling from ASG neurons is specifically required for the retrieval, but not the acquisition, of learned salt avoidance. This highlights 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 flexible behaviors emerge from neuromodulatory networks.

784A Explore novel functions of anti-microbial neuropeptides

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Infections caused by pathogens have been shown to alter the behaviour of animals, in part due to interactions between antimicrobial response pathways and the nervous system. Recent research has shown that the activation of immune cells can modulate neuronal circuits, and that the nervous system in turn can regulate both innate and adaptive immune responses. However, it remains unclear how small, infection-related neurotransmitters could contribute to the neuro-immune interactions.

Here, we selected 10 epidermally expressed antimicrobial peptides (AMPs) in *C. elegans*, aiming at study the biological functions of these neuropeptides in worm behaviours. All these neuropeptides contain several YGGYG motifs, which is considered to have an antimicrobial function. Among them, expression level of *nlp-24*, *nlp-29*, *nlp-31* are known to be up-regulated after fungal infection (N. Pujol *et al.* 2008). We have generated knockout mutants for each of these antimicrobial peptide genes using Crispr, and failed to observe any effect of these mutations on worm locomotion, or the response to gentle or harsh touch. However, several AMP mutants had significant effects on egg-laying behaviour. Specifically, we discovered that *nlp-27, nlp-28,* and *nlp-30* mutants exhibited increased egg-laying rates, an effect primarily due to increased egg-laying within the egg-laying active phase. On the other hand, *nlp-29* mutant showed significantly decreased egg-laying and specifically decreased egg-laying rate within the active phase. These results implicate several AMPs in the control of intensity and/or duration of the active phase of egg-laying. Our next step is to further study if the modulated egg-laying phenotypes caused by the knock-out of the certain neuropeptide will be altered after culturing the worm on pathogenic bacteria. We also aim at discovering the corresponding receptors for these AMPs and further discovering the down-stream signalling pathways that are involved in egg-laying regulation.

785B Natural variation in *C. elegans* thermosensory behaviors

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The *C. elegans* N2 strain has been used as the reference strain to study diverse behaviors in the lab since its isolation in 1951. However, extensive experiments have established that wild strains can differ from N2 in multiple aspects of development, physiology, and behavior. This variation has been exploited to not only describe the genetic architectures underlying specific behavioral outputs but to also identify causal genetic variants that affect nervous system function. A limited number of previous studies have examined natural variation in thermosensory behaviors in *C. elegans*. Here, we tested thermotaxis behaviors of 32 strains from around the world. We found that JU322 and the N2-derived CC1 strains show distinct negative thermotaxis behaviors as compared to N2. While JU322 appears to be largely atactic, CC1 exhibits enhanced negative thermotaxis. Interestingly, JU323, the reference strain for the JU322 isotype, exhibits N2-like thermotaxis behaviors. We have recently completed whole-genome sequencing of our JU322 and JU323 isolates, and have also generated recombinant inbred lines (RILs) between CC1 and N2. Future work is aimed at identifying the causal genetic variants that contribute to variation in thermosensory behaviors.

786C Regulation of feeding-induced sleep by neuropeptide signaling in *C. elegans*

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Sleep and metabolism are interconnected homeostatic states; sleep cycle can be entrained by feeding cycle, and perturbation of sleep often results in dysregulation in metabolism. However, the neuro-molecular mechanism by which metabolism regulates sleep is not fully understood. We investigated how metabolism and feeding regulate sleep using satiety quiescence behavior as a readout in *C. elegans*, which mimics many aspects of postprandial sleep in mammals. From an RNA interference-based screen of two neuropeptide families, RF-amide (FLPs) and insulin like peptides (INSs), we identified *flp-11*, known to regulate other types of sleep-like behaviors in *C. elegans*, plays the most significant role in satiety quiescence among 28 FLPs screened. A mutation in *flp-11* significantly reduces quiescence, whereas over-expression enhances it. A genetic analysis shows that FLP-11 acts upstream of the cGMP signaling but downstream of the TGFβ pathway, suggesting TGFβ released from a pair of head sensory neurons (ASI) activates FLP-11 in an interneuron (RIS). Then, cGMP signaling acting in downstream of RIS induces satiety quiescence. Among 28 the INSs genes screened, two INS genes, *ins-1* and *ins-7*, known to play a significant role in starvation-associated behavior working in AIA and URX, respectively, are inhibitory to satiety quiescence. Our study suggests that specific combinations of neuropeptides are released, and their signals are integrated in order for an animal to gauge the metabolic state and to control satiety quiescence, a feeding-induced sleep-like behavior in *C. elegans*.

787A Charactering the roles of neuropeptides in non-associative learning

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Non-associative forms of learning (habituation, dishabituation and sensitization) are the simplest forms of learning and are often thought as “cognitive building blocks” for higher learning. To better understand non-associative forms of plasticity and the cellular and molecular mechanisms, our work focuses on neuromodulatory signalling pathways underlying these forms of plasticity. First, we found that *egl-3* and *egl-21* mutants deficient of neuropeptides showed learning deficits in three forms of non-associative learning, habituation, dishabituation, and sensitization,, suggesting neuropeptides play a key role in non-associative learning. Further, we found that FLP-20/FRPR-3 signalling mediates sensitization but not dishabituation of the same response, and that the PDF neuropeptides differentially affect sensitization of response duration and response speed. These data suggest different forms of non-associative plasticity are mediated by different cellular and molecular components. We are in the process of testing mutations in a range of neuropeptide genes to examine how they influence non-associative learning. This project will generate data to better understand how neuropeptide signalling mediates different forms of non-associative plasticity.

788B Genetic Analyses Reveal Redundant Negative Regulators of *Caenorhabditis elegans* Starvation-Odor Associative Learning

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Past experience of an environment can provide valuable information to guide optimal behavioural choices, but only if salient information can be separated from incidental information. In *Caenorhabditis elegans,* paired exposure to a naively attractive

scent and starvation results in subsequent avoidance of the scent for several hours, while the same scent in the presence of food fails to form this aversive memory. To understand how food presence or absence modulates formation of this memory, we examined the effects of genetic and pharmacological perturbations on it. Serotonin has been studied extensively as a satiety signal in *C. elegans*, but we find that while application of exogenous serotonin in place of food is able to prevent the formation of this memory in a manner primarily dependent on the receptor SER-4, loss of function mutations in either the serotonin synthesis gene *tph-1* or in serotonin receptors have only a weak effect in preventing the food-mediated inhibition of memory formation. Mutations in the gene encoding the TGF-β ligand DAF-7, conversely, dramatically reduce the ability of food to block formation of the memory, and *tph-1;daf-7* double mutants form the starvation memory under all but the most plentiful food conditions. Our findings support a model in which TGF-β, and to a lesser degree serotonin signaling, function in concert with an as-yet unidentified mechanism to inhibit the formation of aversive memories by signaling the presence of food.

789C A role for L1CAM/SAX-7 in fluid regulation and vulva development

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Primary congenital hydrocephalus (CH) is a progressive, life-threatening condition that arises from abnormal buildup of cerebrospinal fluid (CSF) in the ventricles of the brain. If untreated, intracranial pressure due to CSF accumulation can result in brain damage and death. While the pathophysiology underlying CH is undefined, the most common cause is loss or impaired function of L1, a transmembrane immunoglobulin cell adhesion molecule with well-defined roles in neuronal migration, synaptic regulation, and axon guidance. It is not known how impaired L1 function causes CH, but the incomplete penetrance and variable expressivity of L1-associated CH within families is indicative of complex genetic interactions. Uncovering L1 functions and mechanisms of action are key to developing novel therapies and preventative measures for CH.

The *C. elegans* L1 homologue, SAX-7, has well-characterized roles in nervous system development and maintenance. Recently, we identified a genetic interaction between SAX-7 and the Ras/MAPK signaling pathway, uncovering novel roles for SAX-7 in fluid regulation and vulval development. *sax-7* null animals with hyperactive Ras/LET-60(gf) display a synthetic phenotype of progressive fluid buildup that often results in mid-to-late larval lethality. Dying animals often show ruptures at the excretory pore, suggestive of increased internal pressure. Consistent with internal fluid build-up, *sax-7 let-60(gf)* animals also show hypersensitivity to hypotonic solutions, their bodies tending to burst shortly after immersion as compared to *sax-7* and *let-60(gf)* single mutants. Our conditional knockout experiments reveal a neuronal requirement for SAX-7 in this process. While *sax-7* null animals do not exhibit vulva abnormalities, loss of SAX-7 synergistically enhances the multiple vulva phenotype (Muv) exhibited by *let-60(gf)* animals, revealing a previously uncharacterized role for SAX-7 in vulva development. Both the *sax-7 let-60(gf)* fluid buildup and Muv phenotypes can be suppressed by knocking out KSR-1, a key protein scaffold that promotes MAPK signaling, consistent with elevated MAPK activity as underlying the *sax-7 let-60(gf)* synergistic phenotypes. Interestingly, CH is sometimes presented in a cluster of diseases known as “Rasopathies,” characterized by elevated Ras/MAPK signaling. Based on these findings, we hypothesize that SAX-7 and Ras/MAPK signaling act synergistically in fluid homeostasis and vulval development.

790A Multicellular rosettes organize neuropil formation

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Neuropil formation requires structural organization in order to support information processing. While the organization of the *C. elegans* main neuropil (nerve ring) has been thoroughly mapped in the larva and adult, how these organizational features emerge from early (pre-neurite) collective cell behaviors is poorly understood. Prior to neurite outgrowth, we find that head neurons and surrounding cells collectively form a ring of multicellular rosettes that organize the topographic neighborhoods of the nerve ring. Neurite bundles that correspond directly to future topographic neighborhoods grow from rosette centers, travel along the ring on “bridge” cells that are simultaneously engaged in adjacent rosettes, and assemble into a scaffold

that physically links adjacent topographic neighborhoods. Laser ablation of rosette cells leads to delays in nerve ring closure, while laser ablation of bridge cells leads to ectopic neurite growth between rosettes. Furthermore, we find that SAX-3/Robo is required for proper rosette formation and neurite outgrowth from rosette centers. Our results reveal how pre-neurite collective cell behaviors support complex neuropil patterning.

791B Forkhead transcription factor FKH-8 is a master regulator of sensory cilia

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Cilia are complex evolutionary conserved eukaryotic structures that, projecting from cell surfaces, perform a variety of biological roles. Cilia are traditionally classified into motile or sensory and hundreds of proteins take part in their composition. This set of genes coding for ciliary components is known as the ciliome. Mutations in the ciliome underlie an ever-growing group of highly pleiotropic multisystemic diseases globally termed as ciliopathies. These diseases are characterized, among other symptoms, by mental retardation, sensory defects and/or metabolic disorders. Despite an estimated 1 in 1,000 people affected by these diseases, the molecular bases of the ciliopathies are still poorly understood.

Proper cilium assembly and functionality requires the tightly co-regulated expression of ciliary components; however, little is known about the regulatory logic controlling ciliome transcription. Most ciliome genes are shared between motile and sensory cilia. RFX transcription factors (TFs) have an evolutionarily conserved role in the transcriptional regulation of both motile and sensory ciliome. In vertebrates, transcription of the motile ciliome is also directly regulated by FoxJ1, a Forkhead (FKH) TF. However, to date, TFs working together with RFX in the transcription of the sensory ciliome are unknown in any organism.

We have identified FKH-8, a FKH TF, as a terminal selector of the sensory ciliome in *C. elegans*. *fkh-8* expression is restricted to the sixty ciliated sensory neurons of *C. elegans*, it binds the regulatory regions of the sensory ciliome, it is required for correct ciliome gene expression and cilium morphology and acts synergistically with DAF-19/RFX TF. Accordingly, *fkh-8* mutants display a wide range of behavioral defects in a plethora of sensory mediated paradigms, including olfaction, gustation, and mechano-sensation. Importantly, ciliome expression defects in *fkh-8* mutants can be rescued by mammalian FoxJ1 but not other FKH TFs.

Thus, we have identified, for the first time, a TF that acts together with RFX TFs in the direct regulation of the sensory ciliome. Moreover, together with previous work, our results show that FKH and RFX TFs act together in the regulation of both motile and sensory cilia, suggesting this regulatory logic could be an ancient trait pre-dating functional sub-specialization of cilia. Finally, our results could help better understand the biological basis of orphan ciliopathies.

792C A *C. elegans* model for human PACS1 syndrome

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PACS1 syndrome (also known as Schuurs-Hoeijmakers syndrome) is a human neurogenetic disorder characterized by distinctive dysmorphic facial features, intellectual disability, and developmental delays. All known PACS1 patients have de novo mutations causing an R203W amino acid substitution in a highly conserved position of the PACS1 protein. While PACS1 (Phosphofurin Acidic Cluster Sorting Protein 1) was first identified by its role in sorting the proprotein convertase furin to the trans-Golgi network, most understanding of PACS1 function has been attained through studies of cultured cells. To better understand how the R203W disease variant changes PACS1 function *in vivo*, we have modeled PACS1 syndrome in *C. elegans*. The single *C. elegans pacs-1* gene encodes a protein that shares all conserved domains, and is 27% identical/46% similar to human PACS1. The furin-binding region is especially well conserved between *C. elegans* and humans (45% identical/70% similar) with the disease variant site, R203, absolutely conserved and denoted as R116 in *C. elegans*. We edited the endogenous *C. elegans pacs-1* gene to generate a PACS1 syndrome model expressing PACS-1(R116W). Using drugs to probe neuronal function, we found that *pacs-1*(R116W) animals are resistant to the paralyzing effect of the acetylcholine esterase inhibitor aldicarb. Consistent with its neuronal function, we also found that expression of PACS1 is enriched in the nervous system. Our *C. elegans pacs-1*(R116W) provides the first germline-expressed model of PACS1 syndrome that can be screened for potential chemical therapeutics and further characterized to understand molecular mechanisms of the PACS1 syndrome variant.

793A Novel neurodevelopmental genes in *C. elegans*

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Ongoing research with *C. elegans* continues to uncover fundamental principles of nervous system development and function. Neuron migration, axon guidance and dendritic spine development are essential to establish functional neural circuits. Our goal here is to study cellular and molecular mechanisms that mediate neuronal development, including that of dendritic spines. For this, we are characterizing several genes named *mau* (maternal-effect uncoordinated), which are defined by mutations isolated in a screen for maternal-effect viable mutations (Hekimi et al., 1995; our unpublished results). *mau* mutant animals display morphological and behavioural defects that vary in penetrance and expressivity. Our phenotypic analyses of *mau* mutants reveal that their locomotion is abnormal and variable over time, with episodes of paralysis, spasms and altered body postures. *mau* mutants have defective axon guidance, including left/right guidance choice at the ventral midline. Moreover, *mau* mutants display defects in dendritic spines number and distribution, as well as in synaptic transmission. We are progressing towards molecularly identifying and characterizing the *mau* mutants, which will provide insights on how these novel genes contribute to neuronal development, including of dendritic spines *in vivo*.

794B A novel function for the kinetochore machinery in neural circuit assembly

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The central nervous system is a complex network of neurons and supporting cells. Its assembly is a highly orchestrated event that involves neurite outgrowth, fasciculation (axon bundling) and synapse formation. The molecular mechanisms that drive and coordinate the building of this network during development are poorly understood. As neurons develop, the neuronal microtubule cytoskeleton is organized and sculpted by the cell machinery to form axons, dendrites and the neural network. Not much is known about nature of microtubule regulators that facilitate the assembly of neural circuits. We have uncovered a cell division-independent function for the kinetochores, conserved multiprotein structures that connect chromosomes to spindle microtubules, in the assembly of neural circuits. Here we show that core microtubule-coupling machinery at the kinetochore, the 10-subunit KMN network (KMN: Knl1-complex/Mis12-complex/Ndc80-complex), is redeployed post-mitotically to pattern the nerve ring, considered the “brain” of *C. elegans*, which consists of a dense network of axon bundles and synapses. KMN proteins are enriched in the extending dendrites and axons of the nerve ring. Post-mitotic degradation of the KMN proteins in developing neurons resulted in a disorganized nerve ring and axon morphology and fasciculation defects. We are currently developing genetic tools and quantitative fluorescence microscopy methods to gain further insight into how KMN proteins facilitate neuronal network formation. This unexpected finding for a post-mitotic function for the cell division machinery in developing neurons provides molecular insights into how specific microtubule regulators shape the nervous system.

795C DYF-4 regulates patched-related/DAF-6-mediated sensory compartment formation

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Coordination of neurite extension with surrounding glia development is critical for neuronal function, but the underlying molecular mechanisms remain poorly understood. Through a genome-wide mutagenesis screen in *C. elegans*, we identified *dyf-4* and *daf-6* as two mutants sharing similar defects in dendrite extension. DAF-6 encodes a glia-specific patched-related membrane protein that plays vital roles in glial morphogenesis. We cloned *dyf-4* and found that DYF-4 encodes a glia-secreted protein. Further investigations revealed that DYF-4 interacts with DAF-6 and function in a same pathway as DAF-6 to regulate sensory compartment formation. Furthermore, we demonstrated that reported glial suppressors of *daf-6* could also restore dendrite elongation and ciliogenesis in both *dyf-4* and *daf-6* mutants. Collectively, our data reveal that DYF-4 is a regulator for DAF-6 which promotes the proper formation of the glial channel and indirectly affects neurite extension and ciliogenesis.

796A The conserved transcription factor UNC-30/PITX1-3 coordinates synaptogenesis with cell identity in *C. elegans* GABA motor neurons.

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During nervous system development, it is critical for neurons to establish functional synapses. This process relies on the ability of a presynaptic neuron to synthesize, package, and release a specific neurotransmitter, and the ability of a postsynaptic neuron to present the correct neurotransmitter receptor. However, the molecular mechanisms that coordinate these distinct events, occurring at a pre- and a post-synaptic cell, are poorly understood. The nematode *C. elegans* represents a powerful model to study synapse development due to its known connectome, powerful genetics, and single-cell resolution analysis. The evolutionarily conserved transcription factor (TF) UNC-30/PITX1-3 has been shown to control neuronal communication between nerve cord GABAergic motor neurons (MNs) and body-wall muscle by directly activating the expression of GABA biosynthesis genes (e.g., *unc-25*/GAD, *unc-46*/LAMP, *unc-47*/VGAT) in the presynaptic side. Our preliminary data shows that animals lacking *unc-30* gene activity display defects in GABA-Receptor (GABA-R) clustering in the postsynaptic side, although UNC-30 is not expressed in muscle. Intriguingly, the same GABA-R clustering defect is observed in animals lacking the short isoform of *madd-4*/Punctin (*madd-4S*), a secreted synaptic organizer produced by GABA MNs. Thus, we hypothesize that UNC-30 controls the establishment of functional synapses by activating GABA biosynthesis genes and *madd-4S*. We found that *madd-4S* expression is reduced in GABA MNs of *unc-30* mutant animals. Chromatin immunoprecipitation followed by sequencing (ChIP-Seq) suggests UNC-30 controls *madd-4S* directly, a notion we confirmed by deleting the UNC-30 binding site in the context of the endogenous *madd-4S* locus. Besides acting as an activator of GABA synthesis genes and *madd-4S*, our preliminary data also suggests UNC-30 is required to prevent the adoption of alternative neuronal identities in GABA MNs. We found that several genes normally expressed in cholinergic MNs (e.g., *madd-4L*, *glr-5, unc-53*) become ectopically expressed in GABA MNs of *unc-30* mutant animals. These findings together with ChIP-Seq data suggest an additional role for UNC-30 as direct repressor of alternative identities. Since these analyses rely on a null *unc-30* allele that disrupts gene activity throughout all life stages, ongoing experiments will determine whether UNC-30 is continuously required to maintain proper gene expression in GABA MNs. Altogether, these findings suggest UNC-30/PITX1-3 is a transcriptional link for the coordination of synaptogenesis with cell identity features in *C. elegans* GABA MNs.

797B Ubiquitin ligase activity inhibits CDK-5 to promote axon termination

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Cdk5 is an atypical cyclin-dependent kinase that is involved in neurodegenerative disease, nervous system development and neuronal plasticity. It also has important non-neuronal roles in the DNA damage response, immune response, angiogenesis and cancer. Cdk5 is positively regulated primarily by its activators, P35 and P39. How Cdk5 is negatively regulated remains relatively poorly understood, in particular in post-mitotic neurons and in the nervous system *in vivo*.

Here, we identify the E3 ubiquitin ligase RPM-1 as a negative regulator of CDK-5 in *C. elegans*. This discovery originated from *in vivo* affinity purification proteomics with an RPM-1 biochemical 'trap' that enriched ubiquitination substrates, which included CDK-5. A combination of both biochemical and genetic findings demonstrated that RPM-1 ubiquitin ligase activity restricts CDK-5. Co-immunoprecipitation using endogenous proteins tagged by CRISPR/Cas9 engineering confirmed that CDK-5 binds to RPM-1. CDK-5 also binds to FSN-1, the F-box protein that is the substrate recognition module of the RPM-1 ubiquitin ligase complex. These results suggest that CDK-5 is an RPM-1 ubiquitination substrate. Genetic results indicated that RPM-1 inhibits CDK-5 to promote axon termination in both ALM mechanosensory neurons and SAB motor neurons. Outcomes from CRISPR gene-editing and cell-specific rescue experiments showed that RPM-1 restricts the kinase activity of CDK-5 cell-autonomously in neurons.

Thus, we have identified RPM-1 as a ubiquitin ligase that inhibits CDK-5 *in vivo* in the nervous system. We further demonstrate that CDK-5 kinase activity needs to be restrained for proper axon termination. Understanding how Cdk5 is inhibited could have important implications given its role in neurodegenerative disease and emerging links to neurodevelopmental disorders, including intellectual disability.

798C Kinesin-13 mediated regulation of dendritic branch remodeling during the development of PVD neuron

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The neurological function of an organism depends on the intact neural circuitry composed of polarized neurons that undergo extensive structural changes during development. This is accompanied by reorganization of the underlying cytoskeleton, calibration of the polarized transport, and changes in the transcriptome. Unlike axons, dendrites undergo major structural changes throughout the lifetime of an organism but the role of cytoskeleton dynamics in this remodeling process is not clearly understood.

Using PVD neurons of *C. elegans*, we are investigating how the cytoskeleton changes during their dendritic development. The dendritic arbor of PVD neurons is extensive with orthogonal branches in anatomy, hierarchy, and cytoskeletal constitution. We found that the loss of the Kinesin-13 family microtubule depolymerizing enzyme *klp-7* leads to an increased number of quaternary branches. The live imaging revealed that the PVD dendrites are in continuous flux during the L3 to L4 stage of development. The tertiary and quaternary branches display distinct phases of growth and shrinkage. Loss of *klp-7* disrupts the equilibrium of the dynamic events of branch remodeling. Live imaging of the EBP-2::GFP revealed that although the relative orientation or dynamics of microtubules did not change due to loss of *klp-7* function, a significant number of dynamic microtubules were mislocalized to the secondary dendritic branches. Furthermore, the axonal cargo, RAB-3 was also mislocalized to dendritic branches in the *klp-7* null mutant. Relative enrichment of KLP-7 at the incipient branch points indicates its role in limiting branch initiation and microtubule ingression to higher-order branches.

These results highlight the balancing act of microtubule homeostasis that allows the organism to maintain an appropriate dendritic arbor. Our paradigm of live imaging can be adapted to explore other cytoskeletal molecules and their regulatory pathways.

799A Intraspecific evolution of QR.pax final position in *Caenorhabditis elegans*

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The QR neuroblast migrates anteriorly in the first stage larva of *Caenorhabditis elegans*, while undergoing three rounds of division. The two daughter cells of QR.pa, QR.paa and QR.pap (henceafter called QR.pax), stop their migration in an anterior body position and acquire a neuronal fate. Mentink et al. (Dev Cell 2014) found that QR.pax migration stops upon expression of the Wnt receptor MIG-1, which surprisingly is not induced by positional clues but by a position-independent timing mechanism. We recently showed that the final position of QR.pax is sensitive to environmental perturbations and body size. Furthermore, there is natural variation on the final position that is not explained by natural variation in body size (Dubois et al. Development 2021). Among the isolates, CB4932 and JU1242 exhibit an opposite phenotype but are genetically close. The main phenotypic difference between them is the final position of QR.pap, which is much more anterior in CB4932. The aim now is to find the genetic basis for this displacement of QR.pap. To do so, we generated and phenotyped 200 Recombinant Inbred Lines (RILs) between the two parental genotypes. A Bulk Segregant Analysis on the RILs pinpointed a tight region in the chromosome IV associated with a difference in QR.pap final position. The region contains about 50 Single Nucleotide Polymorphisms (SNPs) and Indels that are different between the two strains (and N2). By building Introgressed Lines, we confirmed that the chromosome IV is sufficient to explain the difference in QR.pap position. The analysis of heterozygous progeny revealed that both alleles are semi-dominant. Overall, this study shows natural variation of QR.pax final position in *C. elegans* and examines the genetic basis of a substantial QR.pap displacement present in a wild isolate.

800B The *C. elegans* Hox gene *ceh-13/labial/Hox1* controls motor neuron terminal identity

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The phylogenetically conserved Hox gene family is known for its early roles in embryonic patterning along the anteroposterior axis. However, whether Hox genes exert later roles during development and post-embryonic life, remains unclear. In the context of the nervous system, Hox genes control fundamental processes occurring during early development, such as neuronal specification and axon guidance. On the other hand, we know much less about the function of Hox genes in the last steps of neuronal development, during which neurons obtain their terminal identity features, such as expression of neurotransmitters, receptors and ion channels. Among the six *C. elegans* Hox genes, the function of *ceh-13/labial/Hox1* remains poorly understood, in part due to the early embryonic/larval lethality observed in *ceh-13* null mutant animals. Here, we generated a conditional allele based on the auxin-inducible protein degradation system, enabling post-embryonic CEH-13

depletion. This allele also serves as an endogenous *ceh-13* reporter, enabling us to establish, with single cell resolution, the expression profile of *ceh-13* in post-mitotic larval motor neurons of the ventral nerve cord. Using *ceh-13* null mutants, we identified three terminal identity genes (*acr-2/CHRNA1, unc-53/NAV1, unc-129/GDF10*) as CEH-13 targets, suggesting that it controls the establishment of motor neuron terminal identity. We also observed lifelong expression of *ceh-13* in motor neurons, suggesting that it is required to maintain terminal identity features in these cells. Lastly, we found that expression of a transgenic *ceh-13* reporter is significantly reduced in nerve cord motor neurons upon either genetic removal of *ceh-13* during early development or inducible CEH-13 depletion at post-embryonic stages, raising the possibility of transcriptional autoregulation as a mechanism for lifelong *ceh-13* expression. These findings advance our current understanding of *ceh-13/labial/Hox1* function in *C. elegans* and further suggest that Hox proteins control both early and late steps of neuronal development.

801C *ccd-5,* a novel *cdk-5* binding partner, regulates *C. elegans* ventral nerve cord pioneer axon guidance

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During nervous system development

, axons navigate complex environments to reach synaptic targets. Growth cones of early extending axons must interact with guidance cues in surrounding tissue, while later extending axons can interact directly with earlier “pioneering” axons, “following” their path. This is how the ventral nerve cord (VNC) is formed in *Caenorhabditis elegans*. The first axon to extend along the VNC is that of the AVG neuron, which pioneers the right VNC axon tract. Motoneuron and interneuron axons of the motor circuit follow the AVG pioneer axon. In the absence of AVG, many VNC follower axons are misguided, causing locomotion defects. The mechanisms for AVG axon guidance are largely unknown.

To uncover genes controlling AVG axon guidance

, we conducted an enhancer screen in a sensitized *nid-1* mutant background. NID-1 is a basement membrane component enriched along the VNC. In the absence of *nid-1*, the AVG axon is misguided at a low penetrance. From this screen, we previously identified several genes, including *aex*-3, a *rab-3* guanine exchange factor. *aex-3 and rab-3* genetically interact with *unc-5*, a receptor for the guidance cue UNC-6/netrin.

Here we describe a new gene in this pathway:

ccd-5 is a putative cdk-5 binding partner that acts in a pathway with *cdk-5, aex-3, unc-18,* and *unc-5*. *ccd-5 nid-1* double mutants exhibit elevated navigation defects of AVG, command interneuron, and motoneuron axons. Navigation defects of interneuron and motoneuron axons significantly correlate with AVG pioneer axon defects. This suggests that most of the follower axon defects observed are a secondary effect of the AVG defects, and that *ccd-5* specifically affects pioneer axon navigation.

To determine the functional effects of these navigation defects

, we used a multi-worm tracker to assess locomotion, responsiveness, and habituation. We found the *ccd-5* single mutants have no significant behaviour defects, and *ccd-5 nid-1* double mutants are less responsive to mechanosensory stimuli than *nid-1* single mutants. These surprisingly minor behavioral defects indicate either a high tolerance for axon guidance defects within the motor circuit and/or an ability to maintain synaptic connections among commonly misguided axons.

802A Membrane-anchored UNC-6/Netrin reveals roles of both close- and long-range interactions in regulating VD growth cone dorsal outgrowth

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The UNC-6/Netrin guidance cue directs dorsal-ventral axon pathfinding and is expressed in ventral cord motor neurons. Classically, UNC-6 was thought to form a ventral-to-dorsal gradient that was interpreted dynamically by growth cones. Our previous VD growth cone imaging studies indicated that the mechanism is likely more complex, involving discrete aspects of growth cone polarity coupled with regulation of growth cone protrusion. This polarity/protrusion model parts from the classical gradient model in important ways. First, growth cone polarity is separable from growth cone protrusion (Gujar et al.,

2019). Second, UNC-40/DCC and UNC-5 receptors both have roles in growth cones that grow dorsally away from UNC-6 (Norris and Lundquist, 2011; Norris et al., 2014).

To further test the polarity/protrusion model, we constructed a membrane-anchored UNC-6 molecule by adding a C-terminal transmembrane domain through genome editing called *unc-6(lq154)*. This mutant is predicted to lack diffusible UNC-6. The AVM neuron is born laterally and extends an axon ventrally toward UNC-6. As there is no contact between AVM and UNC-6-expressing cells, diffusible UNC-6 is predicted to be required to guide the AVM axon. *unc-6(lq154)* animals display axon guidance defects to the same extent as *unc-6(ev400)* null mutants, consistent with the idea that no diffusible UNC-6 is produced. In contrast, VD/DD axon guidance defects of *unc-6(lq154)* were less severe than those of *unc-6(ev400)*, suggesting that *unc-6(lq154)* retains some function in VD/DD axon guidance. *unc-6(ev400)* VD growth cones were unpolarized, whereas polarity in VD growth cones near the ventral surface in *unc-6(lq154)* was normal. Growth cones further from the ventral surface, in the dorsal half of the animal, were unpolarized in *unc-6(lq154)*. This result suggests that initial growth cone polarity is normal in *unc-6(lq154)*, but polarity is lost as the growth cones migrate dorsally. Possibly, a close-range or contact-mediated interaction of UNC-6 and UNC-5 polarizes the growth cone, but longer-range diffusible UNC-6 is required to maintain this polarity. Preliminary studies indicate that hypomorphic *unc-5* alleles, which are predicted to affect only the long isoforms of UNC-5, show a similar phenotype. This leads to the notion that the short *unc-5B* isoform mediates close-range polarity, possibly a contact-mediated event, whereas the long isoforms might mediate maintenance of polarity over longer distances requiring diffusible UNC-6.

803B Visualization of Synaptic Remodeling

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The architecture of the developing brain is highly dynamic with new synapses appearing as others are removed. Despite the key role of synaptic plasticity in sculpting circuit function, little is known of the molecular pathways that determine whether a synapse will be eliminated or maintained during development. To address this question, we are studying synaptic remodeling in GABAergic Dorsal D (DD) motor neurons in *C. elegans*. DD synaptic remodeling involves the translocation of ventral presynaptic DD terminals to the dorsal side during early larval development. Although DD remodeling was originally discovered by EM reconstruction, dynamic imaging methods with fluorescently labeled synaptic markers are more readily implemented for temporal studies of the underlying cell biological mechanisms. To circumvent the potential problem of over-expression from transgenic arrays, we are testing an endogenously labeled presynaptic marker, GFP::RAB-3. Because it is closely associated with synaptic vesicles, RAB-3 is a reliable marker for the presynaptic apparatus. In this case, we are using cell-specific flippase to limit GFP::RAB-3 expression to DD neurons. From snapshots of GFP::RAB-3 at five different time points in late L1 early L2 *C. elegans* larvae, we have confirmed that DD presynaptic domains are eliminated from the ventral side as new dorsal GFP::RAB-3 puncta emerge. We are now using our native GFP::RAB-3 marker to screen candidate genes for roles in the DD remodeling program. The long-term goal of this work is to identify remodeling genes that also govern synaptic plasticity in the human brain.

804C SYG-2/nephrin mediates incorporation of new synapses into preexisting circuits

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Genetic insults that disrupt synaptogenic proteins have been well established as risk factors for an array of heritable neurodevelopmental and neuropsychiatric disorders, but the mechanisms by which these mutations perturb synapse assembly may differ at various developmental stages. Leveraging the stereotypical neuronal circuitry, genetic tractability, and optical accessibility of the nematode *C. elegans,* we are investigating the molecular mechanisms promoting the incorporation of new synapses into preexisting circuits. A screen in the *C. elegans* DA9 motor neuron revealed that the nephrin family neuronal cell adhesion molecule SYG-2 cell-autonomously regulates the number, size, and spacing of excitatory motor neuron synapses at late developmental stages. We found that SYG-2::GFP localizes to the entire presynaptic domain of DA9, rather than specifically at synapses like other canonical neuronal cell adhesion molecules (such as neurexin), and additionally that SYG-2 acts in the same pathway as NCK-1, a protein involved in actin cytoskeleton remodeling. We hypothesize that SYG-2 functions to regulate the addition of new synapses into preexisting circuits by modulating local cytoskeletal elements through interaction with NCK-1, and we are interrogating this hypothesis using structure/function studies to identify the domain(s) of syg-2/nephrin that regulate its cell-autonomous functions and interact with cytoskeletal remodeling pathways. An integrated understanding

of synaptogenesis at distinct stages of neural circuit development may shed light on how molecular disruptions to synapse formation manifest as neurodevelopmental disorders.

805A Neurexin clustering at synapses is mediated by active zone scaffold intrinsically disordered domains

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Synaptic cell-adhesion molecules (sCAMs) are thought to mediate the formation of neuronal circuits by recognizing appropriate trans-synaptic partners and initiating the assembly of pre- and post-synaptic specializations. Of the sCAMs, neurexins are among the most extensively studied, and all neurexin family members have been identified as risk factors for autism and other neurodevelopmental disorders. The human genome contains three neurexin genes which, through alternative splicing, encode ~4000 long (α), medium (β) and short (γ) isoforms that differ in their extracellular domain but retain an identical intracellular domain. The *C. elegans* genome contains a single neurexin gene nrx-1, which is expressed only as several long (α) and short (γ) isoforms that also share a common intracellular domain. To evaluate whether nrx-1 isoforms are differentially expressed across the *C. elegans* nervous system, we generated promoter reporters for the short (γ) and long (α) isoforms, and found differential expression patterns. We are now using the NeuroPal tool to identify the specific neurons in which these isoforms are expressed. In addition, we have generated long and short isoform-specific knockouts of endogenously-tagged nrx-1 to reveal the contribution of each isoform to overall synaptic expression. We have previously shown that a short (γ) isoform of NRX-1 that doesn't include any canonical extracellular binding domains nonetheless localizes to presynaptic active zones and regulates presynaptic maturation and stability. We now show that expression of the intracellular domain alone, targeted to the membrane with a myristoylation sequence, can rescue the nrx-1 null phenotype (although a cytosolic version does not), indicating that the membrane-bound NRX-1 intracellular domain is sufficient for mediating its presynaptic assembly functions. To identify mediators of NRX-1 localization at synapses, we performed a candidate screen of potential intracellular interactors using endogenously-tagged NRX-1 and identified the synaptic vesicle kinesin UNC-104, as well as active zone scaffold molecules SYD-1 and SYD-2. A deletion of the intrinsically disordered domain of SYD-2, shown previously to mediate its ability to undergo phase separation and thereby recruit additional active zone molecules, affected NRX-1 localization to the same degree as syd-2 null mutants, implicating phase separation as a potential mechanism for NRX-1 recruitment to synapses.

806B A sex-specific switch in glial gene expression is controlled by a cell-autonomous program involving MAB-3 and NFYA-1

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While both neurons and glia undergo sex-specific changes, most attention has focused on sex differences in neurons. In principle, glia could develop sex differences in response to their neuronal partners or they could have their own cell-autonomous programs for sexual differentiation. In *C. elegans*, we discovered that CEPso glia become sexually dimorphic and initiate expression of a transcriptional reporter (*grl-18*pro:GFP) in adult males only. This change in CEPso glia occurs concomitantly with the activation of their neuronal partners, the male-specific CEM neurons. However, by genetically manipulating the sex identity of neurons and glia, we found that this sex-specific change in CEPso glia does not depend on CEM neurons but instead is determined by the sex identity of glia alone. Using candidate and unbiased genetic screens, we identified the timing factors *lep-2*/Makorin and *lep-5* as well as the sex identity factor *mab-3*/DMRT as required for the sexual differentiation of CEPso glia. In these mutants, adult male *grl-18* expression is delayed or absent, respectively. These factors have also been shown to control sexual differentiation of neurons, but we find that *mab-3* acts cell-autonomously in CEPso glia to switch on adult male-specific genes. Finally, we isolated three novel alleles of the transcriptional repressor *nfya-1*/NF-Y, which has not previously been implicated in sexual differentiation of the nervous system. In *nfya-1* mutants, CEPso glia of adult hermaphrodites inappropriately express *grl-18*. Epistasis analysis shows that loss of *nfya-1* completely bypasses the requirement for *mab-3*, suggesting that *nfya-1* may be a novel downstream effector of *mab-3* that regulates sex-specific gene expression in glia. Together, our findings suggest a model in which male-specific expression of *mab-3* in CEPso glia leads to the repression of NFYA-1, thereby allowing male genes to switch on in adults. These results demonstrate that glia do not passively respond to changes in their neuronal surroundings but instead initiate their own program of sexual differentiation.

807C HLH-3 is required for the terminal differentiation of AIM interneurons in adult males

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The transition of juvenile to mature cell identity is often manifested by alterations in their transcriptome, connectivity, and/or morphology. These changes are regulated by developmental timing and sex identity. Our research is focused on characterizing the role of the bHLH transcription factor, HLH-3, in the maturation of sex specific and sex shared neurons that have been co-opted for sex specific functions. Previous work has shown that expression of *hlh-3* in hermaphrodite specific neurons (HSNs) and ventral cord type C neurons (VCs) is necessary to acquire a terminally differentiated state in hermaphrodites (Perez & Alfonso 2020). We also have evidence that a number of male specific neurons, including CEMs and ray neurons, lack terminal identity features in *hlh-3* null mutants (Marquez & Alfonso, unpublished). A growing body of evidence demonstrates that sex-shared neurons in males become altered to function in a sex specific manner upon the fourth larval stage to adulthood transition. For example, sex-shared male AIM interneurons change neurotransmitter identity during sexual maturation. To address whether *hlh-3* has a role in the terminal differentiation of sex shared neurons coopted for sex-specific functions in males, we asked whether AIMs acquire their terminally differentiated state in *hlh-3(lof)* adult males by assessing their neurotransmitter identity. Our data shows that *hlh-3(lof)* adult males retain increased levels of *eat-4(VGLUT)* expression and lack wild type levels of *cho-1(ChT)* expression, that is, adult male AIM interneurons do not acquire a terminally differentiated state in the absence of *hlh-3* function.

808A Decoding pharyngeal neuron fate specification

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The pharynx has its own nervous system consisting of 14 neuron types that form an independent circuit almost completely isolated from the somatic nervous system. We know that the homeodomain transcription factor (TF) *ceh-34* is required to specify all pharyngeal neuron types (see abstract by B. Vidal et al). We hypothesize that there must be other factors operating with *ceh-34* in a target gene-dependent manner, forming a combinatorial code that gives each pharyngeal neuron its unique identity. In support of this notion, we found that *ceh-34* interacts with two homeobox genes, *ttx-3* and *unc-86*, to affect NSM neuron specification.

In order to find additional factors that may collaborate with *ceh-34*, we are using (1) a candidate gene approach based on homeodomain TF expression, (2) we are performing unbiased forward genetic screens and (3) we are dissecting the cis-regulatory region of terminal effector genes. So far mutant analysis of homeobox genes expressed in the pharynx has shown that *ceh-2*, *ceh-7* and *vab-15* affect neurotransmitter identity in specific pharyngeal neurons, making them candidate cofactors for *ceh-34*. Furthermore, forward genetic screens have yielded four mutants with defects in *nlp-8* expression in the I2 neurons. We are in the process of pinpointing the molecular lesion responsible for this mutant phenotype. Finally, dissecting the endogenous *unc-17* cis-regulatory region we have identified a conserved motif required for the expression of *unc-17* in MC and I1 neurons.

We continue to work on these three approaches, which hopefully will lead to a broad understanding of the regulatory code underlying pharyngeal neuron specification.

809B UNC- 70 (Spectrin) acts cell autonomously and non-autonomously to maintain the neuronal microtubule cytoskeleton

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The microtubule cytoskeleton plays a central role in neuron development and functioning. It serves as tracks for cargo transport and offers structural support to the axons and dendrites. Previously we identified a cortical anchoring complex that holds the microtubule cytoskeleton in place in neurons, where UNC-119 functions as a linker between the cortical UNC-44 (Ankyrin) and the microtubule binding UNC-33 (CRMP) (He et al., 2020). Whereas ankyrin proteins are well described scaffolds that connect various membrane proteins to the cortical spectrin-actin cytoskeleton, it is unclear how these interactions relate to the role of UNC-44 (Ankyrin) in maintaining the microtubule integrity.

Here we used a floxed *unc-70* (beta-Spectrin) allele for loss of function studies in a tissue specific manner. Whereas neuron specific depletion did lead to a strong reduction in its binding partner UNC-44 (Ankyrin), this did not lead to major microtubule defects. However, Spectrin depletion in both the PVD neuron and surrounding hypodermis did lead to defects in microtubule cytoskeleton immobilization and polarity organization in axons and dendrites. In agreement, we found that neuron specific depletion had only mild defect on dendritic arbors of the PVD neuron, whereas when co-depleted in the hypodermis the defects were much more pronounced. We are currently investigating whether hypodermal spectrin controls neuronal adhesion proteins such as SAX-7 (NRCAM), which in turn may bind to the UNC-44 (Ankyrin) to connect to the microtubules. Altogether,

we found that UNC- 70 (Spectrin) acts cell autonomously and non-autonomously to maintain the neuronal microtubule cytoskeleton.

810C PTRN-1 (CAMSAP) and NOCA-2 (NINEIN) redundantly mediate MTOC localization and microtubule polarity in dendrites

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In neurons the microtubule cytoskeleton is key for proper axon and dendrite deployment. In axons microtubules are arranged with their plus ends distal to the cell body, whereas dendritic microtubules are predominantly arranged with their minus ends distal to the cell body (invertebrates) or with mixed orientation (vertebrates). This difference in microtubule organization allows for selective transport into axons or dendrites to differentiate these cell extensions. It was recently found in *C. elegans* that during dendrite development of the PVD neuron, RAB-11 positive vesicles function as a non-centrosomal microtubule organizing center (MTOC). These vesicles localize to the growing dendritic tip to nucleate microtubules with their characteristic minus ends distal organization (Liang et al. eLife, 2020). However, the mechanism that localizes these MTOC vesicles to the dendrite tip is poorly understood. Looking for proteins that may act at the microtubule minus ends, we found that PTRN-1 (CAMSAP) and NOCA-2 (NINEIN) act redundantly for distal MTOC localization during dendrite development; their loss of function leads to defects in microtubule polarity establishment. We found that NOCA-2 colocalizes to the MTOC vesicles, and its depletion partially disrupted the recruitment of γ-Tubulin to the MTOC vesicles. CAMSAP proteins are well described microtubule minus-end binding proteins that can stabilize/protect the minus-end from depolymerization. Not surprisingly we observed a punctate PTRN-1 distribution throughout the dendrite but also an accumulation around the MTOC vesicles. Surprisingly we found that during dendrite tip growth the MTOC vesicles and the surrounding Camsap puncta co-migrate with the growing tip, suggesting that these structures are connected. We are currently probing whether sliding of the distal microtubule skeleton is responsible for pushing the MTOC vesicles forward to form and maintain the dendritic minus end distal microtubes.

811A Gene regulatory networks underlying cell fate specification of a *C. elegans* sensory/inter/motor neuron-type

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Neuronal differentiation and specification are coordinated through a cascade of gene expression via interactions between *trans*-acting transcription factors and *cis*-regulatory elements of their target genes. However, the mechanisms by which specific transcription factors determine neuronal cell fate during development are still not clear. In *C. elegans*, the IL1 sensory/inter/motor neurons consist of six neurons that appear to regulate the rate and pattern of foraging movement (Hart et al., 1995). To identify molecular and cellular mechanisms by which IL1s are terminally differentiated, we searched for *trans*-acting factors and *cis*-regulatory motifs that are necessary and sufficient for the specification of IL1s. To investigate *trans*-acting factor, we performed candidate gene searches and forward mutagenesis screens and isolated *nfya-1* mutants which show decreased *flp-3* neuropeptide gene expression in IL1L/R. *nfya*-1 encodes nuclear transcription factor Y alpha subunit, which is evolutionarily conserved. NFYA-1 is localized to the nuclei of IL1s and transient NFYA-1 expression in *nfya-1* mutants restores *flp-3* expression. To identify *cis*-regulatory motifs, we performed promoter analysis of the set of genes that are specifically expressed in IL1s, including *flp-3* (Kim et al., 2004). We identified a motif that is necessary for *flp-3* expression in IL1L/R, DNA sequences of the motif share limited sequence conservation with putative NFYA-1 binding sequences. We also found distinct motifs exist in promoters of other IL1-expressed genes including *unc-8* DEG/ENaC cation-sensitive channel gene and *eat-4* vesicular glutamate transporter gene. Interestingly, the motifs in *unc-8* and *eat-4* promoters affect not only L/R cells but all IL1 cells. Taken together, we propose that NFYA-1 regulates asymmetric neuronal subtypes differentiation between lateral and dorsal/ventral neurons. We are currently identifying the precise function of the *nfya-1* gene in IL1 neuronal specification.

812B Elucidating the role of NHR-25 in shaping and maintaining neuron structure

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The PVD sensory neuron pair offer a powerful model for studying how complex dendritic trees are patterned and their structure may be modified and repaired. We previously demonstrated the cell-to-cell fusion proteins (fusogens) EFF-1 and AFF-1 play key roles in preserving the PVD structure. EFF-1 maintains PVD stereotypic dendritic shape by retracting and fusing excess branching while AFF-1 mediates aspects of its regenerative potential following injury. However, the site of action, regulation, and temporal contribution of fusogens to neuron plasticity and function remain largely unknown.

Unpublished results from our lab (WBPaper00043316, WBPaper00030126) identify a candidate regulatory transcription factor, the nuclear hormone receptor NHR-25, acting upstream to *eff-1* and *aff-1* activities in PVD morphogenesis and repair. We have previously demonstrated that *nhr-25* mutants display aberrant intra-dendrite fusion events, misshaped structures, and persistent excessive outgrowth following injury. Epistatic analysis revealed that *nhr-25* influences intra-dendrite fusions by inhibiting *aff*-1. In contrast, *nhr-25* activates *eff-1* to elicit pruning after injury, outgrowth, and successful regeneration. These genetic results lack spatial and temporal resolution, which we plan to address using the recently improved system for auxin-inducible degradation. By using precise tissue-specific protein depletion techniques we will assay the relative contribution of EFF-1, AFF-1 and NHR-25 to aspects of neuron morphogenesis, maintenance, and repair. We will obtain strains edited to include a degron tag on the endogenous *eff-1*, *aff-1*, and *nhr-25* genes. These tagged proteins will be degraded in the presence of the plant hormone auxin and an adaptor protein, TIR1. Since TIR1 is not endogenous to *C. elegans,* we could remove them solely in the PVD or from other specific tissues selectively expressing TIR1. Observing PVD morphogenesis and injury response and repair in the presence or absence of auxin in such strains will enable us to directly determine the spatial and temporal requirements for NHR-25 and the fusogens EFF-1 and AFF-1 in dendrite morphogenesis and repair.

813C Coordination of neuronal activity and transcriptional programs in motor circuit remodeling

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Neuronal plasticity and circuit stability are fundamental properties of brain development and function. Activity-dependent changes to neuronal connectivity often occur within a defined time window, also known as a developmental plasticity window. How neuronal activity contributes to such precise timing of neural circuit rewiring is a central question in neuroscience. Ultrastructural connectomic studies that began nearly 50 years ago revealed that during the first larval stage, the *C. elegans* locomotor circuit undergoes dramatic synaptic rewiring known as 'DD synapse remodeling' as postembryonic motor neurons are born to establish the mature motor circuit (White et al., 1978). Live imaging studies subsequently showed that presynaptic terminals in DD motor neurons are progressively removed from the ventral side and new synapses are formed at dorsal locations from mid to late L1 stages (Hallam and Jin, 1998). The precise timing of DD synapse remodeling has been shown to depend on several transcriptional programs and can be modulated by neuronal activity. However, it remains unclear which form of neuronal activity affects the time window of this developmental plasticity, and how neuronal activity is molecularly coupled to transcription regulation. To address this, we are using fluorescently tagged reporters for *in vivo* detection of the key transcription factors, such as LIN-14 and UNC-30. To precisely determine L1 developmental stages, we use P cell nuclear migration and divisions using Nomarski optics (Sulston and Horvitz, 1977). We have also generated a nuclear calcium sensor to measure activity in DD neurons during synapse remodeling. We will present our detailed findings on the changes in nuclear calcium dynamics before, during and after DD synapse remodeling.

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814A It takes two: Hox proteins cooperate to specify midbody fates in male CP neurons

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Hox transcription factors are key regulators of anterior-posterior fates in animals; Hox-mediated specification of neuron fates on the A-P axis is essential to nervous system organization and function. In the ventral nerve cord of *C. elegans* males, fates of the nine male-specific CP neurons depend on the activities of the Hox proteins LIN-39 and MAB-5, which are expressed in partially overlapping domains. LIN-39 defines fates of anterior CPs 1-4, including expression of the neurotransmitter genes *tph-1* and *flp-22*; MAB-5 defines fates of posterior CPs 7-9, including expression of *flp-21*. In the midbody, LIN-39 and MAB-5 together define fates of CPs 5-6, which express *tph-1*, but not *flp-22* or *flp-21*. In *mab-5(e1751gf)* mutants, MAB-5 is overexpressed in the anterior ventral cord, leading CPs 1-4 to adopt normally CP 5-6-specific traits: CPs 1-4 lack *flp-22::gfp* expression and express *tph-1::mCherry* at high levels normally seen in CPs 5-6. This is consistent with the idea that, together, LIN-39 and MAB-5 are sufficient to define CP 5-6 fate.

How are LIN-39 and MAB-5 activities coordinated to define a unique neuron fate in their region of co-expression? The *lin-39(ccc16)* mutation sheds light on this question. *lin-39(ccc16)* specifically alters CPs 5-6, such that CPs 5-6 express posterior (CP 7-9-like) traits. In contrast, fates of CPs 1-4, which also normally depend on LIN-39 activity, are relatively unaffected by *lin-39(ccc16)*. We hypothesize that the protein encoded by *lin-39(ccc16),* which is predicted to lack sequences C-terminal to the homeodomain, is able to specify CP fates in the absence of MAB-5 (i.e., in CPs 1-4), but is unable to interact with MAB-5 to define CP 5-6 fates. Consistent with this, *lin-39(ccc16)* males express *tph-1::mCherry* only in CPs 1-4, whereas *lin-39(ccc16) mab-5(0)* mutants express *tph-1::mCherry* in CPs 1-6, suggesting that LIN-39(ccc16) is functional only in MAB-5's absence. To test whether sequences C-terminal to the LIN-39 homeodomain are necessary for interactions among LIN-39, MAB-5, and TALE cofactor CEH-20we used bimolecular fluorescence complementation to query protein interactions *in vivo*. Preliminary results suggest that full-length LIN-39 interacts with MAB-5 and CEH-20, but that LIN-39(ccc16) fails to interact with MAB-5. We hypothesize that sequences C-terminal to LIN-39's homeodomain mediate formation of a complex that contains LIN-39 and MAB-5, allowing them to coregulate targets to define CP 5-6 fates.

815B Neurodevelopmental toxicity assessment after pesticides exposure using *C. elegans*

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Pesticides are widely used in agriculture to kill unwanted insects, worms, and fungi that interfere with crop growth. Although pesticides are developed through stringent regulation processes to have minimal impact on human health, people who use pesticides or regularly come in contact with them have potential health risks such as respiratory diseases, cancers, and neurological diseases. A hazard assessment of pesticides is primarily based on acute toxicity (LD50: Lethal Dose 50%). However, the chronic toxicity of pesticides, especially neurotoxicity, has not yet been carefully tested since it is more difficult to determine. Pesticides also induce oxidative stress, which is closely related to neurotoxicity. Here, we assessed neurotoxicity and oxidative stress caused by various active ingredients in pesticide products, using *C. elegans* as a model system. We found that both neurotoxicity and oxidative stress are increased in a dose-dependent manner by many single compounds. We will continue to screen these single compounds showing high neurotoxicity and plan to quantify the expression levels of oxidative stress-related genes. Also, we will test whether mitochondrial fragmentation occurs in the cells. We hope to understand the neurotoxic mechanisms of various pesticides and our research will provide a reassessment of pesticide safety in an aspect of neurodevelopmental toxicity. This study was supported by the National Research Foundation of Korea (NRF-2019M3C7A1031836, NRF-2019R1A2C1003329).

816C LRON-11 functions in axon guidance within the ventral nerve cord of *C. elegans*

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lron-11 is a member of the extracellular Leucine Rich Repeat ONly (*lron*) gene family in *Caenorhabditis elegans*. The Leucine-Rich Repeats (LRRs) motif facilitates protein-protein interactions. In many organisms, extracellular LRR genes have been implicated in axon guidance, fasciculation and synapse formation. This includes another member of the *lron*family, *dma-1*, which encodes an adhesion receptor that functions in dendrite guidance in *C. elegans*. Therefore, we systematically investigated all members of the *lron* family for involvement in axon guidance. We found substantial axon guidance defects in the ventral nerve cord (VNC) of *lron-11* mutants. We observed defects in motor neurons, PVPR, and command interneurons axons. A common defect was aberrant crossing of the midline. For example, the pioneer PVPR's axon incorrectly crossed the midline in ~15% of *lron-11* mutants. In addition, *lron-11* mutants also showed some commissural defects. Normally, motor neurons send commissures from the ventral nerve cord dorsally, up a specific side of the animal. However, in approximately 50% of *lron-11* mutants, multiple commissures traveled up the wrong side. We also observed motor neuron neurites errantly enter the left VNC tract in ~20% of worms. Since LRON-11 is a single pass transmembrane protein with all its LRR motifs on its extracellular domain, these results suggest that LRON-11 is a putative receptor for axon guidance cues in *C. elegans*. Next, we will use CRISPR and expression constructs to verify whether LRON-11 is expressed in neurons with these defects, to further our understanding of its function in axon guidance.

817A A noncanonical role for Hox in the *C. elegans* ventral nerve cord

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Hox proteins constitute a phylogenetically conserved family of transcription factors that play essential roles during early animal development, such as control of regional identity and cell survival. However, our understanding of Hox gene function during

post-embryonic stages remains rudimentary. To bridge this gap, we focused on the *C. elegans* nerve cord, which is populated by eight different classes of motor neurons (MNs) – the cholinergic DA, DB, VA, VB, AS, VC and the GABAergic DD and VD neurons. By generating an endogenous reporter allele for the mid-body Hox gene *lin-39* (Scr/Dfd/Hox4-5), we found that it is continuously expressed – from developmental to adult stages – in MNs of all these eight classes. Using null and auxin-inducible alleles, we observed that *lin-39* is required not only to establish, but also maintain in the adult the expression of effector molecules that define MN final (terminal) identity, such as neurotransmitter biosynthesis proteins, ion channels and adhesion molecules. Chromatin immunoprecipitation followed by sequencing (ChIP-Seq) for LIN-39 together with mutational analysis of its cognate binding site strongly suggest that it acts directly to activate expression of its target genes. LIN-39 does not act alone. Our genetic and biochemical studies indicate that it collaborates with two terminal selector-type transcription factors: UNC-3/Ebf in cholinergic MN classes (DA, DB, VA, VB, AS) and UNC-30/Pitx in GABAergic MNs (DD, VD). We also found that LIN-39 partners with another Hox gene *(mab-5*/Antp/Hox6-8) to consolidate the identity of both cholinergic and GABAergic MNs. Our genetic and biochemical analyses suggest that the expression of *lin-39* and *mab-5* in adult nerve cord MNs is maintained via direct transcriptional autoregulation. Intriguingly, *lin-39* and *mab-5* can jointly control the expression levels of *unc-3* in cholinergic MNs, thereby generating a coherent feed forward loop-type of mechanism (i.e., Hox and *unc-3* activate the same effector molecules, but Hox proteins also activate *unc-3*) for the establishment and maintenance of motor neuron identity. Based on recent profiling studies showing Hox gene expression in the adult fly and mouse nervous systems, the noncanonical Hox gene function described here may be phylogenetically conserved.

818B Sustained expression of *unc-4/Hox* and *unc-37/Groucho* in postmitotic neurons specifies the spatial organization of the cholinergic synapses in *C. elegans*

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Neuronal cell fate determinants establish the identities of neurons by controlling gene expression to regulate neuronal morphology and synaptic connectivity. However, it is not understood if neuronal cell fate determinants have postmitotic functions in synapse pattern formation. Here we identify a novel role for UNC-4 homeobox protein and its corepressor UNC-37/Groucho in tiled synaptic patterning of the cholinergic motor neurons in *Caenorhabditis elegans*. Using temperature sensitive mutants and the auxin-inducible degron system, we show that *unc-4* is not required during neurogenesis but is required in the postmitotic neurons for proper synapse patterning. In contrast, *unc-37* is required in both developing and postmitotic neurons. The synaptic tiling defects of *unc-4* and *unc-37* mutants are suppressed by *bar-1/β-catenin* mutation, which positively regulates the expression of *ceh-12/Hb9*. Ectopic *ceh-12* expression partly underlies the synaptic tiling defects of *unc-4* and *unc-37* mutants. Our results reveal a novel postmitotic role of neuronal cell fate determinants in synapse pattern formation through inhibiting the canonical Wnt signaling pathway.

819C Molecular mechanisms regulating organization of sensory neuron cilia

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Primary cilia are present on all sensory neurons and are critical for their functions. The organization of neurons and their processes within neuropils has been shown to be important for their functions in multiple organisms. However, whether the organization of cilia within a sense organ is similarly important for sensory neuron function is unclear. In *C. elegans*, the cilia of eight chemosensory neurons of the head amphid sense organs are present within a channel formed by surrounding glia. Previous work from the Sengupta lab showed that these cilia are stereotypically arranged within this channel and form specific contacts with one another. We found that *sax-7* mutants, which have altered amphid neuron dendrite order, retain stereotypical cilia organization within this channel, suggesting cilia arrangement is not a consequence of dendrite order. Cilia-cilia contact appears to be dependent on the presence of ciliary distal segments. Because flagella adhesion during *Chlamydomonas* mating is mediated by glycoproteins, we asked whether glycoproteins might control ciliary organization and cilia-cilia contact in *C. elegans*. Our preliminary results indicate that mutations in an enzyme involved in synthesizing N-linked glycoproteins alters ciliary organization and cilia-cilia contact. In a screen of candidate regulators of cilia organization, we identified the conserved phogrin homolog IDA-1. Mutations in *ida-1* alter cilia position within the amphid channel. IDA-1 localizes to the ciliary base; however, mutations in the BBSome protein BBS-7 mislocalize IDA-1 to the cilia. To determine whether IDA-1 localization is critical for regulating ciliary position, we examined the cilia of *bbs-7* mutants. Our preliminary results indicate that cilia in these animals are also disorganized. Additionally, the cilia-cilia contacts typically seen in wild-type

animals may be altered in *bbs-7* mutants. IDA-1 is a transmembrane protein localized to the membrane of dense-core vesicles (DCVs) and is implicated in regulating DCV cargo release. Mutations in *unc-31* disrupt DCV exocytosis, however, the cilia of *unc-31* mutants appear largely wild-type, suggesting that *ida-1* control of ciliary organization is independent of its role in DCV signaling. Our current experiments are aimed at further exploring the molecular mechanisms underlying ciliary organization and establishing the contribution of cilia-cilia contact for regulating sensory neuron functions.

820A EOR-1/PLZF and EOR-2 Inhibit Expression of the RIM or RIC Neuronal Cell Fates

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The development of each of the 302 neurons in the *C. elegans* nervous system is tightly regulated, but mechanisms underlying specification of these neuronal cell fates are incompletely understood. For example, the tyraminergic RIM and octopaminergic RIC interneurons are involved in diverse sensorimotor behaviors, including reversals, head movements, and feeding, but the factors guiding the development of these neurons are largely unknown. To identify regulators of RIM/RIC neuronal development, we used genetic screens for genes that when mutated cause extra GFP-positive cells in *tdc-1::GFP* transgenic worms, which express GFP specifically in the two RIM and two RIC neurons. We screened approximately 67,000 genomes and isolated 3 alleles of *eor-2* (*egl-1* suppressor/DiO uptake defective/*raf* enhancer). *eor-2* encodes a co-factor of the transcription factor EOR-1, a homolog of the promyelocytic leukemia zinc finger (PLZF). We found that *eor-1* and *eor-2* act in the same genetic pathway to prevent the generation of extra GFP-positive cells. A *ced-3(lf)* mutation results in 8-9 *tdc-1:GFP* positive cells, and the addition of the *eor-2(n5163)* mutation, which generates a premature stop codon in the fifth exon of the 17 exons in *eor-2*, increased this number to 10-11 cells. This observation suggests that EOR-1/EOR-2 act separately from CED-3 and regulate the fate of specific neurons rather than the deaths of the RIM and RIC sister cells. Next we used RIM- and RIC-specific reporters to ask whether the extra GFP-positive cells in *eor-1(cs28)* loss-of-function mutants were RIM-like or RIC-like. We found that the *eor-1(cs28)* mutants ectopically expressed both the RIM and the RIC reporter, and that the ectopic RIM and RIC reporters were expressed in different cells, suggesting that EOR-1 regulates the fate of at least two different cell types to prevent fate transformation to RIM-like or RIC-like cells. Additional experiments using other cell-specific markers suggested that the extra RIM-like cells normally differentiate to become GABAergic RME neurons. Our results demonstrate that the EOR-1 transcription factor and EOR-2 together regulate the cell-fate determination of specific neurons during development. We hope this study will provide novel insights concerning the functions of the evolutionarily conserved PLZF transcription factor in animal development and of the dysfunction of PLZF in human diseases, such as cancer.

821B Microtubule dynamics regulates gap junction trafficking and placement in the motor circuit

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Gap junctions, or electrical synapses, mediate fast communication between neurons but the mechanisms that direct gap junction assembly between specific neurons or within specific subcellular compartments are poorly understood. Gap junction localization ultimately depends on kinesins, which walk along microtubule (MT) tracks to deliver gap junction components to their cellular destinations. We are investigating gap junction assembly in VA motor neurons in which the UNC-4 transcription factor and its corepressor UNC-37 regulate the neuron specificity and placement of electrical synapses. Gap junction trafficking is disrupted in *unc-4* mutants (Palumbos et al, worm meeting 2019). To investigate the mechanism of this effect, we utilized the MT plus-end marker, EBP-2::GFP, to monitor MT dynamics. We determined that the rate of MT polymerization and outgrowth length are reduced in *unc-37* mutant VA neurons. VAB-8, an atypical kinesin that binds MTs but lacks motor activity, is negatively regulated by UNC-4/UNC-37. We are now testing the hypothesis that ectopic expression of VAB-8 in *unc-4* mutant VA neurons disrupts gap junction trafficking by altering MT dynamics.

822C CUT class homeobox genes redundantly control panneuronal identity features in *C. elegans*

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Panneuronally expressed genes, such as genes involved in synaptic vesicle biology, are critical for the proper function of all neurons, but the control mechanisms that direct such genes to all neurons remain poorly understood. Here, we have identified the CUT class homeobox factors as critical regulators of panneuronal gene expression, as demonstrated by the following observations. First, six different CUT factors are expressed neuronally in *C. elegans*: *ceh-48* and *ceh-44* show a panneuronal expression pattern, with little if any expression outside the nervous system, while *ceh-38*, *ceh-21, ceh-39* and *ceh-41* are

ubiquitously expressed. Nervous system expression is observed for all 6 genes throughout all developmental stages starting in embryos and maintained in the adult stage. Second, loss of individual factors does not cause obvious phenotypes, however mutation of CUT genes in different combinations leads to a reduction of expression of panneuronal gene reporters, with the strongest phenotype observed when all six CUT factors are removed in a sextuple null mutant strain. A more extensive neuronal transcriptional profiling in neurons lacking all neuronal CUT genes reveals that these factors are required for the expression of large cohorts of panneuronal genes. We found that CUT factors are required for neuronal function since CUT mutants show defects on synaptic transmission and worm locomotion. Third, the high sequence similarities in their DNA binding domains predict that these factors will recognize the same binding motif. Indeed, available ChIP-seq data for a subset of the CUT factors reveals binding to panneuronal gene promoters at the same locations. Deletion of the shared CUT binding motif from panneuronal gene endogenous reporters disrupts expression, indicating direct binding. To sum up, CUT factors are required for panneuronal gene expression and proper nervous system activity. Their redundant function might have evolved as a safeguard mechanism to prevent profound impacts on neuronal function. Members of the CUT family in other invertebrate and vertebrates also show panneuronal expression, indicating that they could have a conserved role in nervous system specification.

823A Characterizing the nervous system of the nematode *Pristionchus pacificus* - similarities and differences with *C. elegans*

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The nervous systems of many nematodes are remarkably similar despite considerable divergence of their genomes. We are examining the evolution of nematode nervous systems by characterizing the nervous system of the nematode *Pristionchus pacificus (Ppa)* to compare with that of the highly described *C. elegans (Cel),* which boasts a complete cell lineage, and a complete set of neurons and their connectivities in both sexes (Sulston & Horvitz, 1977; Sulston et al., 1983; White et al., 1986; Cook et al., 2019, Nature 571: 63–71). Within a few years, we and others seek to approach a similar level of characterization for *Ppa.* For example, the connectivity and likely homologies for head sensory neurons in *Ppa* have been determined, demonstrating considerable similarities plus a few striking differences, such as the absence of amphid 'winged' cilia found in *Cel* (Hong et al., 2019, eLife 2019;8:e47155). Considerable progress has been made in transgenics, traditional reporters and CRISPR generation of mutants and short knock-ins. To study neuronal specification in *Ppa,* we have epitope-tagged transcription factor genes to examine their expression patterns, including *Ppa* orthologs of *unc-3*, *unc-86* and *unc-42*. We have also found that an antiserum to *C. elegans unc-86* works in *Ppa,* showing a pattern identical to that of the epitope-tagged locus. The pattern of *Ppa-unc-86* expression is remarkably similar to that of *Cel*; one difference is in the absence of clear HSN homologs in hermaphrodites. This is consistent with the absence of HSNs in *Ppa* seen with anti-serotonin. Other differences in serotonergic neurons suggest possible differences in the role of *Ppa-unc-86* in *Ppa* vs. *Cel.* In *Cel,* the 'terminal selector' *unc-86* plays a role in specifying serotonergic fate (Zhang et al., 2014, Development 141: 422-435). For example, in *Cel,* the AIM neurons express both *unc-86* and serotonin; in *Ppa* presumptive AIM neurons lack serotonin but still express *unc-86.* We will present these and other results of our analysis of nervous system similarities and difference in *C. elegans* and *P. pacificus.*

824B Investigating the effects of altered gravity on dendritic structures during development in *C. elegans*

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Space flight has shown that altering gravity can affect many biological processes including muscle and bone development. However, gravity's effect on neuronal development is not clear. Previously, we showed that hypergravity affects axonal development of motor neurons. Here, we will 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. We identified hypergravity-induced abnormal structures in the PVD neuron, particularly 4° branch defects. Since basement membrane protein UNC-52/perlecan is known for its fundamental role for patterning PVD 4° branches, we exposed UNC-52/perlecan mutants to hypergravity. Preliminary results show that unc-52 mutants can suppress hypergravity-induced PVD "L" and "T" shape 4° branch defects. Furthermore, we are exposing *C. elegans* to different gravity forces and gravity time frames. This work will let us know the minimum force and critical time point for inducing PVD dendrite defects.

We plan to expose *C.elegans* to simulated microgravity on a clinostat, and real microgravity aboard the International Space Station. Now we are planning to prepare our next space flight launching in 2021 and 2022.

825C Inositol pentakisphosphate kinase-1 (IPPK-1) is involved in ventral nerve cord assembly in *C. elegans*

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Assembly of the *C. elegans* ventral nerve cord (VNC) involves many features of mammalian neurulation, including regulation by the planar cell polarity (PCP) pathway and rosette mediated convergent extension. The VNC is formed through a stereotypic migration of neuroblasts from either side of the embryo toward the midline, followed by intercalations that constrict the width of the tissue and extending its length. These processes transition the tissue it into a single line of neurons. Failure of this process results in aberrant motor neuron position or spacing defects along the VNC. We have found that mutations in *ippk-1*/Inositol pentakisphosphate kinase leads to broadening of the embryonic VNC and mispositioning of the motor neurons post-hatching. *ippk-1* encodes a kinase which plays a central role in inositol phosphate metabolism and has been linked to multiple developmental processes and conditions, including neural tube defects (NTDs). Here, we describe how the activity of IPPK-1 contributes to its function. Convergent extension during VNC formation is regulated by the planar cell polarity (PCP) and SAX-3/ROBO pathways; loss of either pathway or IPPK-1 results in anterior shifts in neuron spacing. We show that IPPK-1 likely acts in parallel to PCP and SAX-3/ROBO signalling to regulate VNC assembly. Time-lapse fluorescent microscopy was used to examine the role of IPPK-1 in VNC assembly. In *ippk-1* mutants, we found that left and right neuroblasts display defective initial cell contacts just prior to central rosette formation. Additionally, rosette resolution is also delayed leading to persistent cell contacts. To determine if the metabolic product of IPPK-1 activity is important for its function, we injected inositol hexaphosphate (IP6) directly into the gonads of young adult worms. We found that IP6 injection rescues motor neuron spacing defects. These results suggest that IPPK-1-derived inositol phosphate metabolites promote proper convergent extension movements during embryonic VNC development.

826A The PBAF chromatin remodeling complex is required for cholinergic motor neuron subtype identity

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We have previously shown that the evolutionarily conserved COE (Collier, Olf, Ebf)-type transcription factor UNC-3 acts as a terminal selector and determines cholinergic motor neuron (MN) identity in multiple cholinergic MN classes (SAB, DA, DB, VA, VB, AS). UNC-3 directly controls the expression of both shared (e.g., acetylcholine pathway genes) and class-specific terminal identity genes (e.g., ion channels, neurotransmitter receptors). However, *unc-3* is expressed in all these MN classes, leading us to hypothesize the existence of repressor proteins that restrict the ability of UNC-3 to activate these class-specific genes more broadly. To test this hypothesis, we performed a forward genetic screen using the UNC-3 target gene *glr-4*, which encodes a glutamate receptor subunit selectively expressed in SAB MNs. We found that *pbrm-1*, the sole *C. elegans* ortholog of the evolutionarily conserved chromatin regulator BAF180, selectively prevents expression of a transgenic *glr-4* reporter in DA, VA, and AS classes, resulting in mixed MN identity. Similar results were obtained when we monitored endogenous *glr-4* expression via RNA fluorescent in situ hybridization and a reporter allele. Since PBRM-1/BAF180 is a subunit of PBAF, a chromatin remodeling complex of the SWI/SNF family, we reasoned that animals lacking gene activity for other PBAF subunits might display similar MN phenotypes. We indeed found that loss of *swsn-9* (*C. elegans* ortholog of human BRD7 and BRD9), *swsn-7* (*C. elegans* ortholog of human ARID2), and *phf-10* (ortholog of human PHF10) results in gain of *glr-4* expression in these three MN subtypes. Rescue and RNAi experiments using cholinergic MN-specific promoters (*cho-1* and *lin-39*) further demonstrated that these four PBAF components, despite their ubiquitous expression, act cell-autonomously in post-mitotic MNs. Finally, we found that the transcription factors MAB-9/Tbx20 and UNC-4/UNCX represses *glr-4* expression in AS and DA/VA neurons, respectively. To account for the observed specificity of PBAF-mediated *glr-4* repression in select MN classes, we hypothesize that PBAF is recruited by MN class-specific transcription factors (e.g., MAB-9, UNC-4) to repress UNC-3 target genes. Altogether, we provide novel insights on the epigenetic mechanisms that generate neuronal diversity by uncovering a previously unrecognized, neuron-specific role for the PBAF chromatin-remodeling complex in selective repression of terminal selector target genes.

827B DIP-2 and SAX-2 play synergistic roles to maintain *C. elegans* neuronal morphology

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Many *C. elegans* neurons project monopolar or bipolar neurites. However, it is unknown how the stereotypical morphology established during development is maintained throughout life. Several factors have been found to regulate the morphology of various types of neurons. Among these, DIP-2 (Disco-interacting-protein 2) (Noblett et al 2018) and SAX-2 (sensory axon guidance defect) (Zallen et al 1999) are critical for the maintenance of neuronal morphology. DIP-2 represses ectopic neurite outgrowth from neuronal soma, and suppresses injury-induced axon regrowth. SAX-2 also represses ectopic neurite outgrowth. Both proteins are expressed in the nervous system, and act cell-autonomously to repress ectopic neurites in neurons. DIP-2 is a member of a conserved family of proteins implicated in acyl-CoA metabolism. Our genetic studies between *dip-2* and the Kennedy phospholipid synthetic pathway show that DIP-2 acts downstream of the phospholipid pathway. SAX-2 is a member of the Fry/Furry family of proteins implicated in cytoskeletal dynamics (Nagai et al., 2013). By knock-in of fluorescent protein tag to endogenous locus, we show that DIP-2 displays diffuse cytosolic expression in neurons, whereas SAX-2 localizes to cytoplasmic puncta whose identity is currently being investigated. To understand potential interactions between these pathways influencing neuronal morphology, we have analyzed *dip-2* and *sax-2* double mutants and found that these animals display synergistic defects in neuronal morphology, as well as synergistic developmental and reproductive defects. Exploiting these synergistic defects, we conducted genetic screens and isolated multiple suppressors of the phenotypes of *dip-2*, *sax-2*, or both. We are currently mapping these suppressors to better understand how the DIP-2 and SAX-2 pathways act to maintain neuronal morphology.

828C Specific N-glycans fine-tune somatosensory dendrite patterning

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Dendrites are essential for the transmission and processing of stimuli through the nervous system, and their development requires the precise orchestration of many proteins. For example, the *C. elegans* PVD somatosensory neurons require a conserved cell-adhesion 'menorin' complex - comprised of skin-derived MNR-1/Menorin and SAX-7/L1CAM, muscle secreted LECT-2/Chondromodulin II, and the transmembrane receptor, DMA-1/LRR-TM, in PVD - for their stereotyped arborization. We found that a key enzyme in the N-glycosylation pathway, AMAN-2/Golgi alpha-mannosidase II, plays a role in fine-tuning PVD dendrite patterning. *aman-2*/GM-II encodes an evolutionarily conserved enzyme required for the formation of complex and paucimannose N-glycans. Mutations in *aman-2*/GM-II result in dendritic trees with reduced complexity, and enhance the severity of dendrite defects in hypomorphic alleles of members of the menorin complex. AMAN-2/GM-II requires enzymatic activity in PVD to form higher order branches, suggesting that N-glycosylation of a menorin complex component in PVD itself may be significant. Consistent with this hypothesis, we find that DMA-1/LRR-TM is glycosylated *in vivo* with primarily high-mannose/hybrid N-glycans, and that DMA-1/LRR-TM carries larger, abnormal N-glycans in *aman-2/GM-II* mutants. Importantly, we determined that the presence of abnormal N-glycans, rather than the absence of wildtype N-glycans, is the root cause of the observed defects, and likely leads to altered protein-protein interactions. Lastly, we found that specific N-glycosylation sites in DMA-1/LRR-TM are important for PVD dendrite morphogenesis. Collectively, our findings suggest that specific N-glycan structures fine-tune dendrite patterning, possibly by regulating complex formation of the DMA-1/LRR receptor with other components of the menorin complex.

829A Specificity in Glia-Neuron Interactions

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Nervous systems consist of two cell types, neurons and glia, that physically and molecularly interact. One site where glia associate closely with neurons is the neuron receptive endings (NREs), where neurons receive input. Glia can actively regulate NRE shape and function, with consequences on neuron activity and animal behavior. Each glia can associate with multiple NREs. However, whether or how a single glia regulates different associated NREs remains a crucial outstanding question.

Unlike most organisms, *C. elegans* has invariant glia-neuron cell-cell contacts and each cell is born of invariant lineages. This makes it a uniquely tractable system to investigate specificity of individual glia-NRE interactions. To examine this, we focused on the *C. elegans* amphid sheath (AMsh) glia, which interacts with the NREs of 12 different sensory neurons, including the thermosensory AFD neuron, odor-sensing AWC neuron, and taste-sensing ASE neuron. Ablation of AMsh glia impacts most associated NRE shapes and/or function.

We found that AMsh glia localizes the cation chloride cotransporter KCC-3 to a molecular micro-domain only around AFD-NRE, hinting at non-uniform interaction between AMsh glia and different NREs. Indeed, while AMsh glial KCC-3 regulates AFD NRE shape and associated thermosensory behavior, it notably does not affect ASE or AWC NRE shape, or ASE-mediated behavior.

To probe how this specificity is achieved, we sought to determine how AMsh KCC-3 localizes to a glial micro-domain. Surprisingly, our genetic and laser ablation studies reveal that the AFD neuron does not recruit KCC-3 localization. Instead, disrupting microtubule-based cilia, present on all amphid NREs, is required to localize glial KCC-3 around AFD-NRE. We are currently investigating the role of individual amphid NREs on KCC-3 localization. Our structure-function studies also identify a 89 amino-acid sequence on KCC-3 that guides its localization. Current studies aim to localize this further and determine interacting partners.

These studies show that *C. elegans* AMsh glia regulate different NREs differently by localizing specific regulatory molecules to single NRE contact sites, and aim to shed light on the underlying molecular mechanism.

830B *unc-44* (Ankyrin) is required for axon stability in *C. elegans*

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After an axon reaches and forms synapses with its target, it must become stable and persist potentially for the life of the organism. It is becoming clear that regulation of the axonal cytoskeleton is necessary for this process; mutations in known cytoskeletal components like unc-44 (ankyrin) cause highly disordered nervous systems in C. elegans. Axons in unc-44(-) animals branch, sprout ectopic growth cones, and exhibit severe degeneration. Historically, unc-44 has been categorized as being required for axon outgrowth. Using longitudinal and time-lapse imaging during larval development, we show that UNC-44 is not required for proper outgrowth in GABA neurons. In unc-44(-) animals these axons extend normally toward their targets, but continue to sprout growth cones. These defects increase in frequency as the animal ages, implying a defect in maturation. We recently showed that the expression of a long, 6,994aa isoform of UNC-44 is required for axon stability, but it is still unclear how this protein stabilizes the axon. Using CRISPR, we have shown that only the C-terminal ~850aa are required for UNC-44's neuron maturation function; deleting this region phenocopies unc-44 null mutations. This region contains three domains conserved in nematodes. Based on further truncations it appears that these act combinatorially; deleting individual conserved domains still yield nulls. We are currently mutagenizing this region further and using protein biochemistry to precisely map the sequences in UNC-44's C-terminus and the binding partners that are required for neuron maturation.

831C Systematic analysis of CAMs expressed in ray neurons in *C. elegans.*

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Although cell adhesion molecules (CAMs) have been studied for many years as essential players in neuronal connectivity, we have yet to break the code by which cell adhesion molecules help neurons find appropriate partners. While some CAMs such as *sax-3*/ROBO and *unc-40*/DCC have been heavily researched, many CAMs' functions remain unknown. We recently identified the expression patterns of 100 neural CAMs across 173 neurons and 64 muscles in the *C. elegans* male tail. This data showed that A type and B type ray neurons, which are adjacent sensory neurons derived from the same progenitor cells, do not share expressions of most CAMs. There are nine pairs of rays in the male tail, with each ray being composed of two different types of sensory neurons (RnA and RnB) and a structural cell. The A- and B-type neurons make different connections, which also differ from ray to ray. To investigate whether cell type-specific CAMs expression is important for ray neuron development and connectivity, we tested all viable mutants of CAMs expressed in RnB neurons.

Mutants of *unc-40* and *sax-3* showed a significant defect in both anterior-posterior and dorsal-ventral axon trajectory, and *fmi-1*/Flamingo showed a defect in dorsal-ventral and diagonal axon trajectory. In contrast, the other 6 CAMs mutants didn't have any defects in axon pathfinding. We also tested the possibility that CAMs work redundantly by testing multiple mutants and found a deletion in *hmr-1B/N-cadherin*, which does not lead to any morphological defect in a single mutant, enhanced the defect of the *sax-3* mutants. *lron-7/OPTC* and *lron-13* mutations, which do not affect RnB morphology in the single mutants, slightly suppressed the defect of *sax-3* and *fmi-1*. These results suggest that CAMs across gene families function combinatorially to regulate the RnB morphology.

We employed fluorescent transgenes for the synaptic vesicle protein RAB-3 driven by a B-type neuron-specific promoter to visualize the synaptic connectivity. The quantification of signal intensity of RAB-3::mCherry showed that *unc-40*, *sax-3*, *fmi-1, lron-7*, *lron-13*, and *nrx-1/neurexin* mutants showed significantly low RAB-3 signal intensity. The function of lron-7 and lron-13 in synaptic formation has not been reported before.

832A A Rab-like GTPase Restricts Dendritic Branching

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Dendrite development depends on numerous 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 pathway consists of several factors that function from different tissues to promote PVD dendrite development and have been heavily studied. However, much less is known about factors that restrict branching. During a genetic screen, we have identified a locus that encodes for a putative, uncharacterized Rab-like GTPase, which we name *rabl-3*. Time course analyses determined that the number of branching points was significantly increased in adult animals, suggesting *rabl-3* suppresses dendrite growth. A transcriptional reporter shows expression in the epidermis from early embryonic through adult stages and transgenic expression of a *rabl-3* cDNA in the epidermis is sufficient to rescue the mutant phenotype, possibly in manner that is independent of the GTPase activity of RABL-3. We found that a rescuing N-terminal translational RABL-3 fusion displays intracellular, perinuclear localization, indicative of typical Rab GTPases. Genetic analyses show that the Menorin pathway is largely epistatic to *rabl-3*, indicating it may function in the same genetic pathway. In addition, RNAi knockdown of *rab-5* phenocopies the mutant *rabl-3* excessive branching phenotype, suggesting sorting into the early endosome could play a role in suppression of dendrite branching. Current experiments aim to (1) determine whether mutations in *rabl-3* affect localization of components of the Menorin pathway, (2) determine the subcellular localization of *rabl-3*, and (3) identify potential molecular interactions to build a mechanism by which RABL-3 restricts dendritic branching.

833B Forward Genetic screening to identify novel regulators of neuronal Microtubule cytoskeleton

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A neuron has a polarized structure with a long axon and several dendrites which is established and maintained by microtubule cytoskeleton. Microtubules are heteropolymers of α and β-tubulin dimers and have intrinsic polarity with a fast growing plus end and a slow growing minus end. We are using Touch receptor neurons (TRN) of C. elegans, to understand the basis of this organization and regulation of neuronal microtubules.

One of the major factors that depolymerize microtubules is the kinesin-13 family protein KLP-7. The loss of klp-7 causes excess stabilization of microtubule cytoskeleton leading to ectopic neurite extension in touch neurons and other neurons as well (Ghosh-Roy et al 2012; Puri et al 2019). We found that this phenotype in klp-7(0) can be reversed by a microtubule-destabilizing drug colchicine or in backgrounds lacking either alpha or beta tubulin. We hypothesized that the suppressors of klp-7(0) neuronal phenotype might encode for novel regulators of the neuronal microtubule cytoskeleton. With this idea, we did a forward genetic screen in klp-7(0) mutant using Ethyl Methane Sulfonate(EMS). We isolated 26 mutants that suppress the ectopic posterior extension phenotype of ALM neuron in klp-7(0), out of 12,422 F1's from a clonal screening.

To identify the exact causal mutant, the suppressors were crossed four times with (N2) Bristol to collect 10 individual recombinants. We sequenced the pooled lysates of these recombinants and the FASTQ sequences of Whole Genome Sequencing (WGS) data were analyzed on the Galaxy user interface using MimodD tools. The list of SNP variants was used to generate mapping plots, which gave putative homozygous EMS SNPs for a given suppressor. These SNPs affect genes that encode for tubulin subunits, RNA-binding protein, Ca2+ dependent secretion activators, kinases and ECM components. We will present a comprehensive analysis of the characterization of a few mutants to unravel their roles in regulating microtubule cytoskeleton.

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834C A secondary structural motif in the *kpc-1* 3'UTR promotes dendritic transport of transcripts and local translation to regulate dendrite branching and self-avoidance of a nociceptive neuron

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Mechanical stimuli on the skin of *C. elegans* are detected by the dendritic arbors of PVD nociceptive neurons, which provide uniform sensory coverage outside the head region across the entire animal. Through genetic screens, we isolate three mutants that display profound dendrite branching and self-avoidance defects in PVD neurons and through whole genome sequencing, we identify the responsible mutations in the *kpc-1* gene. Compared to wild-type animals, a strong *kpc-1* mutant allele exhibits a significantly lower number of secondary dendrite branches whereas a weak *kpc-1* mutant allele displays tertiary dendrite self-avoidance defects. Although *kpc-1* was previously implicated in dendrite branching and self-avoidance, the mechanism by which *kpc-1* regulates the process is unknown. Here, we show that the *kpc-1 3′ UTR* is required for the *kpc-1*'s function in dendrite branching and self-avoidance. The *kpc-1* 3'UTR facilitates *kpc-1* RNA localization to branching points and contact points between sibling dendrites. Using fluorescence recovery after photoconversion, we show that the *kpc-1* 3›UTR promotes local translation in the distal segment of PVD dendrites. We identify an important secondary structural motif in the *kpc-1* 3'UTR required for tertiary dendrite self-avoidance. We demonstrate that over-expression of *kpc-1* leads to greater self-avoidance without limiting initial dendrite outgrowth, supporting a direct role of *kpc-1* in self-avoidance. Animals with *dma-1* receptor over-expression display similar secondary dendrite branching and tertiary dendrite self-avoidance defects that are suppressed with *kpc-1* over-expression, which suggests that DMA-1 is a potential KPC-1 target that is down-regulated by KPC-1. Our results support a model where KPC-1 is expressed at the branching points and the contact points between neighboring dendrites to locally down-regulate DMA-1 receptors to promote dendrite branching and self-avoidance.

835A The development and functions of GLR glia.

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C. elegans glia can broadly be divided into three classes: 46 sensory-neuron associated glia (sheath and socket cells), four nerve-ring wrapping glia (CEPsh cells), and six GLR (Glia Like cells of the Nerve Ring) glia. While roles of *C. elegans* sensory organ glia and nerve-ring wrapping glia have been characterized, little is known about the development and functions of GLR glia. The GLR cell bodies lie in a six-fold symmetry just posterior to the nerve ring and extend anterior leaf-like processes that form a seal around the pharynx, isolating the nerve ring from the body cavity. These processes lie in close proximity to the muscle arm plate in the nerve ring, where neuromuscular junctions (NMJ) form with head motor neurons. Based on their morphological characteristics, position, and gene and neurotransmitter expression patterns, GLR roles in NMJ development, activity and motor behavior are likely.

To aid our studies and to understand the molecular basis of the development and functions of GLR glia we first performed GLR-transcriptome analysis. In order to identify genes controlling GLR fate specification and differentiation we are following a candidate gene approach in which mutants and RNAi for transcription factor genes enriched in the GLR transcriptome are tested for defects in GLR gene expression and morphology. We have so far discovered two transcription factors that are important regulators of GLR cell fate specification and differentiation. Apart from exposing regulators of GLR development, newly made conditional mutants in these genes will also be used to inform us on possible functions of these cells in NMJ control.

To directly examine GLR functions in NMJ, we have generated a stable transgenic line in which GLR glia are ablated at the L1 stage. GLR-ablated animals display various motor defects including reduced speed, extended locomotory pausing and increased reversal frequency. A significant overrepresentation for genes related to NMJ synaptic transmission among the most highly enriched GLR-genes further supports our hypothesis that GLR glia could have important functions at the NMJ. We are currently following a candidate gene approach and cell specific rescue experiments to study the involvement of these genes in the observed motor-behavior defects. Progress in these studies will be presented.

836B A negative feedback mechanism regulates DLK-1 signaling in ciliated sensory neurons

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The conserved Dual-Leucine zipper Kinases (DLKs) are neuronal sensors to many forms of stress and traumatic injury. When activated under stress conditions, DLKs act as MAP3Ks upstream of JNK or p38 MAPK signaling to trigger neuroprotective responses. Constitutive activation of DLK, such as via overexpression, can lead to neurodegeneration in different disease models. Previous studies have revealed that under normal conditions, DLK protein activity is controlled by E3 ubiquitin ligase PHR(PAM/Highwire/RPM-1) mediated degradation and chaperones. However, it remains to be discovered whether there are other mechanisms regulating endogenous DLKs. To search for new regulators of *C. elegans* DLK-1, we performed a forward genetic screen using a GFP knock-in that tags endogenous *dlk-1*. We isolated a class of mutants showing ectopic accumulation of DLK-1 at dendritic endings of ciliated sensory neurons. We determined that multiple mutations cause loss-of-function in IntraFlagellar Transport (IFT) genes and impair cilia architecture and function in sensory neurons. The ectopic accumulation of DLK-1 at defective cilia activates signal transduction cascade, as we observed elevated expression of the transcription factor CEBP-1, a key downstream target of DLK-1 pathway, in many sensory neurons. However, the increased CEBP-1 expression shows selective dependency on *dlk-1*. To probe into the neuron-type specificity, we carried out quantitative analyses of CEBP-1 levels in several types of sensory neurons. We found that IFT defects in AWB and AWC cause increased CEBP-1 expression dependent on *dlk-1,* whereas similar IFT defects in ASI and ASH induce CEBP-1 elevation independent of *dlk-1.* Moreover, in AWC *cebp-1* further represses expression of wild type DLK-1 but not a kinase-dead DLK-1, suggesting a negative feedback control of DLK-1 signaling via CEBP-1. Additionally, we found that in AWC neurons overexpression of DLK-1 causes dendrite overgrowth by activating CEBP-1. Together, we propose that in ciliated sensory neurons, DLK-1 signaling is regulated under a cell-specific feedback mechanism, which is coupled with IFT and may contribute to the maintenance of neuronal morphology.

837C Molecular topology of an entire nervous system

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Neurons are the fundamental structural and functional units of the nervous system. Although all neurons share many common features, they also display remarkably diverse morphological and functional characteristics. To uncover the underlying genetic programs that specify individual neuron identities, the CeNGEN consortium produced scRNA-Seq profiles of > 100,000 cells from the L4 stage *C. elegans* hermaphrodite, including all neuron classes and several non-neuronal cells (e.g., glia, muscle, hypodermis, reproductive tissues). In addition, we identified distinct subclasses for 10 of the 118 anatomically-defined classes. Our results suggest that individual neuron classes can be solely identified by combinatorial expression of specific gene families. For example, each neuron class expresses unique codes of ~23 neuropeptide genes and ~36 neuropeptide receptors thus pointing to an expansive “wireless” signaling network. To demonstrate the utility of this uniquely comprehensive gene expression catalog, we used computational approaches to identify cis-regulatory elements for neuron-specific gene expression. Because our scRNA-Seq data match the single cell resolution of the wiring diagram, we also sought to correlate expression of cell adhesion proteins with neuron-specific fasciculation and connectivity in the nerve ring. We expect that this neuron-specific directory of gene expression will spur investigations of underlying mechanisms that define anatomy, connectivity and function throughout the *C. elegans* nervous system.

These data are available at cengen.org and can be interrogated with the web application CengenApp at cengen.shinyapps.io/CengenApp.

838A Genetic analysis of dendritic tiling and field size

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Neurons rely on dendrites for the acquisition of sensory and synaptic input from their particular receptive fields. Findings of aberrant dendritic morphology in disorders such as autism spectrum disorder (ASD) and schizophrenia highlight the importance of understanding how complex dendritic arbors are developed and maintained. The goal of this project is to characterize mechanisms of dendritic outgrowth and regulation of field size using the multi-dendritic FLP and PVD

mechanosensory neurons of *C. elegans* as a model. The dendritic arbor of FLP covers the head of the worm while the arbor of PVD covers the body. Here, we provide a detailed characterization of FLP and PVD field size throughout development and demonstrate that field size is regulated at least in part by contact-mediated repulsion. Additionally, using an unbiased forward genetic approach, we isolated a mutant allele, *dz197*, in *unc-33/Collapsin Response Mediator Protein (CRMP)*, which differentially effects FLP and PVD receptive field sizes. Previous studies of UNC-33/CRMP null mutants have shown that it is required for establishing microtubule polarity in PVD by binding microtubules and through interaction with a protein complex, anchoring them to the membrane. However, these functions are specific to the UNC-33 long isoform, and no clear roles have been attributed to the medium and short UNC-33 isoforms. We found that *dz197* mutants display distinct phenotypes from UNC-33 null mutants both in FLP and PVD morphology as well as in locomotion. Unlike null mutants, *dz197* mutants can be rescued by expression of UNC-33S. We hypothesize that differences between phenotypes in *dz197* mutants and UNC-33 null mutants are due to the *dz197* mutation disrupting oligomerization between various UNC-33 isoforms and that oligomerization is required for microtubule binding. Comparisons between *dz197* and null mutants may reveal specific roles for UNC-33 in establishing dendritic field size and may reveal differences in field size regulation between FLP and PVD.

839B Molecular Mechanism of Coordinating Cilia Intersection and Elongation

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Cilia and flagella are motile or non-motile cellular organelles that track their surroundings and/or coordinate cell/organism motility. Even though multiple motile cilia are bundled together on a cell surface, non-motile cilium elongates from the cell surface as one cilium. Non-motile cilia from separate cells appear to form joint cilia by extending in parallel to each other. The non-motile cilia of C. elegans PHA and PHB sensory neurons, for example, protrude from the end of the dendrite but they extend side by side and intersect in the middle part of the cilia, reaching the same length. However, the molecular mechanisms underlying how adjacent cilia achieve the same length and converge remain a mystery. In the current study, we employ C. elegans to investigate the molecular mechanisms underlying these characteristics. We discovered that several genes, including arl-13, are needed for the correct convergence of PHA and PHB cilia. We next created a list of genes expressed in either of these two ciliated cells (PHA or PHB cells). For example, the conserved potassium voltage-gated channel gene egl-36 is expressed in PHB cells and not in PHA cells, and we found that it localizes at the base of the cilia. We generated egl-36 mutations in combination with other ciliary gene mutants because the disruption function of egl-36 alone does not cause gross ciliary defects. In these double mutants, we observed short cilia in both PHB and PHA sensory neurons. We are gathering more data for this study right now, and we will present our findings at the conference.

Key words: cilia, cilia biogenesis, arl-13; egl-36

840C A homeodomain transcription factor required to specify all pharyngeal neurons

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In addition to their central nervous system (“brain”), all complex multicellular organisms contain an enteric nervous system that controls the activity of fore-, mid- and/or hindgut. The foregut in *C. elegans* is the pharynx, which contains its own nervous system, the pharyngeal nervous system, composed of only 20 neurons belonging to 14 different types. These neurons form a self-contained circuit that controls pharyngeal pumping. Most pharyngeal neurons directly connect to end organs and can be considered polymodal, with sensory-inter-motor characteristics, a feature that is reminiscent of primitive nervous systems. Thus, understanding how pharyngeal neurons are specified during development might shed light on fundamental aspects of neuronal development and evolution.

ceh-34, a homeodomain transcription factor of the Six family, is continuously expressed in all pharyngeal neurons and no other neurons outside of the pharynx. Remarkably, we have found that in *ceh-34* mutants, pharyngeal neurons are generated, but they all fail to express a wide array of neuron-type identity genes, including neurotransmitter pathway genes, indicating that the communication within the circuit is completely disrupted. Also, the anatomy of the pharyngeal neurons (i.e. axon pathfinding) seems to be highly disorganized. These results show that *ceh-34* acts as a pharyngeal neuron master regulator (“terminal selector”) that defines the identity of individual neurons and assembles them into functional circuitry. Moreover, a conditional AID-based allele demonstrates that *ceh-34* is continuously required during the life of the worm to maintain pharyngeal neuron identity. We hypothesize that *ceh-34* acts together with other transcription factors to form a combinatorial code that gives each pharyngeal neuron its unique identity and we have started to identify such factors (see abstract by B.

Gulez et al). We find that *ceh-34* expression is regulated by the pharynx organ selector *pha-4* indicating that this organ selector utilizes distinct downstream selector genes to pattern distinct tissue types within the pharynx.

842B Coordinated regulation of synaptic genes during development: a tale of a transcription factor and an mRNA export complex

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Synapses are assembled during neuronal development and consist of a pre- and postsynapse, which are built from hundreds of proteins. While the molecular composition and architecture of pre- and postsynapses has been widely explored, much less is known about how synaptogenesis is regulated at the level of gene expression. Is there a mechanism that coordinates the expression of functionally related proteins such that they are ready to assemble into higher order structures concomitantly? In order to identify new players in presynaptic gene expression, our lab has conducted genetic screens and identified mutations that affect two subunits of the THO Complex (THOC), an RNA-binding complex implicated in mRNA export (Maeder et al., 2018). We have previously shown that in dopaminergic neurons, THOC is the primary machinery used for the export of synaptic transcripts. Mutation of THOC results in retention of these synaptic transcripts in the nucleus, while non-synaptic transcripts are largely unaffected and are exported normally. To date, it remains unclear how THOC is able to select such a specific set of targets for RNA export. Are there proteins that interact with THOC to instruct this behavior? Mass spectrometry studies conducted in mammalian systems revealed a novel interaction between EVI1/*egl-43* and THOC (Ivanchoko et al., 2019). EGL-43 possesses 6 zinc fingers, all of which are highly homologous to the zinc fingers of EVI1. We performed ATAC-seq on sorted worm neuronal nuclei and identified putative regulatory regions of synaptic genes. Interestingly, we found that many of these regions contained the consensus DNA sequence that is recognized by EVI1. Using CRISPR/Cas9, we have tested the requirement of some of these binding sites and find that they are required for normal presynaptic gene expression. Depletion of EGL-43 through RNAi or auxin-mediated degredation similarly resulted in loss of synaptic markers in PDE. Using single molecule pulldown, we were able to detect weak but significant binding between EGL-43 and THOC. Taken together, our data suggests that EGL-43 could potentially be a link between THOC and its synaptic targets.

References:

Maeder et al., 2018, Cell 174, 1436–1449

Ivanchoko et al., 2019, Nucleic Acids Research 47, 1225–1238

843C β-tubulin BEN-1 has a key role in regulating DLK-1 signal transduction

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The conserved mitogen activated kinase kinase kinase (MAPKKK) DLKs play critical roles in neuronal process growth, synapse formation and axon regeneration. DLKs also act as sensors to mediate neuronal responses to different stresses, including microtubule (MT) disruption. However, it is not clear in which contexts DLKs sense MT stress and which signaling transduction mechanisms control the activity of DLKs to mediate specific functional outcomes. In *C. elegans* the DLK ortholog DLK-1 is normally expressed in most neurons at low levels. From a forward genetic screen for mutants displaying aberrant DLK-1 levels or localization, we identified a mutant that exhibited aggregated DLK-1 in neuronal soma. Using whole genome sequencing and genetic mapping, we determined that this mutation affected *ben-1*, one of six β-tubulin isotypes in *C. elegans*. Levels of the transcription factor CEBP-1, a downstream target induced by DLK-1, were elevated in neurons of *ben-1* mutants. These findings suggest that altering *ben-1* function activates *dlk-1*, resulting in *cebp-1* dependent transcription. A *ben-1* transcriptional reporter is exclusively expressed in neurons. BEN-1 is known to convey the sensitivity to benomyl[1], a microtubule-targeting compound used for killing parasitic nematodes and treating cancers. We found that benomyl treatment also caused neural DLK-1 aggregation, CEBP-1 elevation, as well as neuronal defects such as impaired synaptogenesis. Altogether, our findings

suggest that changes in neuronal β-tubulin BEN-1 containing MT cytoskeleton elicit specific stress responses to activate *dlk-1/ cebp-1* signaling.

1. Driscoll, M., Dean, E., Reilly, E., Bergholz, E., and Chalfie, M. (1989). Genetic and molecular analysis of a Caenorhabditis elegans beta-tubulin that conveys benzimidazole sensitivity. *J. Cell Biol*. 109, 2993-3003.

844A A combination of artificial intelligence and *C. elegans* in identifying neuronal mitophagy inducers

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Mitochondrial dysfunction, due to defective mitophagy, triggers the progression of Alzheimer's disease (AD). The identification of potent mitophagy modulators is critical for the development of novel therapeutic intervention strategies. Here, we describe a high-throughput machine-learning approach combined with laboratory validation using a *C. elegans* model in identifying robust neuronal mitophagy inducers. Lead compounds inhibit memory loss in both *C. elegans* and mouse models of AD. Collectively, our findings demonstrate a conserved mechanism of memory loss in both Aβ- and Tau- AD models that is mediated by defective mitophagy, while our highly accurate *in silico* screening platform paves the way for identifying potent mitophagy modulators to promote neuronal health and brain homeostasis during aging.

845B Interactome analysis of *C. elegans* synapses by TurboID-based proximity labeling

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Characterizing the proteome of subcellular structures and the interactome of specific proteins provides entry points to probe molecular function. Several methods for screening protein-protein interactions (PPIs) have proven useful, including affinity purification and two hybrid screens. However, these methods have limitations, including poor detection of transient or weak PPIs, high false positives, and difficulty detecting PPIs in specific cell types or subcellular compartments. Many of these limitations can be overcome using proximity-dependent protein labeling (PL). We optimize a PL protocol for *C. elegans* using TurboID, a recently improved *E. coli* promiscuous biotin ligase. We analyse the proteome of individual tissues including the nervous system, intestine, hypodermis and muscle, and, to demonstrate single cell resolution, of the pair of AFD neurons. We observe enrichment of tissue-specific proteins, and identify both known and previously unknown AFD-specific proteins. To characterize the interactome of a specific protein, we knocked TurboID into the endogenous *elks-1* gene, which encodes a presynaptic active zone protein. ELKS-1-TurboID highlights both characterized and previously uncharacterized synaptic proteins. We use our data to define non-specific contaminants that should help interpretation of *C. elegans* PL data. TurboID proximity labeling provides a powerful tool to investigate *C. elegans* biology.

Research support: This work was supported by an Advanced ERC Grant (269058 ACMO) and a Wellcome Investigator Award (209504/Z/17/Z) to MdB and an ISTplus Fellowship to MA (Marie Sklodowska-Curie agreement No 754411).

846C Exercise using an Acoustic Gym can rescue neuronal loss in worms

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Parkinson's disease (PD) is the second most prevalent neuronal disorder that affects the older population at a higher rate. PD is characterized by a loss of dopaminergic (DA) neurons in the substantia nigra part of the brain. Currently there is no cure for PD. Regular long-term exercise has been proposed to be an attractive option in alleviating PD symptoms. However, the will and ability to exercise decreases significantly in the older population due to poor health and physical weakness. It is still challenging to identify beneficial exercise -related factors as a possible way of reducing neuronal loss in the aged brain. Here we introduce a microfluidic device integrated with Surface Acoustic Wave (SAW) technology as a potential treatment to reduce DA neuronal loss in a *Caenorhabditis elegans* PD model. Loss of DA neurons can be partially suppressed in worms overexpressing human α-synuclein by SAW-induced swimming exercise in a controllable manner. We propose that this platform will help us identify potential drug targets of PD and compounds that can enhance the beneficial effects of exercise.

847A Deep learning tools for *C. elegans* whole-brain imaging

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Advances in deep learning and computer vision have revolutionized analysis of neuron activity and behavioral data. Here we present a deep learning (DL) toolbox – a collection of three deep learning methods to solve challenging problems in *C. elegans* whole-brain imaging.

First, accurate segmentation of densely packed nuclei in fluorescence channels is critical for downstream tasks such as cell tracking, signal extraction and identity annotation. While many deep learning methods are available for 2D images, 3D segmentation methods for highly anisotropic images are not available. We combined a well-known DL framework for instance segmentation in 2D images with optimal transport based clustering to produce 3D segmentations in anisotropic images. Comparison against other methods on experimental and synthetic datasets show that our method is more accurate (5-8% higher F1 score) and more robust across a range of baseline cell signals and image noise levels (6-17% higher F1 score). Further DL method is 3.7 times faster than previous method.

Second, whole-brain imaging in freely moving worms is currently not widespread because of the requirement of custom designed microscopes with low-magnification behavior tracking and high-magnification fluorescence imaging channels. We developed and optimized a fast DL framework (30-644 times smaller model size and 5-8 times faster than previous methods) to directly predict the worm pose (skeleton) from fluorescence channels. Fast inference of pose using only fluorescence channel enables worm-tracking thus eliminating the need of separate behavior channel. We show that predicted worm pose can be used for behavior analysis and cell-tracking in videos. Further, eliminating the need of custom microscopes will enable more labs to do whole-brain imaging in freely moving animals.

Third, we developed a DL framework for restoring low signal-to-noise ratio (SNR) fluorescence images (acquired at low laser power/low exposure time) to high SNR images. Low laser power imaging eliminates photo-bleaching of fluorophores, light damage to worms and enables long-term neuron activity imaging across days. Image restoration can be performed on both GCaMP and RFP channels. We show that restored images provide cleaner calcium signals compared to traditional de-noising methods (13-30% smaller mean-absolute error compared to clean trace). Additionally, restored images also improve cell detection, tracking and identity annotation tasks.

Methods in our toolbox can be easily adapted for similar tasks in other organisms.

848B Chemical *in vivo* activation of *C. elegans* neurons using a histamine-gated cation channel

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The ability to non-invasively activate and inactivate neurons in intact behaving animals provides powerful methods to study the functions of neural circuits. Recently, transgenic expression of a *Drosophila melanogaster* histamine-gated chloride channel (HisCl1) in *C. elegans* has enabled hyperpolarization of specific neurons in response to histamine (Pokala et al., 2014). The small molecule histamine is used as a transmitter in many species however *C. elegans* lack endogenous histamine signaling. We will present data to show that modification of the pore domain of this histamine-gated channel renders it selective to cations rather than anions. We find that cell specific expression of this modified channel, which we named HisCat1, enables histamine induced activation of neurons and can elicit specific behaviors. Pan-neuronal expression of HisCat1 induced uncoordinated seizure like behavior, whereas expression in cholinergic neurons caused hypercontraction and inhibited locomotion in animals exposed to histamine. When we expressed HisCat1 in serotonergic neurons, histamine exposure induced persistent pharyngeal pumping in the absence of food, consistent with the serotonin release. Finally, we demonstrate that HisCat1 can be used to depolarize non-neuronal tissues as expression in body-wall muscle induces calcium influx in response to histamine. Chemical activation using the HisCat1 channel provides a useful complement to existing optogenetic tools. Neuronal activation using HisCat1 appears to be stable over long durations, allowing prolonged activation that is more difficult to achieve with existing optogenetic methods. Furthermore, the HisCat1 channel can be combined with Channelrhodopsin and optical sensors such as GCaMP, so light and histamine can be used to activate and monitor several neurons independently in the same experiment. Therefore, HisCat1 is an effective means to non-invasively activate neurons in *C. elegans* and increases the existing toolkit of light induced sensors and ion channels.

Pokala, N., Liu, Q., Gordus, A., Bargmann, C.I., 2014. Inducible and titratable silencing of Caenorhabditis elegans neurons in vivo with histamine-gated chloride channels. Proc. Natl. Acad. Sci. U. S. A. 111, 2770–5.

849C The Role of Alzheimer's disease relevant Tau modifications in Neurodegeneration and Mitochondrial dysfunction

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Background:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder whose pathological hallmarks include intraneuronal neurofibrillary tangles (NFTs) composed of the microtubule-associated protein Tau. Tau isolated from AD brain exhibits abnormally high levels of post-translational modifications (PTMs) including phosphorylation and acetylation at specific epitopes that increase with disease severity and age. In addition, mitochondrial dysfunction is an early feature of AD, and abnormal, toxic tau PTMs may contribute to disease pathogenesis. A major bottleneck in understanding the mechanisms behind the neurotoxicity of pathological forms of Tau is the lack of genetically tractable models that can recapitulate the effects of Tau PTMs in a short time frame without artifacts associated with Tau overexpression.

Method:

Human 0N4R Tau (wild type) was expressed in touch receptor neurons through single-copy gene insertion. Mutations were introduced into the single-copy tau transgene through CRISPR-Cas9 genome editing, including T231E, to mimic phosphorylation of a commonly observed pathological epitope, and K274/281Q, to mimic disease-associated lysine acetylation. We then assessed their impact on age-dependent response to light touch, neurodegeneration, and mitochondrial parameters such as abundance, morphology, trafficking, and turnover, using fluorescent biosensors including mito-mKeima.

Result:

Unlike existing tau overexpression models, *C. elegans* single-copy expression of wild type human tau did not elicit overt pathological phenotypes at baseline. However, strains expressing disease associated PTM-mimetics (T231E and K274/281Q) exhibited reduced touch sensation and neuronal morphological abnormalities that increased with age. Remarkably, the PTM-mimetics selectively impaired mitophagy following mitochondrial oxidative stress, but had no effect on macroautophagy, and furthermore reduced mitolysosomal trafficking.

Conclusion:

Single copy expression limits pathological phenotypes to strains expressing disease-associated Tau mutants. In addition to overt pathology, these mutants eliminate oxidative stress-induced mitophagy and reduce trafficking of mitolysosomes. Our findings highlight a selective mechanism through which disease-associated Tau PTMs may suppress compensatory responses to mitochondrial stress that occur with age and provide a new perspective into the pathogenic mechanisms underlying AD.

Published paper:

Guha, S., S. Fischer, G.V.W. Johnson, and K. Nehrke. 2020. Tauopathy-associated tau modifications selectively impact neurodegeneration and mitophagy in a novel *C. elegans* single-copy transgenic model. Mol Neurodegener. 15(1).

850A A deep learning approach to calcium imaging analysis

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Analysis of calcium imaging recordings is tedious, repetitive and time consuming. In this new project, we propose to develop a machine learning-based automated tool for the analysis of recordings from *C. elegans* neurons, thereby reducing noise, time loss and experimenter bias. We are specifically training our model to segment time-lapse fluorescence recordings of the RIA interneuron in semi-restrained animals. Our tool can segment animals and track their head movements as well as identify the three compartments of the RIA neurite. This prototype tool demonstrates the potential of deep learning to accelerate and improve data acquisition from time-lapse fluorescence recordings and other imaging data.

851B Optogenetic tools for manipulation of cyclic nucleotides, functionally coupled to CNG-channels

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The cyclic nucleotides cAMP and cGMP are ubiquitous second messengers that regulate numerous biological processes by activating e.g. protein kinases (PKA and PKG) or cyclic nucleotide gated channels (CNGCs). In eukaryotic GPCR signalling, cAMP is generated predominantly by membrane-bound adenylyl cyclases (mbACs), which are located in microdomains together with GPCRs, PK(A) and their targets. The existing optogenetic toolbox in *C. elegans* is restricted to soluble adenylyl cyclases (i.e. microbial photoactivatable adenylyl cyclases (PACs) from *Euglena* (euPAC) and *Beggiatoa* (bPAC), and the synthetic phytochrome-linked cyclases IlaC22 k27 and PaaC), the membrane-bound *Blastocladiella emersonii* guanylyl cyclase opsin (BeCyclOp) and hyperpolarising rhodopsins (e.g. *Natronomonas pharaonis* halorhodopsin - NpHR). Yet missing are membrane-bound photoactivatable adenylyl cyclases (mbPACs) and hyperpolarizers based on K^+-currents. To obtain mbPACs, we mutated 2-3 key amino acids in the active site of *Blastocladiella* and *Catenaria* CyclOps, which are particular in combining a rhodopsin and a guanylyl cyclase domain. For characterization of photoactivatable nucleotidyl cyclases, we expressed the proteins alone or in combination with CNGCs ("two-component optogenetics") in muscle cells and cholinergic motor neurons. To investigate the extent of optogenetic cNMP production and the ability of the systems to de- or hyperpolarise cells, we performed behavioural analyses (locomotion, muscle contraction), measured cNMP content *in vitro*, and compared *in vivo* expression levels. We implemented *Catenaria* CyclOp as a new tool for cGMP production, allowing fine-control of cGMP levels. We established the mbPACs YFP-BeCyclOp(A-2x) and YFP-CaCyclOp(A-2x), based on mutated versions ("A-2x") of Be and Ca CyclOp, enabling more efficient and specific cAMP signalling compared to soluble bPAC, despite lower overall cAMP production. For hyperpolarization of excitable cells by two-component optogenetics, we introduced the cAMP-gated K^+-channel SthK from *Spirochaeta thermophila* and combined it with bPAC, BeCyclOp(A-2x), or YFP-BeCyclOp(A-2x). As an alternative, we implemented the *Blastocladiella emersonii* cGMP-gated K^+-channel BeCNG1 together with BeCyclOp. In summary, we established a comprehensive suite of optogenetic tools for cNMP manipulation, useful for applications in many cell types, including sensory neurons, and for potent hyperpolarization.

852C Simultaneous measurements of membrane voltage and intracellular Ca^{2+} of AWA neurons by a gene encoded voltage indicator and GCaMP.

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Measurement of neuronal activities in non-invasive and unanesthetized condition is important for understanding neuronal function in intact animals. Ca^{2+} imaging by fluorescent gene encoded calcium indicators (GECI) are a powerful way to measure neuronal activities in *C. elegans*. Although Ca^{2+} imaging revealed important aspects in neuronal functions, the measurement of neuronal membrane voltage is important to understand the neuronal functions. Furthermore, the relations of change of membrane voltages and changes of Ca^{2+} has not been fully understood. Recently, several types of gene encoded voltage indicators (GEVI) that are derived from 7TM proteins used for optogenetics has been developed to measure changes of membrane voltage in living animals. Even though the fluorescence of these GEVIs is dim, they showed fast time constants and relatively high fluorescent change depend on voltages. Among those GEVIs, we use paQuasAr3 for the voltage measurement, because it shows relatively higher fluorescence with other superior characteristics.

Since AWA, one of the olfactory sensory neurons, which is responsible for diacetyl sensation, was reported to show all-or-none action potentials (Liu et al. 2018), we firstly analyzed AWA voltage changes induced by diacetyl. We found that fluorescence of paQuasAr3 expressed in AWA cell body is changed in response to diacetyl stimulation with high reproducibility. At the beginning of the stimulation, the transient increase and decrease of fluorescence intensity was observed, whereas the relatively higher fluorescence intensity was sustained during the stimulation. To elucidate relations between the Ca^{2+} responses and the voltage responses, we made wild-type animals expressing paQuasAr3 and GCaMP6f in AWA neurons, and measured

both fluorescence at a cell body simultaneously. We found that the changes of paQuasAr3 started faster than the changes of GCaMP. These analyses will give insights on the neuronal functions in informational processing.

853A Chemical profiling of *C. elegans* single neurons using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)

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Characterizing single cell heterogeneity helps reveal the chemical and functional complexity in nervous systems and aids in understanding the neurochemical basis of differential neuronal activity. While the nervous system in *Caenorhabditis elegans* is anatomically and genetically well-defined, the chemical profiles of their single neurons are less well-known. A non-targeted, single-cell chemical profiling of *C. elegans* neurons of interest is desirable to link the chemical complexity to neuronal activities. Mass spectrometry (MS) has been used to detect proteins, lipids, peptides, and metabolites in single cells. We adapt an optically guided, single cell MS technique, microMS, to profile dissociated and diffusely dispersed *C. elegans* single neurons. Dissociated single cells from the pan-neuronal RFP reporter *rab-3p::*NLS::RFP were spread across glass slides, fluorescently imaged, and coated with a chemical matrix. MicroMS identified and converted single cell locations in the fluorescent image into instrument stage positions. The stage positions enable automated single cell sampling with a matrix-assisted laser desorption/ionization MS (MALDI-MS) instrument. We successfully applied lysis and cell dissociation protocols to *C. elegans* and located individual cells using fluorescent microscopy. Preliminary results showed lipid and peptide-like signals from single neurons and we are currently optimizing the sample preparation to increase mass spectral quality.

854B pOpsicle: An all-optical reporter system for synaptic vesicle recycling using pH-sensitive fluorescent proteins

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pH-sensitive fluorescent proteins have been widely used in various model organisms to study vesicle release and recycling. When targeted to the lumen of synaptic vesicles (SVs), these proteins are quenched due to acidification, which is essential for filling SVs with neurotransmitter. Activation of synaptic transmission leads to vesicle fusion with the plasma membrane and exposition of these fluorophores to the extracellular medium resulting in an increase in the emitted fluorescence. Subsequent acidification of SVs can thus be tracked by tagging of an integral SV protein to pH-sensitive proteins. Previous work by the Bargmann lab demonstrated that synaptic transmission and SV recycling in sensory neurons of live worms could be estimated by usage of the green-fluorescent pHluorin (1). However, while sensory neurons can be stimulated by application of the respective stimulus, this is not possible in motor neurons. To investigate the refilling of SV pools in this neuron type, we combined optogenetic methods to stimulate neuronal transmission with the novel red-fluorescent pH-sensitive protein pHuji (2).

pHuji was inserted into the luminal loop of the integral SV protein synaptogyrin (SNG-1). This construct was co-expressed in cholinergic neurons with the blue light-gated ion channel channelrhodopsin-2 (ChR2). When supplemented with its fluorophore *all-trans* retinal (ATR), ChR2 can be used to depolarize neurons. We observed an increase in pHuji fluorescence after blue light illumination, only when animals were treated with ATR. Furthermore, the decay of pHuji fluorescence after stimulation is delayed in mutants of known SV recycling factors such as UNC-26 (synaptojanin) and UNC-57 (endophilin). Also, the increase in pHuji fluorescence is abolished in *snb-1* (synaptobrevin) SV fusion mutants. These results suggest that the rise and decay of fluorescence is an indicator for SV fusion and recycling. We now plan to investigate putative recycling factors through this assay we termed ‘pOpsicle’ for “**p**

H-sensitive **op**

togenetic reporter of synaptic ve**sicle**

recycling") by using semi-automatization based on microfluidic devices. We further want to explore differences in the recycling dynamics of cholinergic and GABAergic motor neurons and possibly other cell types. In sum, pOpsicle allows for a non-invasive and easily applicable investigation of the SV cycle.

(1) Ventimiglia, D. and C. I. Bargmann (2017) Elife 6, e31234.

(2) Shen, Y et al. (2014) J Cell Biol 207(3): 419-432.

855C Quantitative peptidomics in *C. elegans* via targeted mass spectrometry of neuropeptides

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Neuropeptides are a class of bioactive peptides that obtain their biological activity after cleavage from larger precursor proteins. Once released, these peptides are able to regulate standard physiological functions such as digestion and reproduction but also exert long-term effects in more complex processes, including behavioral adaptation, memory processes and aging. Hence, neuropeptides are often an interesting entry point in an effort to study and better understand these types of behavior. Current strategies for studying relative differences in the neuropeptidome often rely on RNA sequencing, which remains completely blind to the real abundancies of actual neuropeptides, as these result from extensive post-translational processing. On the other hand, current mass spectrometric methods for neuropeptide identification are discovery-driven and lack robustness and reproducibility, hampering quantitative studies.

We here present a mass spectrometry-based approach for the relative quantification of the neuropeptidome in *C. elegans*, using a parallel reaction monitoring method. Our current method can detect and quantify 288 mature neuropeptides, this corresponds to 67% of the (known and predicted) neuropeptidome of *C. elegans.* When applied to wild type controls, our method identified 178 neuropeptides with a 96% overlap between samples. Insofar as we are aware, we hereby are able to provide the most extensive method to map the peptidome of an animal. We are validating the method for differential studies, delivering a promising avenue to finally enable the detection and differential analysis of neuropeptidomic variations over different conditions.

856A Introducing optoSynC – a novel optogenetic tool for synaptic silencing

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The investigation of neuronal circuits as well as molecular and cellular functions of neurons requires the control of activity in a spatio-temporal precise manner. The field of optogenetics opens the pathway to stimulate or inhibit specific types of neurons with light. In the last years, several tools have been developed that allow inhibition of specific neurons on time scales from milliseconds to minutes or long-term silencing. However, these optogenetic tools come at the cost of fast induction or reversibility of the altered neuronal function. Thus, a tool which allows for fast, long-term and spatially restricted neuronal silencing, while still allowing for fast reversibility, is of substantial need. Here, we developed an approach to achieve these goals. Using the ability of the *Arabidopsis thaliana* cryptochrome 2 (CRY2) to form homo-oligomers, we designed an optogenetic tool to cluster synaptic vesicles (SVs) and thus inhibit their function acutely. The tool, called OptoSynC (optogenetic synaptic vesicle clustering), comprises CRY2, fused to the synaptic vesicle intrinsic membrane protein synaptogyrin (SNG-1). The efficiency of OptoSynC was evaluated at the behavioral level. Blue light illumination of pan-neuronally expressed OptoSynC significantly reduced swimming cycles by 80% within seconds. Termination of blue light for more than 15 minutes allowed worms to recover their initial swimming behavior. In addition to behavioral assays, inhibition of synaptic transmission could be demonstrated by electrophysiology experiments. Using a combination of optogenetic activation of neurons with the red-light activated channel Chrimson and blue-light activated inhibition using OptoSynC, we could show the effect even in a single neuron, PVD, required for nociception.

While behaviorally, OptoSynC evokes drastic effects, its mode of action has yet to be revealed, as it either inhibits synaptic transmission by SV clustering in the reserve pool, or it might clog-up release sites at the presynaptic terminal due to clustering of SNG-1 protein already present in the plasma membrane. Therefore, we currently employ electron microscopy to shed light on this mechanistic detail.

With further optimization of this tool and the knowledge of its underlying mechanism OptoSynC can be a potent tool for synaptic silencing, that might also be used for the research of how SVs are guided to the plasma membrane or in which precise sequence of events SV recycling proceeds.

857B 3DeeCellTracker, a deep learning-based pipeline for segmenting and tracking cells in 3D time lapse images

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Optical monitoring of cell movements and activities in three-dimensional space over time (3D + T imaging) has become substantially easier due to recent advances in microscopy technology. However, the development of software for segregating cell regions from the background and for tracking their dynamic positions remains a bottleneck in the field. Individual laboratories still need to develop their own software to extract important features from 3D + T images obtained using different optical systems and/or imaging conditions. Moreover, even when identical optical systems are used, optimization of many parameters is often required for different datasets. We developed a software pipeline, 3DeeCellTracker, by integrating multiple existing and new techniques including deep learning for the first time for tracking. With only one volume of training data, one initial correction, and a few parameter changes, 3DeeCellTracker on a desktop PC with GPU successfully segmented and tracked 100–200 head neurons in both semi-immobilized and "straightened" freely moving worms, ~100 cells in a naturally beating zebrafish heart, and ~1,000 cells in a 3D cultured tumor spheroid. While these datasets were imaged with highly divergent optical systems, such as spinning confocal, SCAPE, and 2-photon microscopes, our method tracked 90–100% of the cells in most cases, which is comparable or superior to previous results, and we extracted complex patterns of calcium dynamics in the worm brain neurons and the rhythmic patterns in synchronization with heart chamber movement in the zebrafish heart cells. Thus, 3DeeCellTracker could pave the way for revealing dynamic cell activities in image datasets that have not been analyzed previously.

858C Fast deep learning correspondence for neuron tracking and identification in *C. elegans* using semi-synthetic training

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The nervous system of the nematode C.elegans is well characterized, such that each of the 302 neurons is named and has stereotyped locations across animals(Witvliet et al., 2020). The capability to find corresponding neurons across animals is essential to investigate neural coding and neural dynamics across animals. Despite the worm's overall stereotypy, the variability in neurons' spatial arrangement is sufficient to make predicting neural correspondence a challenge.

We present an automated method to track and identify neurons in C. elegans, called "fast Deep Learning Correspondence" or fDLC, based on the transformer network architecture(Vaswani et al., 2017). The transformer has shown great success in natural language processing tasks by modeling the dependencies between words in a sentence. We reasoned this same architecture would be well-suited to extract spatial relationships between neurons in order to build a representation that facilitates finding correspondence to neurons in a template worm. The model is trained once on empirically derived semi-synthetic data and then predicts neural correspondence across held-out real animals via transfer learning. The same pre-trained model both tracks neurons across time and identifies corresponding neurons across individuals.

Performance is evaluated against hand-annotated datasets, including NeuroPAL(Yemini et al., 2020). Using only position information, the method achieves 80.0% accuracy at tracking neurons within an individual and 65.8% accuracy at identifying neurons across individuals. Accuracy is even higher on a published dataset(Chaudhary et al., 2021). Accuracy reaches 76.5% when using color information from NeuroPAL. Unlike previous methods, fDLC does not require straightening or transforming the animal into a canonical coordinate system. The method is fast and predicts correspondence in 10 ms making it suitable for future real-time applications.

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859A In vivo modeling of tau polymerization using *Caenorhabditis elegans*

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Alzheimer's disease and other related disorders are tauopathies that can result in cognitive and movement deficits. AD and ADRDS share a commonality in their presentation; the microtubule-associated protein tau is aggregated in areas of the brain that then exhibit high levels of neuronal and synaptic degeneration. Not all people will develop AD or ADRDs, despite tau being present in all neurons. What can drive tau to become aggregated and/or neurotoxic is poorly understood. Here we use an interdisciplinary approach to develop an animal model that allows *in vivo* visualization of tau polymerization. We aim to understand what events can initiate tau polymerization, and then to assess the physiological consequence as a function of aging. To do that, we are expressing GFP-tagged tau proteins throughout the nervous system. We are currently comparing wild-type full-length tau, a disease-relevant tau variant (P301S), and a synthetic tau variant that spontaneously aggregates (3PO). We find that GFP appearance is smooth and filamentous and localizes to axons and cell bodies in htau40 and P301S animals. GFP in 3PO animals, however, is punctuated and preferentially localizes to cell bodies. With aging, the punctuate appearance of GFP in htau40 and P301S start to show inclusion of GFP that could be correlated with the presence of aggregation when using conformation-specific antibodies for tau aggregates. The development of an in vivo model for tau polymerization has the potential to enhance our understanding of tau polymerization kinetics and how it might vary depending on the tau isoform or disease-relevant variant expressed.

860B An aggresome-like collection mechanism functions in neuronal expulsion of disease-aggregates

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The accumulation of aggregated proteins is associated with aging and neurodegeneration. Accumulation and subsequent spread of misfolded proteins causes toxicity that can induce loss of neurological function. Given that aggregate accumulation and spread is a prominent feature in neurodegenerative disease pathology and functional decline, a major goal in aging biology is thus to understand the mechanisms of how cells deal with protein aggregation, accumulation, and spread.

In mammalian biology, cells can handle aggregated proteins to maintain proteostasis via numerous pathways -- central aggregate collection, degradation via the ubiquitin-proteasome system or autophagy-lysosome pathway. In addition, neurons can identify, collect, and eject aggregates in large membrane-bound packages, "exophers". Mammalian and fly neurons also throw out aggregated-trash, which contributes to aggregate spreading via an unknown mechanism and is thought to promote pathology in human neurodegenerative disease.

While we have documented the dynamic aggregate movement from the soma into the exopher domain, followed by a dramatic budding of neuronal contents into the exopher as key hallmarks of exopher formation, we know little about the molecular requirements for these complex tasks.

I will describe the novel aggresome-like organelle in *C. elegans* neurons, an organelle that hosts disease aggregates. We discovered that many proteins important for aggresome-formation are also important for trash-expulsion via the exopher mechanism. Together, both aggresome-formation and exophergenesis-mechanisms represent intriguing molecular targets for improper aggregate handling and aggregate spread as seen in neurodegenerative disease pathology.

861C C. elegans precision AD models confirm transcriptional disruption of autophagy by APOE4 but not APOE3, and help to characterize E4-specific drugs

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Apolipoprotein E (APOE) is a multifunctional protein that transports lipids and also helps to repair brain injuries. By far the strongest genetic risk factor for late-onset Alzheimer's disease (AD) is the inheritance of APOE ε4 alleles (APOE4), increasing AD likelihood by at least 4-fold per ε4 copy – a risk elevation for which mechanisms remain unclear. The APOE genotype influences clearance of protein aggregates from the brain, in particular peri-neuronal aggregates containing amyloid-β and intra-neuronal tau aggregates – two hallmark aggregates for AD. We found that under neuronal stress, APOE4 protein translocates to the nucleus and competes with the transcription factor EB (TFEB) for binding to CLEAR enhancer sites in DNA, thus impeding transcription of TFEB targets, many of which are part of the lysosomal/ autophagy pathway. APOE4, but not APOE3, binds to CLEAR sites and prevents transcription of key autophagy proteins p62/SQSTM1, LAMP2, and LC3B. In the absence of these gene products, the autophagic aggregate-clearance response to starvation/neuronal stress is blunted, resulting in an APOE4-specific increase in protein aggregation. Although C. elegans lacks an APOE ortholog, it expresses the hlh-30 ortholog of TFEB for which the CLEAR binding-motif targets are almost perfectly conserved. It therefore provides a model in which APOE4 may compete for CLEAR sites free of any lipid-transport or other function. We devised a novel protocol to introduce human APOE4 protein directly into transgenic C. elegans models of AD. In adult worms expressing human Aβ42 in muscle, β-amyloid accumulation is increased by APOE4 relative to E3; whereas worms expressing Aβ42 in neurons showed less chemotaxis after E4. We also identified a novel therapeutic small molecule targeting APOE4 protein preferentially over APOE3, which alleviates the age- and aggregation-mediated chemotactic decline in worms expressing neuronal Aβ42.

862A Identifying *C. elegans* genes that suppress neurodegeneration induced by an expanded GGGGCC repeat

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Amyotrophic Lateral Sclerosis (ALS) is characterized by the progressive degeneration of neurons, resulting in chronic muscular atrophy. In 25-40% of familial ALS cases, an inappropriately expanded GGGGCC repeat is found in the first intron of the *C9ORF72* gene, resulting in neuron degeneration and eventually death. We generated a new *C. elegans* model expressing 30 copies of GGGGCC nucleotide repeats at high levels specifically in 8 sensory glutamatergic neurons. Back-filling of the PHA and PHB phasmid sensory neurons with a fluorescent dye revealed that 80% to 90% of 30x(GGGGCC) animals lose neurons or have defective neuron process, but 3x(GGGGCC) animals were relatively unaffected. Given the highly penetrant defect we were able to use this simple assay and perform an unbiased screen to identify genetic suppressors of neurodegeneration. Using EMS, we identified 28 potential suppressor lines in which degeneration of phasmid neurons was partially rescued (between 30 to 70% intact neurons). Of these 28 lines, 23 of them are clearly independent isolate, suggesting they contain unique suppressors mutations. As a secondary screen of the suppressor and to facilitate gene identification, we established a genetic scheme allowing for each line 1/ to test if the EMS hit affected a gene rather than the 30x(GGGGCC) insertion and 2/ Establish independent sub lines carrying the suppressors, named Suppressor lines, and independent lines that derivate from the Suppressor line, named Sibling lines. These Sibling lines have a similar genetic backgrounds i.e. other genes affected by the EMS treatment but will fail to rescue the 30x(GGGGCC) related neuron degeneration, meaning they don't carry the suppressor anymore. These different lines will be sequenced and by using the subtraction method (https://doi.org/10.1534/g3.117.300135) we will establish a list of candidate genes that suppress the neuron degeneration caused by GGGGCC nucleotide expansion. Further tests, using mutants of these candidate genes, will then allow to have crucial insights on genetic modifiers and pathways allowing to alleviate neuron degeneration in ALS.

863B Enhanced functional restoration through axon regeneration by swimming exercise

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Axonal regeneration is a promising approach to overcome impaired functionality due to axonal injury. In mammals, central nervous system has poor regenerative capacity due to both extrinsic and intrinsic factors. The regenerative capacity also declines significantly with ageing. Therefore, functional axon regeneration in adulthood is challenging and needs more understanding. The pharmacological manipulations are not very successful for functional restoration whereas rehabilitation and physical activity shows improvement. As physical exercise has complex systemic effects, understanding the downstream

effectors of physical exercise that is relevant for axon regeneration might be useful. Studying this using simple model organism has several advantages.

Using posterior gentle touch circuit neuron (PLM) of *Caenorhabditis elegans*, we are studying effect of swimming exercise on functional restoration after laser assisted axotomy. We found that a single swimming exercise session of 90 minutes, which is an established paradigm of exercise in worm (Laranjeiro et al., 2017; Laranjeiro et al., 2019) improves functional recovery irrespective of age. However multiple swimming session is required for older worms (A5 stage). Anatomical correlation showed that swimming session improves regrowth initiation, regrowth length and functional connections. We found that the energy sensor kinase AMPK/AAK-2 plays an essential role mediating swimming benefits. Characterizing tissue specific requirement, we found that it has both cell autonomous (PLM neuron) and non-autonomous (muscle) requirement. Pharmacological activation of AMPK/AAK-2 showed enhanced functional restoration similar to swimming. We are studying the downstream molecules and their specific roles in various tissues for swimming mediated functional enhancement which will be helpful for better implementation of this approach.

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864C The role of the extracellular matrix in maintaining neuronal architecture against increased mechanical stress

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Little is known about the mechanisms that protect the long-term architectural integrity of the nervous system: formed during embryogenesis, it will later face the stresses of maturation, growth, body movements and accidents. The cell adhesion molecule SAX-7/L1CAM is one of the neuronal maintenance factors identified in *C. elegans*. Our group has demonstrated that SAX-7S acts post-developmentally to preserve the organization of specific neuronal ganglia and fascicles (Desse et al., bioRxiv). Notably, maintenance defects resulting from the loss of *sax-7* can be suppressed by paralysis, highlighting that the mechanical stress associated with body movements may lead to progressive neuronal disorganization. Neuronal disorganization in *sax-7* mutants is also supressed by the loss of function of the gene *mig-6*/papilin, indicating that these two genes affect the maintenance of neuronal organization in opposite ways. MIG-6 is a conserved extracellular matrix glycoprotein containing a "papilin cassette", an assembly of domains also present in extracellular matrix remodeling enzymes. We found that the state of the extracellular matrix is altered in *mig-6* mutants, suggesting that a balance between adhesion and flexibility of the peri-neuronal environment is important to maintain the integrity of the nervous system. We therefore hypothesize that *mig-6* mutants, which under normal conditions have unaltered neuronal organization, may become defective following increased body movements. To test this, we subjected worms to high mechanical stress and observed that neuronal organization is better preserved in *mig-6* mutants compared to wild-type animals under these conditions. This effect is concomitant with a change in the distribution of EMB-9/collagen type IV, one of the main basement membrane proteins. Understanding the fundamental maintenance mechanisms of neuronal architecture could help identify molecular pathways involved in the development of neuropathologies.

865A Neuro-epidermal adhesions protect hyper-fragile axons from mechanical strain

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Axons, the longest neuronal process, withstand significant strain as a consequence of organismal development and movement. Axonal resilience to mechanical forces results from a cooperation between the axon and its surrounding tissue, although a full understanding of the underlying mechanisms is elusive. Here, focusing on the *C. elegans* posterior lateral mechanosensory neuron (PLM), we reveal a neuroprotective function of the skin hemidesmosome component LET-805/myotactin and an axonal-intrinsic membrane skeleton molecule UNC-70/β-spectrin. We show that a gain-of-function mutation in *let-805*/myotactin causes incomplete embedment and attachment within the skin of hyper-fragile PLM axons lacking UNC-70/β-spectrin, creating hinge points along the axon which eventually lead to axonal breaks in late stages of development. Consistent

with this model, mutants in *him-4*/hemicentin or *mec-5*/collagen, which prevent attachment and embedment of the PLM axon within the skin, eliminate these hinge points and prevent axonal damage in *let-805;unc-70* double mutants. Finally, we show that attachment of the PLM axon to the skin via LET-805/myotactin is progressive and required for the correct ensheathment of the axon, a feature replicated in regenerating axons following injury. In conclusion, we demonstrate that the uniform coupling of mechanosensory neurons to the skin during development requires LET-805/myotactin and functions to protect axons from damage induced by mechanical strain.

866B Exploring neuron death in a *C. elegans* model of Alzheimer's disease

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Alzheimer's disease (AD) is the leading cause of dementia worldwide and has been characterized pathologically as gross loss of neurons and neuronal connections in areas of the brain that control memory and executive function. Genetic variations in a number of genes, including amyloid precursor protein (*APP*) and apolipoprotein E (*APOE*), have been shown to influence both age of onset and severity of AD. Both an additional copy and gain-of-function mutants in *APP* can cause earlier onset of AD, while the e4 allele of *APOE* (*APOE4*) hastens and exacerbates AD compared to its other alleles (*APOE2* and *APOE3*). How these genes influence neuronal health in AD has yet to be determined. Our lab has developed a *C. elegans* model of AD that expresses both *APP* and *APOE4*. These worms exhibit specific, age-dependent neurodegeneration and cell death that can be readily observed using fluorescent microscopy. Combined with behavioral observations, we determined that expression of *APOE4*, but not *APOE3*, leads to death of the command egg laying neuron, HSN, by middle age. Further, genetic manipulation and time-lapse microscopy suggests neurons are not dying via apoptosis. We are currently exploring additional cell death pathways, as discovering the underlying neurodegeneration mechanisms may help identify novel therapeutic targets.

867C Effect of Base Excision Repair, Ung-1 Deletion, on Tau Pathology in *C. elegans*

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Base excision repair (BER) is a DNA repair pathway known to play an important role in removing DNA damage resulting from oxidative stress. DNA repair efficiency decreases with age, and oxidative DNA damage is associated with aging and neurodegenerative diseases such as Alzheimer's disease. Alzheimer's disease (AD) pathology primarily consists of extracellular amyloid-beta plaques and neurofibrillary tangles of hyperphosphorylated tau protein. The aim of this work is to characterize the effects of defective base excision repair on tau pathology in *C. elegans*, in order to test the hypothesis that defects in BER can be a disease modifier of AD. Defective base excision repair has been previously studied in *C. elegans* using the uracil DNA glycosylase deletion mutant *ung-1*. For tau pathology, the strains BR5270 (pan-neuronal over-expression of the F3 pro aggregation fragment of human Tau protein) with BR5271 (pan-neuronal over-expression of F3 pro-aggregation fragment of human Tau with mutations that abrogate aggregation) as a control have been previously used. To study the effect of defective base excision repair on tau pathology in *C. elegans*, the *ung-1* strain was crossed with pro- and anti- aggregate tau strains to generate tau strains with defective base excision repair. The effects of defective base excision repair on tau pathology in these strains were characterized using chemotaxis assay for memory, TMRE staining for mitochondrial function, pharyngeal pumping for nervous system function, and lifespan. Preliminary results suggest that deletion of the *ung-1* gene may exacerbate memory (3 biological replicates) and pharyngeal pumping (12 tech replicates, 4 biological replicates) in the pro-aggregate tau strain of C. elegans. The pro-aggregate tau strain with *ung-1* deletion showed significantly reduced TMRE uptake compared to *ung-1* strain. Preliminary lifespan data has not shown a decreased lifespan for pro-aggregate tau + *ung-1* compared to pro-aggregate tau alone. These results provide insight into how defective base excision repair may affect tau pathology in *C. elegans*. Future experiments will delve deeper into the mechanisms of these effects, with the goal of better understanding BER as a disease modifier of AD.

868A Relation between endogenous TAU levels and neurodegeneration in *C. elegans*

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Numerous debilitating neurodegenerative disorders, such as Alzheimer's disease (AD), Frontotemporal Degeneration (FTD), and Progressive supranuclear palsy (PSP), display patterns of degeneration as the disease progress. For each disorder, neurons generally begin to die in one area. Next, a unique spatiotemporal, patterned wave of degeneration spreads across the brain.

A major hypothesis raised to explain the neurodegeneration in these diseases is that toxic forms of the protein TAU aggregate and spread across synapses of brain regions during disease progression. Despite evidence of TAU aggregation and spread across age-related neurodegenerative disorders, the cellular and molecular underpinnings of pathogenesis and its spread remain elusive.

Previously, our lab generated models of age-related degeneration in *C. elegans* through pan-neuron expression of the human AD-risk genes APP and/or APOE4. In these models, we identified subsets of neurons that degenerated, becoming dysmorphic and/or dysfunctional, while others remained healthy and functional with age. This selective, age-related pattern of degeneration suggests that certain factors may confer vulnerability or protection to neurons in these models. To determine whether TAU represents a vulnerability factor, we are testing whether endogenous levels of PTL-1, the worm version of TAU, play a role in the death of these neurons, as well as others that we have not investigated yet. To tease out patterns of degeneration, we are interrogating behaviors governed by well-defined neuronal circuitry. Our preliminary results suggest that, with some exceptions, the relative levels of endogenous PTL-1 correlate to age-related loss of neuronal integrity in these models.

We seek to determine how widespread this pattern is in worm and search for additional factors that modulate neurodegeneration.

869B Age-related neuronal changes, lifespan pathways and maintenance of neuronal architecture

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The age-dependency of cognitive decline is well-known in humans, however the mechanisms by which nervous system 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. Furthermore, our neuroanatomical analysis of long-lived mutants reveals that neuronal morphological alterations can be robustly delayed in some long-lived mutants, but not all, indicating that delayed age-related neuronal change is not always coupled with lifespan extension, consistent with findings on healthspan analysis (Bansal *et al*, 2015). We are dissecting the molecular pathways responsible for delaying age-related neuronal alterations in long-lived mutants and their interplay with neuronal maintenance molecules. One of these, SAX-7, is homologous to the L1CAM family of cell adhesion molecules in mammals, where it functions to preserve cognitive abilities in adults. We find that the loss or gain of function of *sax-7* affects neuronal structures in distinct ways that inform us on the process of neuronal aging. Our findings indicate that the interaction between molecular mechanisms dedicated to the lifelong maintenance of neuronal architecture and lifespan determination are key to age-related neuronal change. 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.

870C Effects of Purple Pitanga extract in *C.elegans* transgenic strains for Alzheimer´s disease

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Purple Pitanga- PP is a fruit known by some properties that include autophagy induction, oxidative stress resistance and lifespan increase in *C. elegans*. These properties make PP a candidate for screenings against neurodegenerative disease such as Alzheimer´s disease (AD). In this study we evaluated the beneficial effects of PP in two *C. elegans* transgenic strains for AD- like model. CL2006 [pCL12(unc-54/human Abeta peptide 1-42 minigene) + rol-6(su1006)] and CL2355 [pCL45 (snb-1::Abeta 1-42::3' UTR(long) + mtl-2::GFP] transgenic worms were submitted to a chronic treatment (from the L1 stage) with the PP aqueous extract at the concentrations of 100, 250 and 500µg/mL gallic acid equivalents during 48 hours at 20ºC. After this period, we verified that the extract did not cause reduction in survival rate, body area and length. In these worms the amyloid beta (Aβ) aggregation is manifested by increased paralysis which impairs locomotion and reduces longevity. Notably, the treatment with PP was able to reduce the paralysis rate in CL2006 (all concentrations) and CL2355 (250 and 500µg/mL) and extend the longevity in this last one (250µg/mL). Once the locomotion and egg-laying are coordinated by the cholinergic system, we exposed worms to the cholinergic agonist levamisole and scored the eggs laid after 1 hour to exposure. We

observed that in WT (N2) the exposure to levamisole promoted the increase of egg-laying. In CL2006 and CL2355 the egg-laying is higher in relation to N2 probably because these strains present uncoordinated and reduced locomotion, which may promote increase in the eggs laid in early reproductive cicle. Notably, the treatment with PP was able to restore the egg-laying induced by levamisole (500µg/mL) in CL2006. As previously cited, PP presents antioxidant properties and one transcription factor involved in stress resistance in *C. elegans* is DAF-16. After 30 minutes, PP exposure induced the nuclear localization of DAF-16 in TJ356 strain (zIs356 [daf-16p::daf-16a/b::GFP + rol-6(su1006)]) at all concentrations tested. The chronic treatment with PP improved worms locomotion and lifespan. This effect may be conferred by DAF-16 activation which can induce the transcription of important genes that can protect against Aβ toxicity in *C. elegans.* We will pursue which DAF-16 target genes are upregulated by PP and which extract components are accountable for these effects.

871A Expression of *trx-1* correlates with intrinsic regenerative capacity

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A conditioning lesion of the peripheral sensory axon triggers robust central axon regeneration in mammals. Lesion conditioning could be utilized to drive powerful therapies for neuroinjuries. Despite being studied for >30 years, lesion conditioned regeneration remains poorly understood, and progress is severely limited by low throughput in vertebrate models. To expedite research in the field, we are developing a model for lesion-conditioned regeneration in *C. elegans*. Our model employs green fluorescent protein (GFP) to label the ASJ neuron. When we condition the neuron, we see increased fluorescence in the ASJ, indicating a correlation between GFP expression and regenerative capacity. Meanwhile, following prior work from our laboratory, disruptions to the sensory pathway can also chronically condition the neuron, increasing regenerative capacity without the need of a conditioning lesion. We saw increased fluorescence in chronically conditioned strains, further supporting the correlation between GFP expression and regenerative capacity. We used ethyl methanesulfonate to stochastically introduce mutations into a chronically conditioned strain and selected for offspring with decreased ASJ fluorescence, indicating a mutation in a gene potentially in the conditioning pathway. We isolated twelve strains, originating from six distinct F1s. Six of these strains show reduced frequency of ectopic axon outgrowths compared to the pre-mutagenized strain. A reduction in ectopic outgrowths suggests a disruption in the conditioning pathway, as previously characterized by our laboratory. To quantify the fluorescent correlation, we imaged the mutagenized strains with calibrated fluorescent beads and compared fluorescent intensities of the ASJ neurons to strains with increased outgrowths and regenerative potential. We found significantly decreased brightness in cell bodies and dendrites in strains with reduced regenerative potential compared to chronically conditioned strains. This correlation provides a powerful proxy for evaluating regenerative capacity and to identify genes potentially implicated in the lesion conditioned pathway.

872B Modifiers of TDP-43 Toxicity

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Protein toxicity is thought to underlie several, yet incurable, age-related neurodegenerative diseases, including Parkinson's disease and 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 in the disease mechanism. How protein toxicity triggers cell-and physical dysfunction and leads to degeneration is still not understood.

This project aims to find disease mechanisms and uncover targets to suppress ALS-related TDP-43 toxicity. For this aim, a combination of genetic- and phenotypic screens in a Caenorhabditis elegans model for disease are being used. We make use of a C.elegans strain with overexpressed human TDP-43, which shows several cellular- and behavioral ALS disease phenotypes, including age-related motor impairment is used as a model. We performed a genetic screen, which identified 22 mutant animals that show a suppression of this impairment. The strongest suppressor mutant, called MOTT-22 (Modifier of TDP-43 Toxicity 22), was selected for further experiments. We are currently verifying and characterizing a candidate gene that may be responsible for the suppression of motor impairment in MOTT-22. After finding a candidate gene for MOTT-22, gene functions in the cell will be studied to find new mechanisms involved in protein toxicity.

873C The Effects of Cytokine Proteins on the Notch1 Signaling Pathway of Neurogenesis in *Caenorhabditis elegans*.

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In patients suffering from neurodegenerative diseases (NDs), the process of repairing damage to the central nervous system and regenerating neurons is impaired. In these patients, increased levels of cytokine proteins are observed and correlated with increased neuroinflammation. Long-term neuroinflammation such as that seen in patients with NDs is known to impair neurogenesis. Neurogenesis is a complex process and involves various pathways such as the highly conserved Notch1 Signaling Pathway. Using synchronized *C. elegans*, we first examined how exposure to various inflammatory cytokines impacted overall levels of Notch1. Noting a reduction in Notch1 levels following specific cytokine exposure, we next aimed to look closer at regulators of the Notch1 pathway. Adm-4 is a functionally redundant gene to a Notch1 regulatory gene and may be impacted by the presence of cytokine proteins. Using synchronized *C. elegans* GFP tagged at adm-4, we quantitatively observed how exposure to various inflammatory cytokines affected adm-4 expression, specifically in the nerve ring. Understanding more about how the Notch1 Signaling Pathway is impacted during neuroinflammation may allow for scientists to one day learn how to better promote neuroprotection or even reverse early neurodegeneration.

874A A novel pathway links Microbiota-induced neuroprotection to the innate immune response in *C. elegans* models of Alzheimer's Disease

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The human microbiota plays vital roles in metabolism, tissue growth, and the immune system. Changes in the composition of microbiota are linked to different diseases including neurodegenerative disorders. Alzheimer's disease (AD) is a common progressive neurodegenerative disease which development involves both genetic and environmental factors. Many studies show that microbes are critical for AD development. However, the underlying mechanisms remain unclear. Our study employs transgenic *C. elegans* models of AD overexpressing amyloid β-peptide 1–42 ($A\beta_{42}$) or aggregate-prone human Tau to study the links between the human microbiota and neurodegeneration.

Our lab has identified several bacteria strains isolated from the human microbiota that are protective against neurodegeneration in *C. elegans* AD models. We then looked for mRNA targets whose expression levels are changed in worms exposed to different neuroprotective bacteria through RNA-sequencing. One gene that we are interested in is F54D5.4. The expression of F54D5.4 is significantly increased in worms fed with neuroprotective bacteria strains. Furthermore, knocking down F54D5.4 in *C. elegans* AD models promotes neurodegeneration. Thus, we will further study the functions of F54D5.4 and determine specific bacteria factors that regulate its expression.

F54D5.4 expression is upregulated in response to pathogens, suggesting that it may play a role in the innate immune response. Consistent with this idea, we find that F54D5.4 mutant animals are more sensitive to the bacterial pathogen *P. aeruginosa* PA14. Therefore, F54D5.4 is vital for the innate immunity to pathogen infections. However, whether F54D5.4 influences neurodegeneration by modulating innate immune function is unknown.

The immune system is involved in AD development. However, the mechanisms remain elusive. The F54D5.4 regulation may represent a novel pathway that links Microbiota-induced neuroprotection to the innate immune response.

875B Tissue inhibitor of metalloproteinase regulates extracellular beta-amyloid accumulation

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The hallmarks of Alzheimer's disease (AD) pathology are beta-amyloid (Aβ) plaques in the extracellular space and intracellular neurofibrillary tangles of tau. Several collagens (IV, VI, XVIII, and XXV) are found in the deposited amyloid plagues of AD patients. Some SNPs in collagens (XXV) are associated with AD as risk alleles, while other collagens (VI) seem to protect neurons from Aβ toxicity. However, the role of collagens or the extracellular matrix (ECM) in Aβ pathology is unclear. Current transgenic Aβ *C. elegans* show Aβ intracellularly, but an extracellular Aβ model is missing.

To model Ab in the extracellular space *in vivo*, we developed a new transgenic strain carrying superfolder green fluorescent protein (sfGFP) linked to human $A\beta_{1-42}$. Using correlative light electron microscopy, we observed that upon induction and secretion, sfGFP::Aβ localizes at the *C. elegans* cuticle, an ECM rich in collagens. Fluorescence recovery after photobleaching revealed two types of immobile structures on the cuticle. To gain mechanistic insights, we used RNAi to screen 2864 genes that either enhance or lower sfGFP::Aβ levels. The screen consisted of three libraries containing gene orthologues of the Human AlzGene database, geroprotective genes, and the matrisome (*i.e.,* all known ECM genes). We identified 152 enhancer and suppressor candidate genes. Strikingly, knockdown of some metalloproteases (MMPs) known to remodel ECM showed higher

sfGFP::Aβ accumulation, while a MMP inhibitor (TIMP) *cri-2* lowered sfGFP::Aβ accumulation. We narrowed our candidates down to four MMP that might be regulated by TIMP. Taken together, we implicated metalloproteases and their inhibitor in reducing extracellular Aβ aggregation in the ECM.

876C Tauopathy Impairs Axon Injury-Induced Autophagic Activity in *C. elegans*

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Autophagy plays a conserved role in maintaining cellular homeostasis by degrading dysfunctional proteins, lipids, and organelles. Autophagic dysfunction has been reported in various neurodegenerative diseases. It can occur at any step of the autophagic process and can contribute to the formation of protein aggregates and ultimately to neuronal death, although it remains unclear whether autophagy impairment is a contributor or a consequence of neurodegeneration. Axonal injury is acute neuronal stress that triggers autophagic responses in an age-dependent manner. In this study, we investigate the injury-triggered autophagy response in a *C. elegans* model of tauopathy. We found that the pro-aggregant F3ΔK280 Tau abolished injury-induced autophagic activity, thereby reducing axon regeneration capacity. Besides, axon trafficking of autophagic vesicles is significantly reduced in animals expressing the pro-aggregant F3ΔK280 Tau, showing that Tau aggregation impairs autophagy regulation. Notably, in the tauopathy model, the reduced number of total or trafficking autophagic vesicles was not restored by the autophagy activator rapamycin. Loss of PTL-1, the sole Tau homologue in *C. elegans*, also led to impaired injury-induced autophagy activation and decreased axon trafficking of autophagic vesicles, but with an increased basal level of autophagic vesicles. Therefore, we have demonstrated that Tau aggregation as well as Tau depletion both lead to disruption of injury-induced autophagy responses, suggesting that aberrant protein aggregation or microtubule dysfunction can modulate autophagy regulation in injured neurons.

877A Loss of *aly* genes suppresses toxicity in transgenic *Caenorhabditis elegans* models of tau or TDP-43

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Both the microtubule-associated protein tau and the DNA and RNA binding protein TDP-43 accumulate into toxic aggregates in multiple neurodegenerative diseases such as Alzheimer's disease and frontotemporal dementia. We have previously used transgenic *Caenorhabditis elegans* models of human tau and TDP-43 to discover novel genes that modulate their toxicity. These transgenic models are characterized by significant locomotor deficits, progressive loss of neurons, shortened lifespan, and accumulation of hyperphosphorylated and insoluble tau or TDP-43 protein. One gene identified as a strong suppressor of tau toxicity in *C. elegans* and mammalian models is the RNA binding protein *sut-2/MSUT2/ZC3H14.* To better understand the mechanism in which *sut-2* suppresses tauopathy, we investigated the *aly* genes (3 genes in *C. elegans*), whose mammalian homolog *ALYREF* has been shown to interact with *MSUT2/ZC3H14* (Morris & Corbett (2018) Nucleic Acids Res 46:6561-6575). Additionally, research in *Drosophila melanogaster* of TDP-43 toxicity showed that loss of the fly homolog *ref1* could suppress TDP-43-induced toxicity (Berson et al (2019) Acta Neuropath Commun 7:65). Therefore we determined whether loss of *aly* genes suppressed tau and/or TDP-43 toxicity in our *C. elegans* models. We found that loss of *aly-1* or *aly-2* alone, but not *aly-3*, caused mild suppression of tau-induced locomotor deficits, while loss of *aly-2* and *aly-3* together caused the strongest suppression of locomotor deficits. Loss of *aly*-2 and *aly*-3 reduced total and insoluble tau protein accumulation as well as neuron loss with age in tau transgenic *C. elegans*. In TDP-43 transgenic *C. elegans*, loss of *aly-2* and *aly-3* also suppressed behavioral deficits induced by TDP-43 but did not significantly alter total TDP-43 or phosphorylated TDP-43 protein levels. The results here suggest that although the *aly* genes modulate both tau and TDP-43-induced toxicity, they do so via different mechanisms.

878B UNC-16 inhibits actin and microtubule dynamics to regulate rate of regrowth and functional regeneration in *C. elegans* neurons.

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Neuronal regeneration after injury depends on the intrinsic growth potential of neurons. We demonstrate that UNC-16, a *C. elegans* JIP3 homologue, inhibits axonal regeneration by regulating initiation and rate of regrowth. UNC-16 inhibits the regeneration-promoting activity of the long isoform of DLK-1 independently of the inhibitory short isoform of DLK-1. UNC-16 promotes DLK-1 punctate localization in a concentration-dependent manner limiting the availability of the long isoform of DLK-1 at the cut site, minutes after injury. UNC-16 negatively regulates actin dynamics and microtubule dynamics post-injury, the former depends on DLK-1 and the latter partially depends on DLK-1. UNC-16 dependent post-injury cytoskeletal dynamics is also partially dependent on CEBP-1. The faster regeneration seen in *unc-16* mutants does not lead to functional recovery. We propose a model where UNC-16 displays its inhibitory role through both cytoskeletal and tight temporal and spatial control of DLK-1. The inhibitory control by UNC-16, the short isoform of DLK-1 and the activity of CEBP-1 balances the intrinsic growth-promoting function of the long isoform DLK-1 *in vivo*.

879C *hnRNPQ/hrp-2* role in splicing and neurodegeneration.

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A correct splicing of mRNA is globally required in all cells, but neurons seem highly sensitive to perturbations with numerous neurological diseases caused by splicing defects, including spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), and dementias. Neurons have features that make them vulnerable to mis-splicing events, such as their postmitotic state and longevity. However, the reason of this peculiar sensitivity of neurons to splicing alterations, why only subclasses of neurons are affected and which step of RNA processing is impaired in these diseases is still debated. We are investigating the molecular mechanisms underlying the neurodegeneration caused by splicing defects in SMA, using a neuron specific silencing approach in *C.elegans*. SMA is a motor neurons (MNs) specific disorder caused by mutations in the Survival Motor Neuron (SMN) gene. RNA-sequencing of iPS-MNs from SMA patients and healthy people allowed us to identify differentially expressed/spliced genes, enriched in RNA motif 7 (CAAAAAG). This motif is specifically bound by hnRNPQ, a protein interacting with SMN in the SMN complex. *hrp-2* is the *C.elegans* homolog of hnRNPQ and we determined that, similarly to *smn-1(KO), hrp-2 (ok1278)* null mutants arrest as larvae, have a severe decrease in lifespan and locomotion defects. *hrp-2* mutants show a reduction of visible D-type MNs and axonal defects, suggesting a specific role in neuron survival. *hrp-2* overexpression in D-type MNs rescued the degeneration of MNs and the defects in locomotion observed in *smn-1* silenced animals. These data suggest a specific involvement of *hrp-2* in MNs survival and in *smn-1* pathway, supporting the role of *hrp-2* as an *smn-1* modifier and as a possible therapeutic target for the disease. Further experiments are ongoing to better understand the genetic interaction between the two genes in MNs and to identify the role of mis-spliced targets in neurodegeneration.

880A TDP-43 promotes pathological tau phosphorylation and neurotoxicity in *C. elegans*

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While the neuropathology of Alzheimer’s disease (AD) is defined by the presence of amyloid beta and tau aggregates, up to 50% of patients also exhibit aggregates of the protein TDP-43. AD patients with TDP-43 pathology have worse cognitive impairment with more rapid decline and some data suggest TDP-43 may synergize with tau pathology. *C. elegans* has been widely used to study tau and TDP-43 proteotoxicity separately and such efforts have led to the discovery of *sut-2,* a gene whose loss-of-function protects against tau neurotoxicity in *C. elegans*. However, little is known about how tau and TDP-43 interact when co-expressed in the same organism or if there are modifiers that may disrupt that relationship, necessitating models of combined pathology. We have previously shown that co-expression of tau and TDP-43 in the same organism has synergistically deleterious effects. To further explore this relationship we developed a new low-expression *C. elegans* model of comorbid tau and TDP-43 and found that even at low levels TDP-43 exacerbates tau pathology, resulting in increased pathological protein accumulation as well as enhanced uncoordinated locomotion and progressive neurodegeneration. We highlight the specificity of this synergy by demonstrating that a strain expressing both wild-type human amyloid and TDP-43 is phenotypically no different from strains that express either protein alone. Finally, in order to further probe the mechanisms underlying the synergy between tau and TDP-43 we crossed tau+TDP Tg with a *sut-2* loss-of-function strain, which largely rescued the enhanced phenotype. The ability of this potent modifier of tau to also have an effect on a model of tau and

TDP-43 co-expression suggests that it is enhanced tau toxicity, and not TDP-43, that contributes to the synergistic tau+TDP Tg phenotypes. Characterizing the neurotoxic synergies between tau and TDP-43 *in vivo* will be critical for understanding and treating mixed pathology AD. Employing a novel model of tau and TDP-43 proteotoxicity, these studies provide initial insights into the mechanisms underlying tau and TDP-43 proteotoxic synergy, and establish the utility and tractability of the tau+TDP Tg model for studying comorbid pathology. Ultimately this model will be useful for identifying additional genetic modifiers of mixed tau and TDP-43 neurotoxicity and aid in a deeper dissection of the biology underlying these phenotypes.

881B The Effect of Cytochrome P450 Metabolites of Dietary Polyunsaturated Fatty Acids on Age-Associated Neurodegeneration.

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By 2035, the population older than 65 is projected to be 21.3% (78 million) increasing from 15.2% in 2016. Most elderly patients eventually develop some degree of cognitive dysfunction, and Alzheimer's disease alone affects almost 33% to 50% of the population over 85. As such, there is an unmet medical need to identify pathways that could curb neurodegeneration. Such a discovery significantly improves the quality of life for elderly patients. Recent research demonstrated that inhibition of cytochrome P450 (CYP) metabolism of dietary polyunsaturated fatty acids (PUFAs) is beneficial for several neurodegenerative diseases, including Parkinson's and Alzheimer's diseases. However, the detailed mechanism by which specific PUFA CYP metabolites responsible for such beneficial effects remains largely unknown. To tackle this problem, we apply a multidisciplinary approach to investigate the underlying mechanism and identify the signaling molecules responsible for the effects of PUFA CYP metabolites on neurodegeneration. We will use *C. elegans* as an animal model because it has been used to investigate neurodegeneration mechanism for more than a decade. Specifically, in this presentation, we will present our efforts in developing and applying different chemical tools to study the mechanism of how PUFA CYP metabolites affect neurodegeneration in different transgenic *C. elegans* strains. We found that inhibition of epoxide hydrolase in *C. elegans* rescues the neurodegenerative phenotypes in the strains expressing either amyloid-β or tau, which are similar to what has been found in the murine Alzheimer's disease amyloidosis or tauopathy models. We also demonstrated that a specific ω-6 PUFA CYP metabolite actually induces neurodegeneration. This result is interesting as most studies suggested that in general, PUFA CYP metabolites are beneficial for neurodegenerative disease. Our studies open new directions for neurodegeneration and *C. elegans* research, and further mechanistic studies are currently being undertaken in our laboratory.

882C RNAi screening of a phosphatase library identifies new modifiers of TDP-43 in a *C. elegans* model of ALS and FTLD-TDP

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Accumulation of hyperphosphorylated TDP-43 protein in neuronal aggregates is the major pathological feature of two devastating neurodegenerative diseases, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTLD-TDP). To study the cellular, molecular, and genetic underpinnings of TDP-43 mediated neurotoxicity in a tractable model system, we have developed a *C. elegans* model of TDP-43 proteinopathy expressing human disease-causing mutant TDP-43 pan-neuronally (TDP-43 tg). These transgenic animals display early, progressive uncoordinated movement (Unc), decreased lifespan, and age-dependent neurodegeneration. Using this model, we have found that phosphorylation of TDP-43 increases mutant TDP-43 toxicity. To identify phosphatases controlling TDP-43 phosphorylation, we have screened an RNA interference (RNAi) library targeting most known or predicted *C. elegans* phosphatases. 167 candidate genes were individually tested for modification of TDP-43 dependent behavioral phenotypes and for changes in the phosphorylation status of TDP-43 by immunoblot. From this primary screen we have identified 15 phosphatase genes that modify TDP-43 phenotypes. Additional work on these genes will provide mechanistic insight into the environmental and cellular triggers of TDP-43 phosphorylation, and provide potential novel avenues for therapeutic interventions into TDP-43 proteinopathies such as ALS and FTLD-TDP.

883A Elucidating Alzheimer's Disease related interactions between amyloid β and the pathogen *P. gingivalis* in the model organism *C. elegans*

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Alzheimer's Disease (AD) is a neurodegenerative disease which causes progressive loss of function in patients. AD affects about 5.8 million people in the US and is currently the sixth leading cause of death, with an estimated 122,000 deaths in 2018. AD

is characterized by abnormal, widespread aggregation of the proteins amyloid β (Aβ) and microtubule-associated protein tau (tau), neurodegeneration, immune system activation, inflammation, and oxidative stress. Aβ localization and aggregation have been linked to a number of AD traits but lack a clear causative role in the disease. Recently, infection by several pathogens have been identified as possible initiators for AD. One pathogen in particular, *P. gingivalis* (PG), has exhibited a number of features capable of inducing AD-like symptoms but its direct interactions with Aβ are poorly understood. We aim to identify and study these Aβ-PG interactions utilizing a novel Aβ expression system in the model organism *C. elegans.* This approach uses the C99 fragment of the amyloid precursor protein, the endogenous *C. elegans* γ-gamma secretase, and a neuron-derived *C. elegans* signal peptide. Aβ is expressed as a part of C99, which is directed to the neuron membrane and cleaved, releasing Aβ extracellularly. This strain was compared to an intracellular Aβ expression strain to observe localization-dependent effects of Aβ on the organisms. We then infected the strains with PG, observing neuron health and overall health over the worms' lifespan. We seek to further characterize the detrimental effects, connecting them to AD traits seen in humans. Additionally, we will screen for genes relevant to the Aβ-PG interactions, such as *C. elegans* immunity pathways.

884B Impact of microbiota on neurodegeneration in *C. elegans* models of tauopathy

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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. *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 desaturate *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.

885C Refining Sugar: A neuroprotective role for high sugar diets despite lifespan and reproductive losses

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High sugar diets are cited as a causal factor in the ongoing increase in obesity rates as Americans consume over 75 g of refined sugar and corn syrup per day on average. Further, obesity is associated with increased risk for multiple negative health outcomes including the neurodegenerative disease Parkinson's Disease. Utilizing *C. elegans* to investigate the effects of two common dietary sugars, glucose and fructose, we investigated the systemic and dopaminergic neuron-specific effects of high sugar diets on bioenergetics and susceptibility to mitochondrial dysfunction. Through the use of *in vivo* fluorescent reporters and Seahorse XF whole worm respiratory analysis, we found that glucose-fed worms exhibited a 14.9% increase in basal oxygen consumption and alterations to dopaminergic neuronal mitochondrial dynamics and morphology. Our study also indicated that unlike exposures beginning during development, chronic adult high glucose and high fructose diets are protective from 6-hydroxydopamine-induced dopaminergic neurodegeneration, despite deleterious impacts on lifespan and reproduction. These findings support the idea that a shift to glycolytic metabolism protects from acute electron transport chain inhibition, while also supporting previously observed adverse impacts of high sugar diets.

886A Uncovering a mechanism behind microbiota-induced neuroprotection in *C. elegans* models of Alzheimer's disease

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Alzheimer's Disease (AD) is the most common neurodegenerative disorder, presently affecting over 46 million people worldwide. The risk of developing Alzheimer's Disease is influenced not only by genetic factors, but also by environment and lifestyle. The multi-factored pathogenesis that leads to AD development poses a challenge for identifying causal factors that promote or protect against neurodegeneration. We use *Caenorhabditis elegans* as a model of AD to measure the impact of

human microbiota species on AD-related phenotypes. Two hallmarks of AD are amyloid-β (Aβ) plaques and neurofibrillary tangles composed of the protein Tau. Using an Aβ-overexpressing *C. elegans* model, we identified several bacterial species that differentially impact paralysis, and are characterizing a group of *Enterobacteriaceae* species that significantly reduce paralysis. To validate these findings, we used another *C. elegans* model that pan-neuronally expresses Tau protein aggregates. We observed decreased neurodegeneration when the animals were exposed to most of the same bacteria protective against Aβ-induced paralysis, providing additional evidence of microbiota-promoted neuroprotection. To explore these effects, we examined global changes in gene expression in animals exposed to neuroprotective bacteria. We identified several biological processes that were differentially regulated in response to the neuroprotective microbiota species, including innate immunity and protein phosphorylation. *C. elegans* orthologs of human tau tubulin kinase isoforms, TTBK1 and TTBK2, were down-regulated in response to neuroprotective microbiota species. RNAi-mediated knockdown of *C. elegans ttbk* genes sufficiently induced neuroprotection in Tau aggregate-prone animals and decreased paralysis in Aβ-overexpressing animals. These findings suggest that bacterial species from the human microbiota can mediate neuroprotection through down-regulation of *ttbk.* Overall, by studying the impact of the human microbiota on models overexpressing Aβ or aggregate-prone Tau, we have uncovered a potential mechanism by which microbiota-mediated neuroprotection can occur.

887B Modeling a neuropathy-associated *GARS* mutation in *C. elegans*

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Charcot-Marie-Tooth disease (CMT) is a heritable peripheral neuropathy that is characterized by motor and sensory defects in the distal extremities. Mutations in five genes encoding aminoacyl-tRNA synthetases (ARSs)—a family of enzymes that ligate amino acids to cognate tRNA molecules during the beginning stages of protein translation—have been implicated in dominantly inherited CMT. One of these implicated enzymes is glycyl-tRNA synthetase (GARS), the enzyme responsible for ligating glycine to cognate tRNA molecules. It has been demonstrated that there is wide allelic and clinical heterogeneity of *GARS*-mediated neuropathy. We recently reported a 12-base-pair, in-frame deletion in *GARS*, (E245_Q248; or ΔETAQ) in a patient with a severe form of peripheral neuropathy that is similar to infantile-onset spinal muscular atrophy. The ΔETAQ mutation ablates enzyme activity *in vitro*, reduces viability in yeast complementation assays, and is dominantly toxic to mouse neurons. To further determine the pathological significance of the ΔETAQ mutation and to establish a pipeline by which disease-causing ARS mutations may be systematically studied in a robust model organism, we employed a CRISPR/Cas9 method to generate a *C. elegans* model of *GARS*-mediated disease. Here, we provide characterization of the first *C. elegans GARS*-mediated neuropathy model, which supports a loss-of-function mechanism and will allow deeper insight into the pathology of *GARS*-mediated disease. This work contributes to our understanding of the role of the GARS enzyme in peripheral neuropathy and provides a framework for a *C. elegans* model system that can be effectively used to study the implications of other ARS mutations of interest.

888C Investigating the role of ubh-1 in maintaining dopamine neuron health

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Oxidative stress is known to be one of the main factors involved in the onset of many neurological diseases. Oxidative stress can lead to the disruption of many metabolic processes in the neuron, including in the ubiquitin-proteasome system (UPS), a cellular quality control system that tags misfolded proteins for refolding or degradation. UCHL-1 plays a vital role in the UPS and defects in UCHL-1 have been linked to the onset of Parkinson's and Alzheimer's diseases. However, the exact function of UCHL-1 in the nervous system has yet to be fully understood. To investigate the role of UCHL-1 in dopamine neurons, we will be using C. *elegans* as a model system. UCHL-1 has three orthologous proteins in *C. elegans,* known as *ubh-1, -2, and-3*. We hypothesize that the protein UBH-1 plays a role in protecting dopaminergic neurons from oxidative stress. To test our hypothesis, we are utilizing RNAi to knock down *ubh-1* in *C. elegans*. To investigate the phenotypic response of knocking down *ubh-1* we will be conducting a basal slowing assay to test the mechanosensory function of *C. elegans'* dopamine neurons, and mitochondrial damage, neurodegeneration, and aging assays, to determine if *ubh-1* affects neuronal health and/or mortality rates in *C. elegans* populations.

889A Trehalose-Vitamin E Nanoparticles attenuate motility impairment and reduced longevity in a *Caenorhabditis elegans* Amyotrophic Lateral Sclerosis model

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the progressive loss of motor neurons. There are several mutations that can lead to the development of this disease, including the SOD-1 gene. Riluzole is the only approved drug to slow the progression of the disease and to promote longer survival. However, its effects are limited. Therefore, it is necessary to develop new therapies for ALS treatment. Previous studies have suggested trehalose and vitamin E, individually, as good candidates for ALS treatment. In order to associate these molecules, we developed nanocapsules and nanoemulsions of trehalose and vitamin E and exposed to *Caenorhabditis elegans* (*C. elegans*) ALS model and evaluated the pharmacological potential of these nanoparticles. Pregnant AM263 [unc-54p::Hsa-sod-1 (WT)::YFP] and AM265 [Punc-54::hSOD1(G93A)::YFP] worms were synchronized. The eggs obtained from these nematodes were transferred to Petri dishes previously dried containing NGM medium and UV-inactivated *E.coli* OP50 with or without treatment. Trehalose/vitamin E nanomaterials were provided by a partner laboratory. The treatment was performed with nanoparticles pipetted on NGM surface. When the worms reached L4 stage, they were transferred to new plates containing or not nanoparticles. This process was performed from day 1 adult until 4th day adult. Nematode paralysis was assessed along longevity; nematodes were considered paralyzed if they did not move when prodded with a wire pick. SOD-::GFP aggregation was observed at 5th day adult through fluorescence microscope. Our data showed that the nanoparticles decreased the number of paralyzed worms and increased their longevity. Besides that, the treatment with Vit. E+ trehalose nanoparticles provided a slight modification in the SOD1 aggregation profile. ALS promote motor neuron disabling, leading to muscular paralysis and death. This way, these results suggest a great advantage of nanoparticles in mitigating the main effects caused by ALS. There are studies reporting the protective effect of sugars, such as sucrose, however, these carbohydrates have many disadvantages. Trehalose is more cost-effective than other sugars, in addition to having neuroprotective and autophagosome modulating activity. In turn, vitamin E is an antioxidant widely used in several pharmacological area including in neuronal studies. In conclusion, the association of these two compounds proved to be efficient in the ALS treatment.

890B An Environmental Contributor of Neurodegeneration impacts lifespan through disruptions in AMPK Signaling in *C. elegans*

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Parkinson's Disease (PD) is characterized by the loss of dopaminergic (DA) neurons. Only 5-10% of PD cases have a direct genetic origin; however, exposure to herbicides, pesticides, and interaction with the soil are all potential risk factors. A soil bacterium, *Streptomyces venezuelae* (*S. ven*), produces a secondary metabolite that causes age- and dose- dependent DA neurodegeneration in *C. elegans*. Studies from our lab determined that exposure to the *S. ven* metabolite causes oxidative stress, mitochondrial fragmentation and enhanced reactive oxygen species (ROS). Upon metabolite exposure, significantly more *daf-16* accumulates within nuclei. Recently, we discovered that exposure to the *S. ven* metabolite is associated with hormetic effects. Specifically, at high concentrations of metabolite (20X), we observe dopaminergic neurodegeneration and enhanced ROS in N2 animals, whereas at low concentrations (5X), the neurons did not display neurodegeneration. We then extended these observations to lifespan studies in N2 animals. Notably, the higher concentration (20X) decreased lifespan in wild-type animals whereas the 5X concentration extended lifespan. *daf-16* mutants were then examined with both concentrations of metabolite in lifespan assays. Notably, *daf-16* mutants displayed no significant differences between solvent and metabolite at both high and low concentrations, suggesting the hormetic response is *daf-16* dependent. Considering *daf-16* is the primary transcription factor of several key aging pathways, we investigated the impact of *S. ven* metabolite on the aging process of *C. elegans* mutants in the insulin signaling pathway. When both *daf-2* and *age-1* mutants were exposed to the 5X concentration of *S. ven*, there was no significance difference between treatments. Yet, when *daf-18* mutants were exposed to the same concentration, lifespan extension remained. These data suggest that the insulin signaling pathway is impacted following chronic metabolite exposure. Additionally, when exposed to the 20X concentration of *S. ven* metabolite, *aak-2* mutants displayed no significant difference between solvent and metabolite. However, when *aak-2* mutants were exposed to the metabolite at the 5X concentration, mutants displayed a decreased lifespan. Taken together, these data indicate that functional *aak-2* might be important for increased lifespan when combating toxicants following chronic exposure.

891C Dynamics of nicotine-induced neuroprotection and the ACR-20 receptor

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Epidemiological studies show that tobacco smoking reduces the prevalence of Parkinson's disease. One explanation for these findings is that tobacco-derived nicotine protects substantia nigra dopaminergic neurons (DNs) from degeneration. To examine this possibility we established, in collaborated with the lab of Guy Caldwell (University of Alabama), a *C. elegans* model for nicotine-induced protection of DNs. Using this model we have shown that chronic nicotine exposure protects DNs against 6-OHDA toxicity via a signaling pathway involving activation of mitochondrial stress. We also identified four nAChR subunits needed for nicotine-induced protection of DNs (Nourse et al., iScience 2021).

ACR-20, one of these "protective" nAChR subunits, was previously shown to form a homomeric receptor (Bauer et al., Mol. Pharmacol. 2014).

To examine whether nicotine-induced protection depends on the ACR-20 homomeric receptor we examined the effects of betaine, an agonist of this receptor. We find that chronic betaine exposure like chronic nicotine exposure protects DNs in a nAChR-dependent manner. Protective effects of betaine are, however, distinct from the protective effects of nicotine; pre-exposure to betaine, unlike similar nicotine exposure, is not protective; while, exposure to betaine during and after 6-OHDA treatment, like similar nicotine exposure, is protective. The protective effects of betaine and the differences in their dynamics, relative to the protective effects of nicotine, are consistent with two distinct nAChRs functioning to protect DNs. In addition, differences between effects of nicotine and betaine suggest that the neuroprotective effects of betaine, unlike these of nicotine, do not involve long lasting gene expression changes. This explanation is consistent with the finding that betaine exposure, unlike nicotine exposure, does not enhance expression of mitochondrial stress markers. Of note, efficacy of nicotine as an agonist for ACR-23, closely related to ACR-20, is very low (Rufener et al., PLOS Path. 2013). This raises a question concerning the role of ACR-20 in nicotine-induced protection. We are now examining the possibility that exposure to nicotine, shown to upregulate *acr-20* expression (Polli et al., Neurotox. 2015), sensitizes DNs to the protective effects of ACR-20 activation during and after 6-OHDA treatment.

892A Downstream effectors of the synergic activation of AMPK by metformin and salicylate to reduce polyQ aggregation

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Huntington disease (HD) is a rare neurodegenerative disorder of autosomal dominant inheritance, characterized by an expansion in the number of CAG triplets in the huntingtin gene's (HTT) first exon. No therapy has been proven effective for HD treatment thus-far, but some compounds, such as metformin, have been reported as potentially beneficial disease-modifiers of this pathology. Previous findings have shown that the synergistic activation of AMP-activated protein kinase (AMPK), with metformin and salicylate, reduces aggregation and neuronal damage in Caenorhabditis elegans models of toxicity induced by polyglutamines (polyQs). Furthermore, strong evidence suggests that activation of AMPK, with just metformin, is neuroprotective in mouse models of HD. AMPK is a master regulator of healthspan and lifespan modulating energy metabolism, stress resistance and cellular proteostasis; via pathways such as autophagy, oxidative stress, and lipid metabolism. For instance, AMPK activates Nrf2/SKN-1, FoxO/DAF-16, and SIRT1/SIR-2.1 signalling pathways; and inhibits the signalling of CRTC-1/CRTC-1, and mTOR/LET-363.

An activation of autophagy has been detected in metformin/salicylate-treated worms promoting proteostasis in a poliQs-induced toxicity context, however, preliminary results in our laboratory indicate that this activation may be independent of AMPK. In this work, we have studied several targets downstream of AMPK, potentially responsible for the protective effect dependent on salicylate and metformin synergistic AMPK activation, and show their relationship with this signalling pathway.

893B Reverse genetic screen of Parkinson's disease-susceptibility genes identifies novel modulators of alpha-synuclein neurotoxicity in *C. elegans*

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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. Here we highlight *seb-3*, an ortholog of human CRHR1, which when inactivated by RNAi leads to increased formation of aSyn inclusions and neurodegeneration in young animals. The nonsense mutation in *seb-3* recapitulated the phenotypes elicited by RNAi while a gain-of function allele of *seb-3* alleviated neurodegeneration as well as formation of aSyn inclusions. Our RNAi data reveal several additional new modulators of aSyn toxicity in dopaminergic neurons of *C. elegans* and provide new potential therapeutic targets for PD.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 844497.

894C The mitochondrial unfolded protein stress response is impacted by alpha-synuclein

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PD pathogenesis is associated with misfolding of a-synuclein (a-syn) and mitochondrial dysfunction. Notably, a-syn was shown to interact with, and disrupt, TOMM20, a mitochondrial outer membrane protein. This interaction causes a mitochondrial protein import block, reduced respiration and enhanced generation of reactive oxygen species in cell culture and mammalian brain studies. Our lab extended these findings by reporting that UPRmt stress was increased following overexpression of a-syn variants within the intestine. This was examined via ATFS-1-dependent transcriptional upregulation with the established reporter for the UPRmt, Phsp-6::GFP. These a-syn variants were wild-type human a-syn (WT), two disease variants (A53T and A30P), and a deletion disrupting an N-terminal mitochondrial targeting sequence (Δ1-32). The disease variants and the N-terminal mutant induced significantly higher UPRmt activity during both larval development and in adults whereas WT a-syn only induced the UPRmt more than controls in larval development. To determine if UPRmt regulation occurs differently for various familial a-syn variants, the transgenic strains were subjected to RNAi of *clpp-1, haf-1,* and *lonp-1* and then re-analyzed for UPRmt stress. Following knockdown of *lonp-1*, all the transgenic worm strains displayed significantly enhanced UPRmt activity compared to their EV RNAi controls. Additionally, all variants, except the Δ1-32 line displayed enhanced UPRmt stress following knockdown of *haf-1*. One striking difference in these data, however, was that WT and A53T a-syn-expressing worm lines displayed increases in UPRmt stress between larval and adult stages, while the other two lines did not display increases in UPRmt stress over time. It is interesting to note that the protein pool for at least a subset of WT and A53T a-syn is likely localized to mitochondria, based on previously published work, while A30P and Δ1-32 a-syn will remain in the aqueous cytoplasm. These studies were extended to neurons, where knockdown of *clpp-1, haf-1, and lonp*-1 was performed exclusively in dopaminergic neurons of animals overexpressing WT a-syn. Enhanced neurodegeneration was observed after depletion of *clpp-1* and *haf-1*, whereas knockdown of *lonp-1* was similar to EV control. In total, these data suggest that WT and A53T a-syn enter mitochondria where CLPP-1 mitochondrial protease and HAF-1 matrix peptide exporter are involved in their degradation and removal.

895A A Genetic Screen for Identification of UPRmt Effectors Associated with *a-synuclein* Neuroprotection in *C. elegans*

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Parkinson's disease (PD) is characterized by neurodegeneration of the central nervous system, leading to impaired motor skills and bodily functions. Here, we look at two components of PD pathology: One is the protein alpha-synuclein (a-syn) that is found within Lewy Bodies and is often prone to misfolding and aggregation. The other is mitochondrial dysfunction via a dysregulated Unfolded Protein Response (UPRmt). Under non-stress conditions, the Activating Transcription Factor Associated with Stress (ATFS-1) is imported into the mitochondria and degraded. During acute stress, mitochondria employ the (UPRmt), that, with the help of ATFS-1, coordinates nuclear expression of chaperones and proteases, that translocate to the mitochondria and remove damaged or unfolded proteins. However, during a long-term genetic stressor such as a-syn, this system becomes dysregulated. We hypothesize that a physical blockade is created when a-syn interacts with the outer mitochondrial membrane protein, TOMM-20, and that this blocks mitochondrial import, leading to proteostatic imbalance. Our research shows that the loss-of-function mutant of *atfs-1* is highly neuroprotective against a-syn-induced neurodegeneration. Therefore, we used *atfs-1(lof)*; a-syn animals in an F3 EMS mutagenesis screen to identify UPRmt effectors associated with a-syn neuroprotection. We have screened through ~3,300 genomes. Populations of animals were examined for changes in neurodegeneration levels using a low power, fluorescent, stereomicroscope and then confirmed for

neurodegenerative changes with compound microscopy analysis. In the *afts-1 (lof)*; a-syn background, more than half of the animals display neuroprotection in the background and we selected mutants where the dopaminergic neurodegeneration level went back to levels similar to a-syn only levels. Through this screen we initially isolated ~70 mutants. These positive candidates were collected, propagated, and screened over multiple generations for consistent neurodegenerative phenotypes, which ultimately provided us with 24 mutants. Of these mutants, at least 11 independent mutations are expected. After outcrossing, DNA was extracted from the mutants and sequenced using the Illumina platform. SNP analysis is currently being performed using GATK. Mutants obtained from this screen may reveal an uncharacterized compensatory mechanism for UPRmt induction.

896B Investigating non-apoptotic roles for *egl-1*

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The Bcl-2 Homology (BH) 3-only protein EGL-1 is a key regulator of apoptosis pathway in *Caenorhabditis elegans* and is conserved across metazoans. Generally, decades of research have shown that expression of *egl-1* is necessary and sufficient to trigger apoptosis in *C. elegans*. However, transcripts of BH3-only proteins are also detected in living neurons of healthy mammalian brains. Non-apoptotic roles and their underlying mechanisms for BH-3-only domain proteins are not well understood. Our lab discovered that *egl-1* is expressed throughout the life of the worm in the URX pair of sensory neurons without inducing apoptosis. This expression depends cell autonomously on neuronal activity and calcium. URX is well known for its function as an oxygen sensor, but it also projects a process into the body cavity neuron where it senses signals from coelomic fluid. We are investigating non-apoptotic roles for *egl-1* expression in URX. Our preliminary results show that both *egl-1* mutation and URX-specific RNAi knock down of *egl-1* impaired URX-related behaviors in worms. Further study of how *egl-1* functions in URX without inducing apoptosis may reveal insight regarding the functions of BH-3-only domain proteins in the mammalian brain.

897C Identification of Metabolic Pathways Involved in Neuronal Regeneration

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The inability of some neurons to sufficiently regenerate is in part due inefficient activation of intrinsic regeneration programs. A regenerating neuron must molecularly reprogram to allow the production of cellular components and meet the increased energy demands as well. Recent evidence highlights the critical role of metabolism in neuronal regeneration. We recently discovered that modifications in O-linked β- N-acetylglucosamine (O-GlcNAc) signaling can strongly enhance axon regeneration. The findings indicate that O-GlcNAc transferase (OGT-1) mutation enhances axon regeneration due to shifts in cellular glycolysis. To identify the important genetic and metabolic factors involved in this effect, we adapted unbiased approach. We performed whole worm RNAseq analysis for wild-type and ogt-1(-/-) or O-GlcNAcase, oga-1(-/-), mutants. A relatively smaller number of genes were differentially expressed in oga-1(-/-) compared to wildtype, but notably they were enriched in carbohydrate, energy, and amino acid metabolism. On the other hand, we identified a large number of differentially expressed genes in the ogt-1(-/-) compared to wild-type. These genes are involved in numerous cellular processes, metabolic and signaling pathways such as development, cell growth and death, and signal transduction, as well as lipid, carbohydrate, amino acid and energy metabolism. Importantly we find differential expression of several genes with known roles in regeneration (including ced-3, daf-18, lin-12, cex-1, fax-1 etc.) confirming the validity of this approach. In addition, we find genes involved in One Carbon Metabolism (OCM), transmethylation pathways (folr-1, sams-5, set-1 etc.) and associated lipid metabolism (fat-7, ckb-2, dod-20 etc.). Performing single neuron regeneration experiments in the major S-Adenosyl Methionine Synthase (sams-1) deletion mutant, we find a significant reduction in regeneration following laser axotomy of the ALM neuron. Importantly sams-1 mutation appears to also block the enhanced regeneration effects of ogt-1 in a sams-1;ogt-1 double mutant. Our findings begin to identify genes and transcriptional regulatory pathways underlying enhanced regeneration via O-GlcNAc signaling. Our results predict that knockdown of OCM or glycolysis genes will either block or reduce, the enhanced regeneration of ogt-1(-/-), highlighting and defining the importance of cellular metabolism in neuronal regeneration.

898A Suppressors of stress-induced glutamatergic neuron degeneration in *sod-1G85R* ALS model

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Amyotrophic Lateral Sclerosis (ALS) is one of the most common adult-onset neurodegenerative disorders that results from the selective and progressive degeneration of cortical and spinal motor neurons. Mutations over 20 genes including RNA binding proteins, FUS and TDP43, and a free radical scavenger, superoxide dismutase 1 (SOD1) cause ALS. Although there is a well-established genetic component of ALS, it is still unclear why mutations in genes encoding functionally diverse proteins cause motor neuron degeneration. Identifying modifiers of neurodegeneration in *C. elegans* ALS models may provide insight into the mechanisms behind the selective and progressive neurodegeneration in ALS. Here, we have undertaken a classical forward genetic screen to identify suppressors of glutamatergic neuron degeneration. After exposure to oxidative stress, SOD-1G85R knock-in mutants have both glutamatergic and cholinergic neuron degeneration (PMC6200258). We mutagenized SOD-1G85R animals and screened for suppressors of glutamatergic neuron degeneration. After whole genome sequencing, we found that an RNA binding protein, hit in three independently isolated lines, suppresses cholinergic and glutamatergic neuron degeneration. Understanding the relationship between SOD1 and RNA binding proteins will provide insight if there are common mechanisms underlying neurodegeneration in ALS and has the potential to facilitate the development of treatments.

899B Rescuing the Paralysed Phenotype of *unc-18 e81* Mutant *C. elegans*

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Mutations in Munc18, an essential protein for several stages of synaptic vesicle exocytosis, have been linked to early infantile epileptic encephalopathy. Null mutations in Munc18 and its homologs in flies (Rop) and yeast (SEC1) are lethal, but null mutations in the *C. elegans* homolog (*unc-18*) are not. Consequently *C. elegans* provide a suitable model to investigate the effects of loss-of-function. We conducted an ethylmethanesulfonate (EMS) mutagenesis suppressor screen of the *unc-18 e81* allele resulting in a novel strain, RESCUE, in which the paralysed phenotype of *unc-18 null* mutants was rescued. Two novel mutations found in the rescue strain, present in the *dgk-1* and *sorf-2* genes were hypothesised to facilitate bypass of *unc-18* function. Diacylglycerol kinase (*dgk-1*) catalyses the conversion of diacylglycerol (DAG) to phosphatidic acid (PA), thus functioning in synaptic transmission. *Sorf-2* encodes the *C. elegans* homolog of WDR81, predicted to function in organelle fusion and endosomal transport. We confirmed that RESCUE worms were significantly better at locomotion compared to *unc-18 null* mutants and statistically indistinguishable from wild-type worms. Introduction of *wild-type dgk-1* into RESCUE worms reversed the improvement in locomotion to *unc-18 null* levels suggesting necessity of the *dgk-1* mutation for the rescued phenotype. Elevating DAG levels in *unc-18 null* mutants failed to improve locomotion, both genetically and pharmacologically, suggesting the *dgk-1* mutation alone is not sufficient for phenotypic rescue. *Sorf-2* RNAi in *unc-18 null* mutants improved locomotion following treatment with the DGK inhibitor II, R59949. Further investigation into the *sorf-2* mutation will lead to a better understanding of the mechanisms through which the two mutations bypass the *unc-18 null* mutation. This will further allow research into WDR81 function, little of which is known as of yet.

900C Specific (co-)transmission of two neuropeptide species from the AVK interneuron

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Communication among cells via neuropeptides is crucial for proper function of the nervous system, as is evident from human diseases caused by disruption of neuropeptide signaling. For example, Neuropeptide Y is involved in a variety of disorders such as hypertension, epilepsy, and obesity (Reichmann 2016; Yi 2018). Across diverse species, many neurons express multiple neuropeptides, raising the question whether these neurons simply co-release cocktails of neuropeptides, or whether some of them may release them separately and specifically. Examples for co-transmission are found in many neuron types, for example mammalian midbrain dopamine neurons, which also release glutamate from separate vesicles. We found that the AVK interneurons of *C. elegans* may specifically (co-)transmit at least two different neuropeptides, FLP-1 and NLP-49, both expressed at high levels in this neuron. However it remains largely unclear whether, in what context and by what mechanisms their release is differentially regulated. FLP-1 and NLP-49 are predominantly expressed in AVK and differentially affect behaviors such as locomotion and egg-laying (Oranth 2018; Chew 2018). We thus examined whether these peptides are differentially regulated. We found from locomotion analyses using the multi worm tracker (Swierczek 2013) that these peptides derived from AVK differentially affect the animal's response to sensory stimuli in a modality-specific manner. We also observed peptide precursors fused with fluorescent proteins and obtained evidence indicative of differential trafficking and regulation of those two neuropeptides in AVK.

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901A Eukaryotic initiation factor EIF-3.G augments mRNA translation efficiency to regulate neuronal activity

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Protein translation is principally regulated at the initiation phase, whereby six multi-subunit translation initiation factors (eIF1-6) ultimately engage elongation-competent 80S ribosomes with the mRNA open reading frame. eIF3 is the largest complex, consisting of 13 unique subunits. While each eIF3 subunit has vital roles in general protein synthesis, recent work has shown that some subunits can drive specialized cellular pathways that enforce when and where translation events occur. Moreover, human genetics studies have revealed prevalent dysregulation of different eIF3 subunits in disease, including neurological disorders. However, very few studies have examined how eIF3 regulates neuronal protein synthesis in living animals.

Here, we report a selective role of the G subunit of *C. elegans* EIF-3 in shaping the motor neuron proteome to control locomotion. EIF-3.G contains an N-terminal domain that binds EIF-3.I, and a conserved zinc finger followed by an RNA-binding RRM at the C-terminus. We find that while EIF-3.G is essential for larval development, a missense mutation (C130Y) in its zinc finger exhibits a highly selective effect in cholinergic motor neurons to dampen overexcitation of the locomotor circuit. *eif-3.G(C130Y)* acts in a gain-of-function manner and requires its RNA binding domain. To systematically identify EIF-3.G mRNA targets in the cholinergic motor neurons, we performed cell-specific seCLIP analysis. Our data reveals that EIF-3.G preferentially occupies the GC-rich 5'UTRs of a specific cohort of mRNAs, many of which perform neuronal activity-dependent functions. We further demonstrate that EIF-3.G exerts translational control over two of its target genes, *hlh-30* and *ncs-2,* through their GC-rich 5'UTRs. Finally, we carried out a genetic screen for genes interacting with EIF-3.G, and find that *eif-3.G(C130Y)* requires the putative phosphatase encoded by *eat-9* and a regulatory factor LIN-66 to modify cholinergic activity. Our work provides the first insight into eIF3 selective translational control in neurons and establishes a system for unveiling protein translation pathways that regulate animal behavior.

902B Encoding principles of a compact sensory system

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Animals critically depend on accurate sensation and processing of environmental cues. This task becomes particularly challenging for animals with a compact and highly interconnected sensory system. Here, we used Caenorhabditis elegans nematodes to investigate how sensory information is encoded within a small nervous system. For this, we generated a strain expressing the genetically encoded calcium indicator GCaMP in all 60 ciliated neurons, and used a fast-scanning confocal system to measure activity simultaneously from all chemosensory neurons while subjecting the worms to various stimuli. We found that the sensory system responds with small, unique, and near-perfectly bi-laterally symmetrical subsets of neurons. Analysis of mutants, defective in neuro-transmitter or neuro-peptide release, revealed that the number of primary neurons which directly respond to the cue is minimal, typically consisting of 2-3 neuron types. These neurons exhibit a range of response dynamics that is both stimulus- and circuitry-dependent, effectively increasing encoding capacity of the compact sensory system. Finally, exposing animals to odor mixtures revealed that the sensory system employs a variety of logic gates including AND, OR, XOR, and NAND to process complex stimuli. Together, here we elucidated the principles that allow a small and compact sensory system to expand its encoding repertoire and to efficiently extract information from the environment.

903C Neural mechanisms underlying temperature-driven host seeking by a human-parasitic nematode

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Skin-penetrating gastrointestinal parasitic nematodes are a major source of "diseases of disadvantage," infecting approximately one billion people. Their life cycle includes an infective third-larval (iL3) stage that searches for hosts in a poorly understood process involving host-emitted sensory cues, including heat. The iL3s of the human-parasitic threadworm *Strongyloides stercoralis* display robust attraction to mammalian body temperatures (37°C). What are the molecular and neural mechanisms that underlie this parasite-specific positive thermotaxis behavior?

Previously, we used CRISPR/Cas9-mediated mutagenesis to demonstrate that positive thermotaxis by *S. stercoralis* iL3s is dependent on the *S. stercoralis* homolog of the *C. elegans tax-4* gene. The *S. stercoralis tax-4* gene is expressed in multiple head neurons in iL3s. Now, in order to identify the primary sensory neurons responsible for positive thermotaxis in iL3s, we again leveraged the genetic similarities between *S. stercoralis* and *C. elegans*. In *C. elegans*, TAX-4+ AFD sensory neurons provide the primary thermosensory drive for thermotaxis navigation. We genetically identified the *S. stercoralis* AFD neurons via a *Strongyloides* homolog of the *C. elegans* AFD-specific gene *gcy-23*, and found that inducible silencing of these neurons suppresses positive thermotaxis in iL3s. Thus, the thermosensory role of AFD is conserved between *S. stercoralis* and *C. elegans* despite species-specific differences in thermal preference and behavior.

Using genetically encoded calcium sensors, we found that thermosensory responses in the *S. stercoralis* AFD neurons are distinct from those in the *C. elegans* AFD neurons. In response to thermal stimuli that mimic those experienced by iL3 during positive thermotaxis, the *S. stercoralis* AFD neurons exhibit a warming-triggered hyperpolarization near ambient temperature followed by near-linear positive encoding of temperatures up to human body temperature. This result is the first direct evidence of differential sensory encoding by homologous neurons in parasitic and free-living nematodes. Finally, we identified three *S. stercoralis* AFD-specific thermosensitive receptor guanylate cyclases that each display an expanded responsiveness to mammalian body temperatures relative to *C. elegans* homologs. Thus, altered thermal encoding in primary thermosensory neurons likely contributes to parasite-specific behaviors. Together, our results provide insight into the molecular mechanisms and neural circuits that allow skin-penetrating nematodes to target hosts.

904A How NLP-3 neuropeptides work with serotonin to activate the *C. elegans* egg-laying circuit

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Mammalian and non-mammalian serotonergic neurons alike typically release specific neuropeptides, though it is not yet understood why or how serotonin and neuropeptide signals might function together. In the model system of the *C. elegans* egg-laying circuit, the Hermaphrodite Specific Neurons (HSNs) co-produce serotonin and NLP-3 neuropeptides to activate egg laying. Previous work[1] has shown that these two signals synergistically enable activity of specific egg-laying muscles; however, because the receptor for NLP-3 has not been identified, it has remained difficult to understand how NLP-3 peptides mediate egg laying behavior alongside serotonin. Using an RNAi screen of 154 G protein coupled receptors that assessed their ability to mediate NLP-3 signaling, we identified a previously uncharacterized receptor, NPR-36, as a putative NLP-3 receptor in the egg-laying circuit. CRISPR knockouts of *npr-36* phenocopy the knockout of *nlp-3*: firstly, in combination with *tph-1* (a mutation that prevents serotonin biosynthesis), both the *nlp-3* and *npr-36* knockouts severely disrupt egg laying behavior. Secondly, *npr-36* knockouts suppress the hyperactive egg-laying phenotype induced by transgenic overexpression of NLP-3 peptides, further evidence in favor of NPR-36 functioning as an NLP-3 receptor. Thirdly, the *nlp-3 npr-36* double knockouts show similar defects to those of either single knockout, consistent with the neuropeptide and receptor acting in the same genetic pathway. In ongoing work, we are using a CRISPR GFP knock-in at the endogenous *npr-36* promoter to identify the cells that express NPR-36. Concurrently, we are applying a range of concentrations of individual NLP-3 peptides to NPR-36-expressing cultured cells and measuring the downstream signaling this induces to determine if one or more of the five NLP-3 peptides activate NPR-36. Our goal is to understand how signals from serotonin and neuropeptides are integrated to activate a defined neural circuit.

1. Brewer et al. (2019) PloS Genet 15:e1007896.

905B The *C.elegans* AWC^{ON} olfactory neuron responds to tangential component of mechanical stimuli and its activation is mediated by TAX-4 cGMP-gated cation channel

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The two major sensory modalities of *C.elegans* are chemosensation and mechanosensation. Calcium imaging experiments have previously suggested that these two modalities are functionally segregated. Here, we demonstrate that AWC^{ON} olfactory neuron, which plays a crucial role in chemosensation, does not only respond to chemicals but also to mechanical stimuli. Touch senses mechanical stimuli, comprising two components: pressure and shear stress. Previous studies assessing mechanosensation in *C.elegans* have always referred to pressure stimuli only.

In this work, we shown that *C.elegans* senses also the tangential component of mechanical stimuli by recruiting the AWC neuron, and we give evidence that this ability may depend on specific odor receptors.

We further show that the mechanosensitivity of AWC neurons has an intrinsic nature rather than a synaptic origin and the calcium transient response is mediated by TAX-4 cGMP-gated cation channel, suggesting the involvement of one or more "odorant" receptors in AWC mechano-transduction. Moreover, calcium events show a bistable neuronal regime structurally different from the typical calcium response to a chemical stimulus. The observed bistability indicates that AWC^{ON} adopts distinct sensory strategies for chemo-and mechanosensation, adapting molecules and receptors to convert chemical and mechanical stimuli into different cellular signaling.

906C Imaging neuronal dynamics during recovery from anesthesia in *C. elegans*

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The innovation of anesthesia with volatile anesthetic agents has made modern surgical practice possible. The anesthetized state is defined by behavioral criteria and is today largely monitored through physiological measurements such as hemodynamics and gas exchange. These measurements are sometimes supplemented by EEG derived metrics, but the relationship between these metrics and the neurophysiological mechanisms of anesthesia is entirely unknown. We have used cellular resolution multi-neuronal functional imaging in *C. elegans* as a powerful model for bridging the gap between single-neuron activity and whole nervous system dynamics under anesthesia. We previously found that the anesthetic state in *C. elegans* corresponds to a loss of synchrony in global neural dynamics as opposed to generalized depression of individual neuron activity. Here we extend this work by examining how the nervous system of *C. elegans* recovers from the anesthetic state of dissynchrony to normal function. Employing a light-sheet diSPIM microscope we can measure the activity of the majority of neurons in the *C. elegans* head as the animal emerges from anesthesia. We observe that correlation in activity between neuron pairs, which is significantly suppressed in the anesthetized state, recovers to near pre-exposure levels in a non-linear, cyclical manner over several hours. Notably, the time required for this recovery is significantly longer then the time needed to induce the neurological anesthetic state. This finding recapitulates the concept of "neural inertia", a proposed hysteresis in the processes of anesthesia induction and emergence well-characterized in humans by the observation that emergence from anesthesia tends to occur at lower concentrations of drug than are required for induction. Because *C. elegans* perform gas exchange through diffusion, the differences we observe in time needed for induction and emergence are consistent with the phenomenon characteristic of neural inertia in humans. This finding further demonstrates the utility of *C. elegans* as a model system for investigating the effects of anesthesia on neuronal function and the physiological basis of those effects.

907A Opponent vesicular transporters regulate the strength of glutamatergic neurotransmission in a *C. elegans* sensory circuit

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Glutamate is a major excitatory neurotransmitter in the nervous system. Well understood mechanisms regulate the strength of glutamatergic synapses by controlling the abundance and composition of post-synaptic receptors. By contrast, less is known about presynaptic mechanisms that control glutamatergic transmission. Neurotransmitters are packaged into synaptic vesicles by vesicular transporters so that they can be released upon depolarization. Despite their critical role in neurotransmission, how such vesicular transporters are regulated *in vivo* remains poorly understood. Our investigation of a chemosensory circuit mediating CO_2-evoked behavior revealed a mechanism that controls the amount of glutamate packaged into synaptic vesicles to set the strength of glutamatergic synapses.

Chemosensory BAG neurons coexpress the vesicular glutamate transporter, EAT-4, and a related vesicular transporter, VST-1. Loss of VST-1 greatly enhances glutamate release from BAGs but does not alter vesicle fusion in BAG neurons, indicating that VST-1 limits how much glutamate is packaged into synaptic vesicles. Excess glutamate release from BAGs has functional consequences. *vst-1* mutants exhibit a severe chemotaxis defect, which is suppressed by loss of the AMPAR-type glutamate receptor GLR-1. This observation indicates that VST-1 regulates neurotransmission at synapses that use AMPAR-type receptors for glutamatergic transmission. Functional imaging of interneurons postsynaptic to BAGs revealed that loss of VST-1 affects a specific node in the chemotaxis circuitry. RIA interneurons, which are normally not engaged by BAGs, become coupled to BAGs

in *vst-1* mutants. Furthermore, ablation of RIAs restores chemotaxis to *vst-1* mutants, indicating that ectopic activation of RIAs causes the chemotaxis defect of *vst-1* mutants.

These data demonstrate that *in vivo* synaptic strengths are set by mechanisms that control loading of glutamate into synaptic vesicles. Our study further suggests that this mechanism is not limited to glutamatergic synapses. VST-1 is expressed in many non-glutamatergic cells, where it is likely to regulate transport of other neurotransmitters into synaptic vesicles, *e.g.*, acetylcholine and monoamines. Finally, our study suggests that the substrate for VST-1 will act as an endogenous small-molecule regulator of neurotransmitter loading into synaptic vesicles. We will describe progress towards identifying this substrate and speculate about its role in synaptic physiology.

908B Multiple GPCRs function in the head mesodermal cell to rhythmically activate it during a rhythmic behavior in *C. elegans*.

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The anterior body wall muscle contraction (aBoc) is a rhythmic muscle contraction that occurs every 50 seconds during the *C. elegans* defecation motor program. During the aBoc step, the pacemaker (intestine) actives motor neurons which leads to release of a FMRF-Like Peptide, FLP-22, from neurons. Previously, we found that FLP-22 does not directly activate the muscle but instead activates the head mesodermal cell (HMC) through the GPCR FRPR-17. Live GCaMP imaging reveals that HMC is rhythmically activated every 50 seconds just after motor neuron activation, and HMC activation is partially dependent upon *frpr-17* function in HMC. HMC is coupled to muscles through gap junctions composed of UNC-9/innexin and *unc-9* mutants exhibit normal HMC activation but are missing most aBoc steps. *frpr-17* or *flp-22* mutations cause a failure of HMC activation about 40% of cycles, however, in the remaining cycles, HMC calcium spike amplitude and duration are similar to wild type controls, suggesting that additional inputs activate HMC to promote aBoc. Selective genetic ablation of HMC reduces but does not eliminate aBoc frequency, suggesting that there are HMC-independent pathways for promoting aBoc. Here, we identify the neuropeptide-like protein, NLP-6 and the GPCR, NMUR-3 as additional components in the circuit that promote aBoc. *nmur-3* or *nlp-6* null mutations do not alter aBoc frequencies or HMC activation, but they significantly enhance the aBoc frequency defects of *frpr-17* or *flp-22* mutants. We found that the frequency of HMC activation is severely reduced in *frpr-17 nmur-3* double mutants. In addition, *nmur-3* mutations do not enhance the aBoc defects of HMC-ablated animals, suggesting that *frpr-17* and *nmur-3* function in parallel in HMC to activate it. By contrast, *nlp-6* mutations strongly enhance the aBoc defects of *nmur-3* mutants or HMC-ablated animals, suggesting that *nlp-6* functions in parallel to HMC to promote aBoc. We propose that HMC promotes aBoc through the activation of both the FRPR-17 and NMUR-3 signaling pathways in HMC, whereas NLP-6 functions through a HMC-independent circuit.

909C Circuit and Molecular Mechanisms of an Associative Learning Task

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The ability of neural circuits to be changed by experience, optimising an organism's behaviour, is key to survival. We are dissecting the circuit underlying aversive olfactory learning to benzaldehyde (BZ), a bacterial metabolite innately attractive to *C. elegans*. When this odourant is paired with starvation, an aversive experience, the behavioural response to BZ switches from attraction to repulsion (Lee 2010, Lin 2010). Furthermore, this switch in behaviour can be overridden in males if a rewarding experience (mates) is present during aversive conditioning with starvation. This process is termed sexual conditioning and it was first shown to drive flexible learned responses to salt (Sakai et al, 2013). Our lab has previously shown that the neuropeptide PDF-1 is necessary for sexual conditioning. Here we show that both PDF-1 and PDF-2 redundantly mediate aversive learning, and seek to identify the PDF circuit for aversive learning.

BZ is sensed primarily by AWC, which synapses onto AIB and AIY to regulate naïve chemotaxis (Bargmann 1993, Chalasani 2007). The switch from attraction to repulsion during aversive learning has been proposed to be driven by changes at the AWC-AIB synapse (Cho 2016). We find that AWC-ablated animals have a dampened response to BZ, but we still observe a switch in response from attraction to repulsion after aversive conditioning, indicating that learning can occur elsewhere in the circuit. We are investigating neurons that are involved in PDF signalling to find the circuit that drives this experience-dependent change in behaviour. We are using an intersectional Cre-Lox strategy and a floxed transgene of PDF-1 cDNA integrated in single copy to return physiological levels of PDF-1 to specific neurons, and thus determine which combinations of neurons are the source of PDF-1 in aversive learning. We will also use an approach that exploits the strength of the Cre-Lox system to restore

PDFR-1 to certain neurons selectively, to determine which neurons are the target of PDF during aversive learning. Then we will image the activity of source and target neurons to determine what changes occur in the circuit during conditioning that lead to changes in behaviour.

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.

910A Wnt signaling regulates a post transcriptional mechanism for synaptic choice

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Coordinated movement depends upon the formation of specific synapses in the motor circuit. In *C. elegans*, the homeodomain transcription factor UNC-4 directs VA motor neurons to connect with AVA interneurons which drive backward locomotion. Backward movement is disrupted in *unc-4* mutants because VA neurons synapse with AVB interneurons instead of with AVA. Previous work demonstrated that UNC-4 antagonizes a posterior EGL-20/Wnt signaling pathway to preserve VA inputs from AVA. In *unc-4* mutants, EGL-20/Wnt drives ectopic expression of the homeodomain transcription factor CEH-12 in posterior VAs which switches synaptic inputs to AVB. We built an endogenous CEH-12::GFP reporter to confirm that EGL-20 is indeed required for ectopic CEH-12::GFP expression in posterior VAs. Surprisingly, *in situ* hybridization (smFISH) detected ectopic *ceh-12* transcripts in *unc-4* mutant VAs throughout the nerve cord. smFISH also revealed that EGL-20/Wnt is not required for *ceh-12* transcription. Thus, our results suggest that EGL-20/Wnt regulates a post-transcriptional mechanism that limits expression of CEH-12 protein to posterior VA motor neurons. We are now investigating the mechanism of Wnt-dependent post-transcriptional control of a key regulator of synaptic choice.

911B A Comprehensive Characterisation of *C. elegans* Neurotransmitter GPCRs Reveals a Novel Adenosine Receptor

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Many G protein coupled receptors (GPCRs) can be activated by neurotransmitters, acting via heteromeric G proteins to control downstream intracellular processes. A GPCR can be simplified as either excitatory (Gαs or Gαq) or inhibitory (Gαi/o). While ligands for many *C. elegans* neurotransmitter GPCRs have been identified, many gaps in our knowledge remain. To address this, we expressed *C. elegans* GPCRs in *Xenopus* oocytes along with G protein-coupled inwardly rectifying potassium channels (GIRKs) to evaluate ligand specificity and G-protein coupling using two-electrode voltage clamp recordings.

Using this approach, we have successfully observed activation of 10 monoamine-activated GPCRs, all with previously identified ligands. Despite this previous knowledge, some results on their G-protein coupling, and the potency of secondary ligands was often conflicting or missing. For example, SER-6 and DOP-4 have previously been shown to couple to Gαs but our experiments indicate Gαq coupling. Activation by two or more ligands was observed for all the GPCRs tested, but the published data does not reflect this phenomenon. Several additional orphan receptors were evaluated, DOP-5, DOP-6, SER-5 and PCDR-1, and no activation was detected. The reason is unknown, but it could be because they require a GPCR partner to form a functional receptor, because they couple to a non-cannonical G-protein, or because we have not tested the correct ligands.

In addition, we have characterized the putative adenosine receptor ADOR-1 and found it to be indeed activated by adenosine. Surprisingly, we obtained evidence suggesting coupling to the excitatory Gαq protein as well as to Gαi/o or Gαs. Further experiments are ongoing to confirm this dual coupling *in vitro* and *in vivo.* Using endogenous fluorescent reporters, we found *ador-1* to be broadly expressed in the nervous system as well as in muscle, including MC/I4/pharyngeal muscle and vulval muscle/HSN. However, despite this expression pattern we did not observe any significant differences in pumping rate or egg laying under normal conditions. In other species, intracellular adenosine levels have been shown to increase after injury or stress, and we observed broad expression of *ador-1* in multiple 'avoidance' neurons (AWB/ASH/ASK). We hypothesise that adenosine may act via a*dor-1* to regulate stress related behaviours or lifespan and we plan to investigate this going forward.

912C Developmental sleep disruption induces neuronal plasticity mediated by conserved autism-associated synaptic adhesion molecules

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Autism spectrum is characterized by altered social and repeated/repetitive behaviors, but also often presents with disrupted sleep behavior, such as late-onset or fragmented sleep. Furthermore, autism and other neurodevelopmental disorders have been linked to changes in synaptic plasticity and excitatory/inhibitory balance, which may mechanistically link sleep phenotypes and other behaviors in these conditions. Still, how genes associated with autism, including those that regulate synaptic function, contribute to plasticity, behavior, and sleep, remains elusive. Previous work from our lab found that the GABAergic DVB neuron undergoes morphologic plasticity in response to experiences during development and in adulthood (e.g., mating, environmental stressors). Here, we utilized genetic, chemogenetic, physical methods to disrupt sleep states in larval development in male *C. elegans,* and quantified DVB neuronal morphology in early adulthood. We found that all methods used to disrupt sleep led to increased DVB neurite outgrowth compared to males with normal sleep. Disruption of sleep specifically during sexual maturation (L4) was sufficient to alter DVB neuronal morphology. Using this novel model of sleep-dependent plasticity, we investigated the role of autism-associated genes in DVB plasticity in response to sleep disruption. We tested the effect of loss of function mutations in the singular *C. elegans* orthologs of the autism-associated genes *nrx-1/NRXN1* and *nlg-1/NLGN3* on DVB plasticity following sleep disruption. Sleep disruption in males mutant for *nrx-1* and *nlg-1* did not show altered DVB outgrowth, suggesting a role for autism-associated synaptic adhesion molecules in regulating sleep-dependent neuronal plasticity. We are currently testing in which neurons *nrx-1* and *nlg-1* are required to restore DVB plasticity after sleep disruption, and whether unique molecular mechanisms underlie the contribution of these genes in sleep-dependent plasticity. This work leverages a simple neuronal system to better define the relationship between sleep disruption, synaptic adhesion molecules, neuronal plasticity, and autism-associated genes, which has important implications for behavior and for the manifestation of neurodevelopmental conditions.

913A Neuromodulation and the relationship between Ca^{2+} transients and neuronal states

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Neuromodulators (monoamines and neuropeptides) shape nervous system function by regulating neuronal depolarization and synaptic strengths. We are studying neuromodulation in the context of nociception, to better understand pain perception and pain treatment strategies. The ASH neuron is a major nociceptor in *C. elegans*. ASH senses 1-octanol and drives an aversive response, modulated by the monoamines serotonin (5-HT, potentiating) and octopamine (OA, inhibitory). To better understand neuromodulation, we are focusing on 1-octanol stimulated Ca^{2+} dynamics in ASH, and the quantitative relationship between Ca^{2+} signals and depolarization amplitudes. We showed that 5-HT potentiates ASH depolarization, but surprisingly, inhibits ASH Ca^{2+} transient amplitudes. These effects, like the 5-HT stimulation of behavior, depend on the SER-5 receptor in ASH. This paradoxical finding is explained by existence of a Ca^{2+}-dependent inhibitory feedback loop: Ca^{2+} inhibits ASH depolarization through SLO-1 IKCa channels, and 5-HT inhibits EGL-19 L-type Ca^{2+} channels in ASH (via SER-5), thus disinhibiting the neuron. We are currently investigating modulation of 1-octanol responses by OA. OA inhibits 1-octanol behavioral responses, and antagonizes 5-HT potentiation, dependent on the OCTR-1 receptor in ASH. OA also inhibits ASH Ca^{2+} transients, representing another paradox: how can OA and 5-HT have opposite effects on ASH-dependent aversive behaviors, but the same effect on ASH Ca^{2+} transients? Our results show that 5-HT and OA utilize distinct signaling pathways in ASH (Gαq for 5-HT and Gαo for OA), and that OA does not inhibit EGL-19. We are currently testing the hypothesis that OA hyperpolarizes ASH through Gαo-dependent activation of IRK K^+ channels. These results further emphasize that neuronal Ca^{2+} transients, as key reporters of neuronal depolarization, are also critical signaling intermediates in and of themselves, with multiple upstream inputs and downstream consequences.

914B $G\alpha_q$ acts via DAG signaling to modulate serotonin motor circuit activity in *C. elegans*

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$G\alpha_q$ signals through the Phospholipase-Cβ (PLCβ) and Trio, a Rho GTPase exchange factor (RhoGEF), but how these two effector pathways promote synaptic transmission remains poorly understood. Here we use the egg-laying behavior circuit of *C. elegans* to show that PLCβ/EGL-8 and Trio/UNC-73 mediate serotonin signaling through related biochemical pathways but by functioning in distinct cells. Using transgenic rescue experiments we find that PLCβ functions in neurons while Trio functions in both neurons and the postsynaptic vulval muscles. Additionally, cell specific activation of Rho GTPase/Rho-1 in the

serotonergic HSN neurons promotes synaptic transmission, associated with increase in HSN activity and egg-laying behavior. While $G\alpha_q$, PLCβ, and Trio mutants all fail to lay eggs in response to serotonin, optogenetic stimulation of the serotonin-releasing HSNs restores egg laying only in PLCβ mutants. We observed vulval muscle Ca^{2+} activity in PLCβ mutants while such activity was eliminated in strong $G\alpha_q$ and Trio mutants. Remarkably, egg-laying circuit activity and behavior defects of $G\alpha_q$, PLCβ, and Trio mutants were rescued by Phorbol esters thought to mimic Diacylglycerol (DAG), a product of PIP2 hydrolysis by Phospholipases like PLCβ/EGL-8. DAG has been proposed to activate effectors including UNC-13, but we find that phorbol esters, but not serotonin, stimulate egg-laying behavior in *unc-13* mutants. Together, these results show that serotonin and $G\alpha_q$ promote egg laying via two parallel mechanisms. Serotonin signaling through $G\alpha_q$ and PLCβ modulates UNC-13 activity to promote neurotransmitter release. Serotonin $G\alpha_q$ signaling through Trio RhoGEF- Rho GTPase and an unidentified, PMA-responsive effector promotes postsynaptic muscle excitability. Thus, one neuromodulator can signal in distinct cells through different effector pathways to activate a motor behavior circuit.

915C Whole-brain imaging with neuronal identities to elucidate the mechanism of a sensory processing

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A neural circuit in the brain processes sensory stimuli such as an odor to elicit a motor command. To understand the mechanism of such a sensory processing, a number of studies have sought to identify a neural circuit which processes a specific sensory stimulus (functional neural circuit). For identification of a functional neural circuit, it is required to conduct two types of experiments throughout the brain: 1) locating neurons that respond to the stimulus, and 2) identifying their cell types (cell-IDs) to estimate their connectivity based on anatomical connections. Locating stimulus-responsive neurons can be achieved by whole-brain calcium imaging, which record all neuronal activities throughout the brain. However, due to the lack of way to efficiently identifying cell-IDs, it has been difficult to identify a complete functional neural circuit with whole-brain imaging and cell identification.

To overcome the limitation, the method for cell identification of all neurons, called NeuroPAL, was developed in *C. elegans* (Yemini et al., Cell, 2020). Together with the connectome information as well as the records of all neuronal activities, we are now able to estimate a functional neural circuit based on cell-IDs of stimulus-responsive neurons.

To identify a complete functional neural circuit by integrating those techniques, we established a whole-brain imaging system combined with efficient cell identification system using NeuroPAL. This system consists of a 3D confocal microscopy, a multi-color imaging system, a microfluidic device, and a pipeline of an image analysis (Chronis et al., Nat. Meth., 2007; Wen et al., eLife, 2021). By using this system, we successfully recorded activities of most head neurons simultaneously with an information of each cell-ID. Now we are identifying a set of odor-responsive neurons using this system with stimulation by a repellent odor 2-nonanone (Kimura et al., J. Neurosci., 2010).

This study aims to identify a complete functional neural circuit for specific sensory processing pathways and it may provide clues for how a sensory stimulus is processed in the whole of a neural circuit. We thank Ev Yemini and Oliver Hobert for the help on NeuroPAL.

916A The regulation of olfactory circuit by EGL-4/PKG

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C. elegans has a simple circuit to process information of attractive odorants. That is basically a network between two pairs of olfactory neurons and several first-layer interneurons, which transmit the signals to downstream command interneurons or motor neurons. However, how the information processing works in the circuit remains elusive. *egl-4* encodes a cGMP-dependent protein kinase (PKG), and the loss-of-function mutations in the gene cause chemotaxis defects. By Ca^{2+} imaging analyses, we revealed that the response property of one of the first-layer interneuron, AIY, to diacetyl is reversed in the *egl-4* mutant, while the responses of two upstream olfactory neurons, AWA and AWC, are largely unchanged. Ablation of AIY improved the chemotaxis to diacetyl of the *egl-4* mutant animals, showing that AIY functions to suppress chemotaxis in *egl-4*. Interestingly, to the stimuli of another odorant, isoamyl alcohol, AIY in the *egl-4* mutant responds almost normally. As expected, ablation of AIY in the *egl-4* mutant did not improve the chemotaxis to isoamyl alcohol. These results implicate that EGL-4/PKG is involved in information processing in an odorant-specific manner and that the role of the AIY interneuron in chemotaxis circuit may be different for each odorant. We speculate that olfactory information of each odorant may be transmitted through different combination of redundant circuitries to induce chemotactic behavior and *egl-4* mutation only

affects a certain circuitry. Such redundant circuitries may ensure the robust chemotaxis of worms and enable worms to perform multiple tasks concurrently. We found that the *egl-4* mutant failed to exhibit chemotaxis to an odorant on plates saturated with another odorant, while the wild-type animals are still able to exhibit chemotaxis in the same condition. This appears to be consistent with the hypothesis. We are now trying to dissect the chemotaxis circuit by imaging analyses and cell ablation experiments and to reveal the neural mechanisms that allow animals to perform complex tasks.

917B The role of network topology in the performance of the circuit for nociceptive behaviors in *C. elegans*

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The nature of the relationship between neural circuits and the resulting animal behavior is a key question in neurobiology. It was previously established that the specific synaptic and cellular properties of neural networks can be widely disparate, yet maintain similar function (Prinz et al., 2004). It is therefore clear that some features of a network's structure are important to retain certain functional features. The sensitivity of such networks to changes in the topology have not been characterized.

To explore the contribution of topology to the network's performance, we focused here on the circuit for nociceptive behaviors in *C. elegans.* The neurons of the circuit are shared between the two sexes, but their connectivity is different (Cook et al., 2019). The behaviors that result from these circuits are sexually dimorphic as well. The distinct network topologies and behavioral outputs make this circuit a good example for exploring the relationship between structure and function in neural networks.

We simulated the response of the nociceptive circuits to external stimuli, in males and in hermaphrodites, using a wide range of realistic values for the circuit's biophysical parameters (synaptic strengths, conductivity, membrane time constants, etc.). We then searched for the parameters' space in which the activity of the motor output neurons in the simulation would match the worms' behaviors in experimental observations.

We found an overlap between the sexes in terms of the synaptic and cellular parameters that allow for the correct behavior of the network. Moreover, our results suggest that the connectivity alone might be sufficient to explain the behavioral differences between the sexes. Notably, more stringent requirements of the models' performance suggests that the connections in this network cannot be all excitatory, as has been commonly assumed, or that additional inhibitory neurons must play an important role in shaping the circuit's response to tail stimulation. Future analysis will further explore the relations between the network's topology and the joint activity patterns of the neurons as measured by calcium imaging.

918C Visualising neuropeptide spatial range of action within the nervous system.

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Past experiences, moods and emotions change our behaviours, partly due to the neuromodulation of underlying circuits from neuropeptides. Neuropeptides are secreted, diffusible proteins that act on G-protein coupled receptors (GPCRs), providing long-lasting modulation to hard-wired neuronal circuits. Most neuropeptide receptor expressing neurons are not synaptically connected to the source, thus, the expression patterns of the ligand and its receptors represent a wireless chemical connectivity map that work in conjunction with traditional synaptic communication. Neuropeptides regulate many global states as well as more discrete modulatory functions such as learning and memory. However, it is unknown how neuropeptides encode such diverse information. For global behavioural state changes, it is thought neuropeptides may activate any target they reach in their diffusion pathway, a process termed volume transmission. For functions that require specific target modulation, such as associative learning, emerging evidence suggests diffusion could be more regulated and controlled (van den Pol 2012), however mechanisms underlying this regulation are unknown. Many affective disorders rely on exogenous application of neuromodulators, therefore improving our understanding of neuropeptide diffusion regulation, could reduce adverse side effects many patients suffer due to off-target activity.

We are employing *C. elegans* to establish an *in vivo* system to visualise neuropeptide based neuronal communication to use as a tool to identify molecular and cellular regulators of neuropeptide diffusion. We are focusing on Pigment Dispersing Factor-1 (PDF-1) and its receptor PDFR-1, a highly conserved neuropeptide involved in a broad array of behaviours. By harnessing GPCR signalling cascades, we will introduce a synthetic pathway that converts the interaction between PDF-1 and PDFR-1 into a fluorescent signal, without interfering with endogenous signalling. We will control PDF-1 expression and secretion in a ligand-null mutant by co-expressing PDF-1 and channelrhodopsin in one neuronal source. This expression pattern will be used as the

basis for a forward genetic screen. This project aims to create a system to visualise neuropeptide modulation *in vivo* which will set the foundation for an in-depth investigation to understand neuropeptide spatial range of action.

919A Glial KQT-2 K+ channels are needed for aversive response to octanol

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KCNQ channels are members of a family of voltage-gated potassium channels that are encoded by five human genes and are evolutionarily conserved across species. KCNQ2 through 5 subunits are primarily expressed in the nervous system, where they modulate cellular excitability. Epilepsy, autism and other neuropsychiatric conditions have been associated with mutations in the KCNQ channels genes expressed in the nervous system. Although strong evidence supports the expression of KCNQ channels both in neurons and in glia, the role of these channels in glial cells is still unknown. In this study, we use C. elegans to investigate the glial function of the nematode KQT channels, which are the homologs of the human KCNQ channels. By RNA sequencing we found that the amphid glia, that encapsulate sensory neurons in the amphid sensory organ, are enriched in kqt-2. Using kqt-2 knock-out and glial-specific knock-down, we found that glial KQT-2 is needed for aversive response to octanol. Furthermore, we showed that the kqt-2 reduced octanol response is rescued by glial-specific expression not only of the nematode KQT-2 but also of the human KCNQ2 and KCNQ3 subunits. Our data support that KCNQ channels function in glia to promote neuronal activity suggesting that diseases associated with mutations in these channels might be contributed by glial KCNQ channels as well. Ca2+ imaging and behavioral experiments with worm and human genes wild type and mutants are in progress to establish via what mechanism glial KCNQ channels regulate neuronal output and behavior.

920B Expansion of Cholinergic Signalling Reveals Polymodal and Novel Ligand Gated Ion Channels Involved in Switching Behavioural States

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Ligand-gated ion channels (LGICs) play important roles in synaptic communication and the regulation of behaviours. The cys-loop superfamily of LGICs, which contains mammalian nicotinic acetylcholine and GABA receptors, has undergone vast gene expansion in nematodes and includes channels gated by classical and non-classical neurotransmitters. Yet the majority of *C. elegans* LGICs remain uncharacterised, with no known ligand or biological function. We have undertaken a deorphanisaiton study of a number of uncharacterised *C. elegans* LGICs, in particular from a subfamily of 12 channels known as the «diverse» group. Using two-electrode voltage clamp recordings from *Xenopus* oocytes injected with worm LGICs, we identified ligands for 5 of the 12 channels in the diverse subgroup. All are inhibitory anion-selective channels, yet despite sharing close sequence similarity, they are gated by chemically diverse ligands.

We identify three receptors for choline, GGR-1, GGR-2 and LGC-40, which despite binding the same ligand differ in their pharmacological and neuronal expression profiles. This may point towards a potential role for choline in the regulation of the nervous system. Interestingly, we found a single channel, LGC-39 to be gated not only by acetylcholine but also by aminergic ligands, in particular octopamine and tyramine. Thus LGC-39 has the capacity to form a polymodal receptor activated by chemically disparate neurotransmitters. The expression pattern of *lgc-39* reveals that it is present in a number of neurons which receive both aminergic and cholinergic input, including AVA, the major synaptic target of the octopamine producing RICs.

Finally, we find that LGC-41 is not activated by any classical neurotransmitter, but is activated by betaine, a metabolite chemically related to choline. LGC-41, along with putative betaine synthesis genes, is widely expressed in the nervous system, in particular in a number of neurons associated with regulating search behaviours including ASI. Strikingly, we find that *lgc-41* and betaine synthesis mutant worms show defects in transitioning from local to global search behaviour in the absence of food. This implicates betaine and its receptor in regulating complex behaviours which rely on the integration of a number of sensory inputs. Taken together our findings highlight the remarkable functional and behavioural diversification amongst the LGICs in *C. elegans*.

921C Identifying Triggers for Pathogen Learning in *C. elegans*

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The ability to discriminate between nutritious and harmful food is essential to survival. As a result, learned avoidance to harmful food sources is conserved from invertebrates to humans. The mechanisms enabling the nervous system to associate sensory cues from a food source with an internal state of sickness to trigger aversive memory formation remain elusive. After prolonged exposure to pathogenic food, *C. elegans* can learn to avoid the pathogen upon subsequent encounter[1]. This learned aversion requires infection; non virulent forms of bacteria are not sufficient for memory formation. In response to exposure to pathogenic food, serotonin is induced in a pair of sensory neurons called ADF and remodels downstream circuits[2]. Learned aversion to pathogen requires serotonin signaling from ADF, suggesting that ADF serves as a site of integration for detecting bacterial cues and internal sickness caused by the pathogen.

We seek to understand how internal state changes the coupling between sensory activation and serotonin release in ADF neurons. As a first step, we are using calcium imaging to examine ADF responses to bacterial cues in both naive and pathogen-exposed animals. ADF responds robustly to conditioned media from both pathogenic and non-pathogenic bacteria in a dose-dependent fashion, and ADF activity can be modulated by previous odor history. We are screening mutants using these quantitative parameters to assess ADF responses to direct chemosensory stimuli, indirect signaling from other sensory neurons, and signaling from non-neuronal tissues indicating bacterial infection. Our goal is to uncover cell-biological mechanisms through which ADF neurons mediate learned pathogenic behavior in *C. elegans*.

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922A Molecular Encoding and Synaptic Decoding of Memory of Chemical Concentration in C. elegans

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Learning the intensity of past stimulus, e.g. a concentration of a chemical substance, is a widely conserved function of the nervous system. However, how the memory is encoded in molecules and decoded through synaptic transmission is not comprehensively understood.

C. elegans memorizes experienced NaCl concentration and migrates toward the learned concentration. The canonical signaling pathway underlying this behavior is the diacylglycerol(DAG)/protein kinase C(PKC) pathway, which functions in the NaCl-sensing neuron ASER. Previous reports observed DAG dynamics during changes in ambient NaCl concentration and revealed that DAG decreased with increasing NaCl and DAG increased in response to NaCl decrease. Subsequently, once activity of PKC-1 is changed, the direction of chemotaxis on the NaCl gradient is biased: when PKC-1 is activated, the worm migrates towards higher NaCl, whereas inactivation causes migration towards lower NaCl. As a consequence, worms migrate towards preferred concentration of NaCl. However, how PKC-1 generates migration bias is yet unknown.

To identify the molecule that acts downstream of PKC-1, we performed nervous system-specific phosphoproteomic analysis, using a nonspecific biotinylation enzyme, TurboID. Among phospho-sites upregulated in pkc-1(gf)-expressing worms, we found a phosphorylation of UNC-64/Syntaxin 1A at Ser65. The Ser65 phosphorylation-deficient mutant of unc-64, unc-64(S65A), showed a migration bias in the same direction as pkc-1(lf). unc-64(S65A) showed reduced release of glutamate following activation of ASER.

Furthermore, we investigated how unc-64(S65A), or a reduction in glutamate release, results in migration bias. As previous studies from our lab showed that the AIB interneurons respond to a change in NaCl concentration via the glutamatergic transmission from ASER, we focused on the response of AIB. Both in unc-64(S65A) and pkc-1(lf), AIB responses were reversed compared to wild type. However, glutamate release from ASER increased by activation of ASER in both wild type and pkc-1(lf), suggesting changes in glutamate release alone cannot explain the reversed AIB response. This led us to examine whether the basal synaptic release, or basal glutamate level may affect the response of AIB. Indeed, direct exposure of AIB neurons to glutamate revealed that the response of AIB to increase of glutamate is reversed according to the glutamate concentration in the external bath solution. We now hypothesize that this is due to a difference of sensitivity between inhibitory and excitatory glutamate receptors.

923B A Dual Role for LAR/PTP-3 in Regulating Long-distance AMPAR Transport and Synaptic Retention Essential for Long Term Associative Memory

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Synaptic AMPA subtype of glutamate receptors (AMPAR) play essential roles in excitatory synaptic transmission, learning and memory. The majority of them are synthesized in the soma of neurons and are transported to far reaching dendrites. Although, recent studies have shown that long-distance synaptic transport is regulated by neuronal activity, we know very little about how AMPAR get delivered or removed by molecular motors. Here we show that loss of the A isoform of the receptor tyrosine phosphatase PTP-3 the *C. elegans* homologue of vertebrate LAR-RPTP, leads to a ~60% decrease in AMPAR transport affecting synaptic delivery and synaptic function necessary for long-term associative olfactory memory (LTAM) in *C. elegans*. We show that PTP-3A is necessary postsynaptically in adult neurons for the regulation of AMPAR transport, delivery and memory. In addition, Fluorescence Recovery After Photobleaching and Photoconversion of tagged GLR-1 reveal, that while loss of PTP-3A leads to defects in transport and local synaptic trafficking of GLR-1, a mutation affecting all PTP-3 phosphatase domains only affects local synaptic cycling and retention. Finally, we show that the N- and C-terminal of PTP-3A have different functions in regulating transport and synaptic retention of AMPARs. Altogether, our results suggest a model in which PTP-3 coordinates transport and delivery of AMPARs to synapses using 2 domains possibly released by synaptic activity and essential for long-term associative learning.

924C Contribution of a *FOXD3/4* ortholog to optimization of avoidance behavior mediated by pre- and postsynaptic gene expression for a biphasic calcium response

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The central neural network optimizes avoidance behavior depending on the nociceptive stimulation intensity is essential for survival. How the property of the hub neurons for selecting behaviors is genetically defined is not well understood. Here we show that the transcription factor *unc-130*, a human *FOXD3/4* ortholog, is required to optimize the avoidance behavior depending on stimulus strength in *Caenorhabditis elegans*. *unc-130* is necessary for both ON-calcium decrease and OFF-calcium increase in AIB, a central neuron of avoidance optimization. Ablation of predicted upstream inhibitory neurons reduced frequency of turn behavior, suggesting optimization needs both calcium responses. At a molecular level, *unc-130* upregulates expression of at least three genes: *nca-2*, a homolog of the vertebrate cation leak channel *NALCN*, *glr-1*, an AMPA type glutamate receptor, and *eat-4*, a hypothetical L-glutamate transmembrane transporter in the central neuron of optimization. *unc-130* shows more limited regulation in optimizing behavior than an *atonal* homolog *lin-32*, *unc-130* and *lin-32* appear to act in parallel molecular pathways. Our findings suggest that *unc-130* contributes to behavioral optimization mediated by pre- and postsynaptic expression to lead biphasic neural responses.

925A A neuropeptide-controlled circuit controls rhythmic anterior body wall muscle contraction

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Neuropeptides play critical roles in controlling behavior, but how neuropeptides pattern the output of neural circuits is poorly understood. Here, we report the identification of a simple circuit composed of the intestine, the motor neuron AVL, and the muscle that controls the rhythmic anterior body wall muscle contraction (aBoc) and we find that this circuit is controlled exclusively by neuropeptide signaling. The neuropeptide like protein NLP-40, which is secreted from the intestine (pacemaker), elicits a calcium transient in AVL every 50 seconds. Genetic ablation of AVL or inhibition of exocytosis from AVL by tetanus toxin (TeTx) abolished 80% of the aBoc events. Live calcium imaging of AVL revealed that *nlp-40* mutations eliminated calcium transients in AVL, whereas null mutations in *aex-2/*GPCR, which encodes the NLP-40 receptor, led to both a reduction in calcium transient frequency and a delay in their onset. These results suggest that AVL is the major target for NLP-40 signaling for aBoc and can be activated by NLP-40 in both an AEX-2-dependent and independent way. We identified two neuropeptides that contribute to aBoc, which are the FMRFamide neuropeptide-like protein FLP-22 and the neuropeptide-like protein NLP-6. *flp-22* mutants reduced aBoc frequency to 60% whereas *nlp-6* mutants had normal aBoc frequency, and *flp-22; nlp-6* double mutants showed severe aBoc defects similar to AVL ablation. Channelrhodopsin-induced AVL activation could robustly elicit aBoc in the absence of *nlp-40*, but failed to elicit robust aBoc in *flp-22; nlp-6* double mutants, suggesting that FLP-22 and NLP-6 secretion from AVL promotes aBoc. FLP-22 or NLP-6 fusion proteins with Venus adopted a punctate pattern of fluorescence in the AVL soma and axon, and there was a significant accumulation of FLP-22 and NLP-6 containing vesicles in axons of animals with impaired pacemaker input (*nlp-40* or *aex-2* mutants), or compromised dense core vesicle secretion (*unc-31/*

CAPS mutants). We found that *lrk-1*/LRRK functions in AVL to promote aBoc to positively regulate the secretion of both FLP-22 and NLP-6, but is not required for AVL activation by NLP-40. We found that AVL-specific inhibition of the PKA pathway, which functions downstream of AEX-2, led to accumulation of NLP-6::Venus at release sites but had no effect on FLP-22::Venus accumulation, whereas inhibition of SNARE-mediated exocytosis (TeTx expression in AVL or *snb-1*/synaptobrevin mutants) selectively increased FLP-22::Venus accumulation. These results suggest that the release of FLP-22 and NLP-6 are regulated by both common and distinct signaling factors. Together, we propose that the differential regulation of FLP-22 and NLP-6 release from AVL by the pacemaker signal ensures that the anterior body wall muscle contraction occurs at the proper frequency and that its timing is coordinated with the defecation motor program.

926B Learning-dependent gain control by asymmetric modulation of the first- and second-order time-differential of stimulus in sensory neurons

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Animals respond to environmental stimuli whose intensity varies by approximately 10^{10}-fold, although neural responses can only change by 10^{2}-fold, which requires proper adjustment of the relationship between the environmental stimuli and the neural response. One example of this adjustment is neural gain control, defined as the change in the slope of a neural response to a stimulus, instead of a general reduction (adaptation) or enhancement (sensitization) of the response. However, these mechanisms are poorly elucidated. Here, we report that the neural gain control in the ASH nociceptive neuron occurs by asymmetric modulation of the first- and second-order time-differentials of sensory stimulus. Previously, we showed that the worm's avoidance behavior to the repulsive odor 2-nonanone is enhanced by pre-exposure to the odor as a type of non-associative learning (Kimura et al., J Neurosci 2010). We now found that the ASH responses, which are activated by increasing the 2-nonanone concentrations (Tanimoto et al., eLife 2017), are modulated by the odor learning. Quantitative odor stimuli analysis revealed that the naive ASH neurons respond similarly to small and large linear increases in odor concentration, whereas the pre-exposed ASH neurons only respond to large increases. Analysis of the stimulus-response relationships suggested that this learning-dependent change is a neural gain control of response. Interestingly, mathematical analysis revealed that the ASH response is approximated by the sum of the first- and second-order time-differentials of odor concentration, and the second-order time-differential is greatly suppressed by learning. We found that the terms of the first- and second-order time differential are expressed by the variable coefficients. To test the validity of this model, we compared it with the first-order time-differential only model and second-order time-differential only model using the Bayesian information criterion (BIC). As predicted, in naive ASH neurons, the model of the sum of the first- and second-order time-differentials of odor concentration was the best fit, and the first-order time-differential only model was the best fit in the pre-exposed condition. These results may suggest that the ASH response is mediated by the long (corresponding to the first-order term) and transiently (the second-order term) activated voltage-gated calcium channels and that the contribution of these channels are modulated by the odor stimulus.

927C Mitochondrial hydrogen peroxide in interneurons induces neuropeptide secretion to regulate the intestinal antioxidant response

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Reactive oxygen species (ROS) are chemically reactive molecules that are formed during oxidative phosphorylation and function as important messengers in cellular signal transduction and homeostasis. We found that endogenously generated hydrogen peroxide (H_2O_2) by axonal mitochondria functions as signaling cue to regulate secretion of the neuropeptide-like protein, FLP-1, from AIY to promote organism-wide protection against oxidative stress. We showed that acute (10 min) exposure to the ROS generator juglone or H_2O_2, or the activation of miniSOG on AIY mitochondria increases the release of FLP-1::Venus fusion proteins from AIY. Mutations in the endogenous mitochondrial peroxiredoxin/*prdx-3*-thioredoxin/*trx-2* antioxidant system, which lead to elevated H_2O_2 levels in AIY axonal mitochondria, increase FLP-1 release from AIY. Mutants with defects in the anterograde transport of mitochondria to axons (*ric-7*) or conversion of superoxide to hydrogen peroxide in mitochondria (*sod-2*) block FLP-1 secretion induced by juglone but not by H_2O_2. *ric-7*, *sod-2* and *trx-2* function cell autonomously in AIY to regulate FLP-1 secretion, and the catalytic activity and mitochondrial targeting of TRX-2 and SOD-2 are essential for their functions in regulating FLP-1 secretion. In addition, H_2O_2 promotes FLP-1 secretion through cell-autonomous activation of the dense core vesicle priming factor protein kinase C (PKC-1). PKC-1 variants lacking a conserved cysteine residue (C524) that is predicted to be sulfenylated by H_2O_2 restore baseline but not juglone- or H_2O_2-induced FLP-1 secretion to *pkc-1* mutants. Finally, H_2O_2-induced FLP-1 release from AIY activates the oxidative stress response transcription factor SKN-1/Nrf2 in

the intestine and protects animals from ROS-mediated toxicity. Together our data reveal a role for endogenous H_2O_2 in linking mitochondrial stress with the oxidative stress response through neuroendocrine signaling.

928A Connectomic comparison of *P. pacificus* and *C. elegans* anterior nervous system structure

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Generating connectomes can lead to an increased understanding of how nervous system structure and connectivity can dictate behavior. To expand upon this growing body of knowledge, here we use connectomics to describe how the nematode nervous system has evolved by comparing the divergent nematode species *P. pacificus* and *C. elegans*. As the complete connectome of *C. elegans* has already been established, we reconstruct serial-section electron micrographs (EM) to generate volumetric reconstructions of multiple *P. pacificus* samples. We extend our analysis of *P. pacificus* nervous system ultrastructure beyond the pharyngeal (Bumbarger et al 2013) and amphidial (Hong et al 2019) circuits to include the complete nerve ring and surrounding anterior nervous system (defined as the tip of the nose to the deirid commissures). We have completed our reconstruction of these samples, and find the overall structure of the nervous system is conserved, and we can unambiguously identify all neurons whose cell bodies or commissures are present within these EM series. Through embryonic lineaging, we observe only one difference in neuron number – the AVH neurons are absent in *P. pacificus* due to an extra cell death. Despite a gross anatomic similarity, approximately 20% of neurons are structurally different between species. These differences (examples in parenthesis) include axonal extensions (ALM), axonal absences (AVM), somatic protrusions (ASJ), dendritic absences (URB), somatic dislocations (IL2), and local branching (RIS). Beyond structural differences at the neuron level, we explored whether fundamental aspects of neighborhood or strata formation, as described by Brittin et al 2021 and Moyle et al 2021, are conserved across species. The number of neighboring or adjacent processes is similar between species and is also nearly equivalent in its constancy. We find, however, that the set of individual neuronal adjacencies differ between species, leading to a more lateralized partitioning of left-right homologous neurons into distinct groups. Together, our results suggest the nerve rings of *P. pacificus* and *C. elegans* are structurally more different than what is seen across development or between the sexes. We continue to analyze our results in the context of synaptic connectivity, behavior, and molecular markers.

929B FSHR-1 regulates cholinergic synaptic vesicle and active zone protein localization to control neuromuscular signaling balance in *C. elegans*

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Excitatory to inhibitory signaling balance is essential to nervous system function. The G protein-coupled receptor FSHR-1 is the *Caenorhabditis elegans* ortholog of mammalian glycopeptide hormone receptors and modulates signaling at the *C. elegans* neuromuscular junction, where excitatory cholinergic and inhibitory GABAergic inputs control muscle excitation. Inhibition of *fshr-1* expression was previously shown to cause reduced muscle contraction and accumulation of fluorescently labeled synaptic vesicles in cholinergic motor neurons. Here, we used a combination of aldicarb-induced paralysis and swimming assays to confirm the neuromuscular defects of *fshr-1(ok778)* loss-of-function mutants, which we found are exacerbated in aged animals, as well as in animals exposed to chronic oxidative stress. Expression of *fshr-1* in all neurons, as well as in cholinergic or GABAergic neurons alone, could restore neuromuscular activity to these animals under normal conditions. Surprisingly, however, this same *fshr-1* re-expression in cholinergic or GABAergic neurons failed to restore wild type synaptic vesicle localization, and endogenous *fshr-1* expression was detected in what appear to be pharyngeal neurons and arcade cells but not in body wall motor neurons, suggesting a complex site of action. *fshr-1(ok778)* mutants also exhibited build-up of the synaptic vesicle priming factor, UNC-10/RIM, and its interaction partner, SYD-2/Liprinα, primarily in cholinergic motor neurons. No such effects were observed for the active zone protein, CLA-1/Clarinet, or the INS-22 dense core vesicle marker. Finally, gain-of-function mutations in *gsa-1*/GaS or *acy-1*/adenylyl cyclase suppressed the reduced muscle excitation of *fshr-1* mutants. Together, these data support a model in which FSHR-1 may act cell non-autonomously, as well as in body wall motor neurons, to promote muscle excitation upstream of GSA-1 and ACY-1, ultimately controlling UNC-10 localization and cholinergic vesicle release. We are currently further exploring FSHR-1 site(s) of action, working to identify potential ligands that may control FSHR-1's neuromuscular effects, as well as investigating potential connections of FSHR-1 to lipid signaling and other stress-related pathways as they relate to neuromuscular and other neuronal signaling events in physiological and stress conditions.

930C Understanding the development, plasticity, and function of synaptic asymmetry in *C. elegans*

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Lateralization of the nervous system is conserved across the animal kingdom from invertebrates to humans. The human brain asymmetrically processes faculties across the left and right hemispheres, and impaired functional lateralization has been implicated in depression, schizophrenia, and autism spectrum disorders. However, whether asymmetric connectivity exists in anatomically symmetric structures and what its function may be remains largely unknown. Serial electron micrograph (EM) reconstruction of the *C. elegans* connectome revealed that the chemosensory ASE neurons exhibit left-right asymmetry in their connection to the odorsensory AWC neurons. While morphologically symmetric, ASEL and ASER exhibit differential gene expression and function. ASEL primarily mediates attraction to increases in Na^+ concentration, while ASER triggers avoidance of decreases in Cl^- concentration. To confirm the EM data as well as study the connection's development, maintenance, plasticity, and function, we created a fluorescent reporter of this connection using *in vivo* Biotin Labeling of Intercellular Contacts (iBLINC). iBLINC analysis confirmed that ASEL tends to form more synaptic contacts onto AWC than ASER. Analysis of connectivity over development revealed that the asymmetry is established by L3 and maintained through adulthood. Genetic conversion of both ASE neurons to an ASEL fate reversed the asymmetry. In contrast, neither changing both ASE neurons to the ASER fate nor symmetrizing the postsynaptic AWC neurons displayed altered synaptic lateralization from wildtype. These findings suggest that pre-synaptic identity contributes to establishment of the asymmetric ASE/AWC connection. Interestingly, the left-right lateralization is reversed when *C. elegans* are exposed to increased NaCl environments. Synaptic number changes progressively over the course of twelve hours and in a dose-dependent manner, suggesting that the lateral connectivity is plastic. Finally, we have identified several genes that are required to establish or maintain the ASE/AWC synaptic asymmetry. Future experiments will characterize the functional significance of left-right asymmetry using Ca^{2+} imaging. In analyzing this connection, we aim to understand fundamental aspects concerning the formation and function of asymmetric connectivity.

931A An adaptive-threshold mechanism for odor sensation and animal navigation

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Identifying the environmental information and computations that drive sensory detection is key for understanding animal behavior. A variety of different coding strategies have been proposed to mediate sensory detection across organisms and systems, including readouts of the signal absolute level, derivatives, or fold change, low-pass-filtered signal variables, and Linear-Nonlinear models. Differentiating between various models can be challenging without a deliberate systematic experimental design. Here we measure responses in the *C. elegans* olfactory neuron AWC^{ON} over a wide range of stimulus conditions. We find that previous sensation models could only match subsets of experimental observations. We formulate an alternative adaptive concentration threshold model in which sensory activity is regulated by an absolute signal threshold that continuously adapts to odor history. The activation threshold is extracted from a non-linear function of the signal followed by a low pass filter. The model fits the measured sensory threshold and latency over a broad stimulus range and accurately predicts sensory activity and probabilistic behavior during animal navigation in odor gradients. At a molecular level, the rate of the threshold adaptation is regulated by EGL-4, a cGMP-dependent protein kinase. The adaptive-threshold model generality was demonstrated by predicting activity of larval zebrafish optic tectum neurons in response to looming visual stimuli. Theoretical analysis shows that the adaptive concentration threshold model is better than the derivative and fold change models in filtering stimulus noise, allowing reliable sensation in fluctuating environments. Our model unifies previous sensation models under one mechanism and demonstrates an efficient encoding sensory mechanism that reconcile apparent tradeoffs between responsiveness, noise filtering, accurate detection and fast response speed.

932B UNC-10 (RIM) and RIMB-1 (RIM-binding protein) localize synaptic UNC-2 (Ca_v2) channels in a differential manner to regulate transmission in cholinergic and GABAergic motor neuron circuits

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Synapses are intricately organized subcellular compartments in which molecular machines cooperate to ensure spatiotemporally precise transmission of chemical signals. Key components of this machinery are P/Q-type voltage-gated Ca^{2+}-channels (VGCCs), specifically UNC-2, that translate electrical signals into a trigger for fusion of synaptic vesicles (SVs) with the plasma membrane. UNC-2 and the Ca^{2+} microdomains it generates must be located in the right distance to the primed SV, to elicit transmitter release without delay. Rab3 interacting molecule (RIM/UNC-10) and RIM-binding protein (RIM-BP/RIMB-1) were shown in different model systems to contribute to the spatial organization of the active zone protein scaffold, and to localize VGCCs next to docked SVs by binding to each other and to the C-terminal region of the Ca_v2 VGCC α-subunit (UNC-2). We asked how this machinery is organized at the *C. elegans* neuromuscular junction (NMJ), and whether it can differentially regulate transmission in circuits composed of different neuron types, specifically cholinergic vs. GABAergic motor neurons. Evidence for such differential functionality of the presynaptic release machinery was provided by earlier work using optogenetic stimulation (1,2) and recordings in distinct mutants or pharmacologically affected synapses (3). *rimb-1* mutants had mild synaptic defects, through loosening the anchoring of the UNC-2 VGCC and delaying the onset of SV fusion, while RIM (*unc-10*) deletion had much more severe defects. *rimb-1* mutants caused increased cholinergic but reduced GABAergic transmission, while overall transmission at the NMJ was reduced, as shown by voltage imaging. The UNC-2 channel could further be untethered by removing its C-terminal PDZ binding motif, and this untethering could be exacerbated by combining the ΔPDZ mutant with the *rimb-1* mutation. Similar phenotypes resulted from acute degradation of the Ca_v2 β-subunit CCB-1, indicating that destabilization of the VGCC complex causes the same phenotypes as its untethering.

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2 Liu *et al.* (2009) PNAS 106(26): 10823-10828.

3 Liu *et al.* (2018) Cell Rep 22(9): 2334-2345.

933C Biochemical characterization of UNC-47 in *Xenopus* oocytes

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GABA is the main inhibitory amino-acid neurotransmitter in mature neurons which can mediate fast and slow neurotransmissions by binding to the GABA receptors $GABA_A$ and $GABA_B$, respectively. In C. *elegans*, the GABAergic nervous system was thought to be composed of only 26 out of a total of 302 neurons and was traditionally defined by expression of three key players: GAD/UNC-25, the enzyme allowing GABA synthesis, VGAT/UNC-47, the H^+ coupled transporter packaging GABA in synaptic vesicles and GAT/SNF-11, the Na^+ coupled transporter recapturing GABA from the synaptic cleft. However, we have shown by immunostaining that 15 new neurons in *C. elegans* are GABA positive but do not always express all of the key players (Gendrel *et al*, 2016). For example, the interneurons AVAs, AVBs and AVJs are GABA-positive but express none of the previous key players even though synaptic vesicles are detected at their synaptic terminals and their post-synaptic partners express GABA receptors, suggesting alternative modes of GABA transport. We are currently in the process of identifying those new GABA transporters but surprisingly the intrinsic properties of the vesicular transporter VGAT/UNC-47 has never been established. We undertook its characterisation in *Xenopus* oocyte. In order to correctly localize VGAT to the oocyte membrane, we are mutating the sorting signal addressing the transporter to vesicle membranes as it was done for the glutamate vesicular transporters VGLUT1/2/3. A GFP-tagged version of the protein will allow us to monitor if the protein is indeed correctly localized at the membrane of the oocytes. Using two- electrode voltage clamp, transporter currents will be recorded in order to determine which ions are being transported with GABA, what is the substrate specificity, as well as the steady-state and pre-steady-state transport kinetics.

934A Expression of mutant human tau protein drives synaptic loss in *Caenorhabditis elegans*

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Tau aggregation is associated with neurodegenerative diseases, and mutations in human tau have been linked to several disorders including FTDP-17, with varying phenotypic effects. How tau aggregation results in altered neuronal function is controversial and poorly understood, given the difficulties associated with modeling tau aggregation and neurodegeneration in animal models. To address this problem, we have generated multiple *C. elegans* lines of transgenic animals expressing different variants of the longest isoform of human tau (htau40) to better understand how aggregation might cause subsequent downstream events leading to increased toxicity. We have produced multiple transgenic lines expressing variants of human tau protein in neurons to compare their effects on neuronal function. When broadly expressed throughout the nervous system

mutated htau40 caused decreased lifespan, as well as a decreased locomotor capability as they age. Decreased lifespan, along with cognitive and motor defects, are associated with human tauopathies, suggesting our model is reproducing consequences relevant to human disorders. When we selectively express mutated htau40 in the GABAergic motorneurons, we find an age-associated decrease in the number of GABAergic motor neuron synapses, importantly, in the absence of other indicators of neuronal degeneration, i.e. axons and cell bodies were still intact. We have also begun testing mutations in *C. elegans* orthologs of known Alzheimer's risk factors and found that loss of the LAR-like receptor tyrosine phosphatase enhances the synaptic loss due to mutations in htau40. Together, these results indicate the utility of our model to understand how tau aggregation impacts neuronal homeostasis and function.

935B Investigating the connection between the DAF-7/TGF-beta signaling pathway and the dense core vesicle protein IDA-1

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The DAF-7/TGF-beta signaling pathway in *C. elegans* is affected by the environment. This includes factors such as inadequate food supply, high temperature, or overpopulation. We previously found that the DAF-7/TGF-beta signaling pathway regulates the abundance of the glutamate receptor GLR-1. Mutants in the DAF-7/TGF-beta signaling pathway have increased levels of GLR-1 and an increased rate of spontaneous reversals, a behavior which is regulated by GLR-1. However, the exact mechanism of how DAF-7/TGF-beta signaling regulates GLR-1 is unknown. We tested to see whether *ida-1*, which is expressed in cells that express *glr-1*, might be involved in this regulation. IDA-1 is normally found on the surface of dense core vesicles (DCV's) and is involved in normal neuropeptide and DCV signaling. To test the involvement of *ida-1* in the DAF-7/TGF-beta-dependent regulation of GLR-1 we assessed spontaneous reversal frequency and GLR-1::GFP levels in single and double mutants. The increased rate of spontaneous reversals and the increased GLR-1::GFP levels that are detected in *daf-7* mutants are blocked in *daf-7 ida-1* double mutants. These results support the idea that IDA-1 does indeed play a role in the DAF-7/TGF-beta-dependent regulation of GLR-1. We then focused on testing whether IDA-1 expression and localization are affected in *daf-7* mutants. We investigated this question using strains expressing IDA-1::GFP. We imaged neurons in the head of *C. elegans* worms of the appropriate genotypes at both the cell body and neurite in order to measure the intensity of the IDA-1::GFP. We found increased levels of IDA-1::GFP in both cell bodies and neuronal puncta in the *daf-7* mutant as compared to wildtype. These results suggest that the DAF-7 pathway regulates levels of IDA-1. The exact mechanism however, is yet to be discovered.

936C Mechanosensory feedback initiates egg-laying circuit activity and behavior of *C. elegans*

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Animals live in complex environments in which decisions about external stimuli are influenced by sensory feedback of internal state, for example hunger, developmental age, or reproductive drive. Our understanding of how animals integrate external and internal sensory information to elicit a context-appropriate, unilateral decision remains incomplete for many such behaviors. This work explores the transition between inactive and active states of egg laying in *Caenorhabditis elegans*. During egg-laying active states, cells of the egg-laying circuit show rhythmic calcium activity that directs egg release. The HSN command neurons show peak activity ~2 s before egg release, followed by the VC motor neurons and the vulval muscles, both of which show peak activity coincident with egg release. However, the signal that initiates these active states remains unknown. Here we test the hypothesis that feedback of egg accumulation in the uterus provides the ultimate initiating signal. We show that acute microinjections mimic the accumulation of eggs, inducing vulval muscle contractility, egg release, and an immediate activation of the other cells of the egg-laying circuit. Surprisingly, injections into double mutants defective in synaptic transmission from the HSNs and the VCs show a normal induction of vulval muscle calcium activity and egg release. Similarly, injections into *unc-13* and *unc-31* mutants defective in small clear and dense core vesicle release also show a normal induction of vulval muscle calcium activity and egg release, consistent with the idea that the postsynaptic vulval muscles themselves are the proximal target of the acute microinjection response. The injection-induced response in the vulval muscle is dependent on L-type voltage-gated calcium channels, as injections into *egl-19* mutants or into animals treated with nemadipine, an EGL-19 channel blocker, show a greatly diminished calcium response. We show that direct prodding of the vulval muscles induces a localized calcium response, suggesting the vulval muscles themselves are directly sensitive to mechanical input. Together our results suggest that the post-synaptic egg-laying vulval muscles detect egg accumulation and signal via retrograde feedback to the presynaptic cells in the circuit to initiate and sustain the active egg-laying behavior state.

937A A central role of AVA in regulating overall motor states activity

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The descending neurons transmit and process neural signals to guide the locomotor behaviour. In the *C. elegans* motor circuit, premotor interneurons AVA and AVB potentiate backward and forward locomotion, respectively. Previously, we and others have shown that AVA hold an unusually depolarized RMP of ~-23mV that implicates tonic activities. We show that a two-pore K^+ leak channel TWK-40 plays a critical role to establish this RMP, and maintaining this RMP is crucial for *C. elegans* to sustain not only reversal, but also forward movement. We demonstrate that AVA's positive role in forward movement requires AVB. Specifically, the chemical synaptic release of AVA is critical for promoting the forward speed, whereas neuropeptide synthesis in AVA and AVB regulates forward bending curvature. These results are in disagreement with the mutual inhibition model of AVA and AVB, but instead, propose an alternative, new model of AVA's central role in controlling both forward and backward movement.

938B Whole Brain Calcium Dynamics During Aversive Memory Recall

Julia Miller[1], Fatema Saifuddin[1], Rashmi Chandra[1], Raymond Dunn[1], Noelle L'Etoile[1] 1) University of California, San Francisco, CA.

Memory provides an important survival benefit across many species, as failure to recognize signals associated with harmful agents can be deadly. For example, the pathogenic bacteria *Serratia marcescens* emits butanone and acetone which attract the nematode *C. elegans*, but if ingested it kills the animal within days. However, worms that survive learn to avoid the bacteria to refrain from further ingestion. We use a spaced cycle odor-training paradigm to induce learning in *C. elegans* by pairing butanone with starvation, and this olfactory memory can last over 16 hours. However, the changes in neural dynamics that allow an animal to make the choice to avoid an odor are still unknown. We want to understand the full complement of neurons, their activity, and the interplay between neuron ensembles that coordinate movement to and away from the odor as a function of memory. As a first step, we will train worms in either butanone or buffer (as a control) in three 80' odor exposures interrupted by 30' feedings in the absence of the odor. We will then image restrained worms that express GCaMP6f in the nuclei of all neurons and present butanone to their nose to ask if butanone exposure elicits calcium changes reflective of the worm's behavior in a chemotaxis assay. Animals trained in buffer chemotax towards butanone, and thus we expect they will show reduced turns and backing during butanone exposure. We predict these behaviors will correlate with lower activity in the neurons AIB and AVA. By contrast, if the animals have learned to avoid butanone, those animals will increase turns and backing, and AIB and AVA will show higher activity under the same odor presentation paradigm. These experiments will also provide us an unbiased approach to identify other neurons important to a learned response. We might predict that the baseline neural dynamics will be the same in buffer and butanone trained animals, in which we may be able to identify the sequence of neurons whose activation elicits butanone attraction in buffer trained animals or repulsion in butanone trained animals. 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 decision-making process and how it changes after learning.

939C PDF-1 modulation of aversion and reward during associative learning

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A central goal in neuroscience is to understand how neural circuits integrate conflicting (rewarding and aversive) experiences that need to be behaviourally resolved during learning. To shed light into the molecular and cellular mechanisms underlying this process we are dissecting the neuronal circuit that regulates sexual conditioning in *C. elegans*.

Previously, the Iino lab (Sakai *et al.,* 2013) and us (Sammut *et al.,* 2015) have shown that *C. elegans* males undergo sexual conditioning, a form of associative learning by which a rewarding experience with mates overrides the behavioural consequences of an aversive association with starvation. Thus, sexual conditioning leads to a switch in behavioural responses to an environmental stimulus from avoidance to attraction.

Our lab also identified the MCM interneurons and PDF-1 neuromodulation as the cellular and molecular regulators of the sexually conditioned behavioural switch (Sammut *et al.,* 2015). These studies showed conditioned responses to salt. Here we show that other stimuli such as benzaldehyde can also be sexually conditioned and this is also regulated by the MCMs and PDF-1.

We find a dual role for PDF-1 in the regulation of aversive learning and sexual conditioning in *C. elegans*. By using a Cre-Lox intersectional strategy we found that PDF signaling encodes both aversion and sexual conditioning by modulating two partially distinct circuits. Within these circuits, we have identified the interneurons RIA, AIY and RIM as target cells receiving PDF-1 neuromodulation to drive sexual conditioning. Expression of the PDF-1 receptor (*pdfr-1*) only in these neurons is sufficient to rescue attraction during sexual conditioning, but not repulsion during aversive learning.

Furthermore, by removing PDF-1 in specific cells, while keeping the endogenous levels of expression in the rest of the circuit, we have identified the MCMs and AVB neurons, as a source of PDF-1 during sexual conditioning. Importantly, PDF-1 release from the MCMs and AVBs is not required during aversive learning. Thus, highlighting the importance of source specificity versus overall neuropeptide levels during fine tune modulation of specific behaviors.

Currently, we are measuring neuronal activity within the chemotaxis circuit to further understand how sensory information is represented after conditioning and PDF neuromodulation to switch odour preferences.

940A LGC-50 - a new serotonin receptor involved in aversive olfactory learning that displays regulated plasma membrane trafficking

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Across animal phyla, monoamines signal through both metabotropic and ionotropic receptors. In worms as well as in humans, metabotropic monoamine receptors, which modulate neuronal activity through G-protein-mediated second messenger pathways, have received most attention. However, expression studies have indicated that the vast majority of *C. elegans* neurons postsynaptic to aminergic neurons do not express metabotropic amine receptors. This implies that synaptic monoamine transmission may be mediated by as yet uncharacterized ionotropic receptors.

We have in our recent work identified endogenous ligands for five new amine-gated ion channels (LGC), all of which are found localised postsynaptically to aminergic neurons. In particular we have shown the serotonin-gated receptor LGC-50 to be a cation channel that is localised in the interneuron RIA, which is strongly innervated by the serotonergic neuron ADF. Previous work has indicated a role for ADF and RIA in aversive pathogen learning, through which animals learn to avoid odours released by pathogenic bacteria following infection. We have also shown that *lgc-50* mutants show a strong defect in pathogen learning, which can be rescued by expression of LGC-50 in RIA. These results suggest that serotonin may act through LGC-50 to modify the strength of specific synapses in the olfactory navigation circuit.

Our recent work also indicated that the plasma membrane localisation of LGC-50 is tightly regulated, and that potential disruption of this trafficking influences memory formation. We have now identified a 17-amino acid long motif in the intracellular M3/4 domain of LGC-50 that conveys this regulated membrane localisation. Further, we have evidence that this motif might act as a binding site for the protein NRA-1 and that this protein-protein interaction might be involved in moving LGC-50 to the plasma membrane during memory formation. LGC-50 thus provides an entry point to define the molecular and neural changes underlying learning and memory in the worm.

941B Neurogenetics of modulatory cholinergic signaling in C. elegans interneurons

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We previously identified a small circuit that integrates both sensory and motor input, involving a glutamatergic interneuron (RIA) that receives cholinergic feedback from head motor neurons (SMDs). RIA is functionally and spatially subdivided into compartments corresponding to sensory input and reciprocal motor domains. Motor input, which is mediated specifically through GAR-3 mAChRs, is received in phase with head movement during locomotion, leading to compartmentalized local calcium events and gait regulation. We found that a novel phenotype we named “head lifting” (movement of the worm’s anterior section along the Z axis) appears to strongly correlate with disruption of RIA compartmentalization. Head lifting arises from bilateral (DV) activation of head muscles and occurs preferentially at particular points in the locomotion cycle. Here, we characterize this phenotype and use it to probe the genetics of compartmentalized signals in RIA.

942C Whole-body neural circuit influences experience-dependent temperature acclimation

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C. elegans stores temperature experiences and can induce temperature acclimation linked with cold tolerance (Ohta et al., *Nature commun*, 2014; Okahata et al., *Science Advances*, 2019). Here we found that a neural circuit containing ASJ sensoryneuron, PVQ and RMG interneurons, a head-to-tail neural circuit, is a central circuit in temperature acclimation. CREB/CRH-1 functions to regulate temperature acclimation in the ASJ thermosensory neuron and RMG head interneuron. Presynaptic signal enhancement by PKCgf revealed PVQ tail interneuron function as a bridge between the ASJ and RMG via glutamatergic signaling. Ca^{2+} imaging demonstrated that activities in ASJ, PVQ and RMG fluctuated with temperature changes, suggesting that these neurons are involved in temperature signaling. To identify the upstream sensoryneuron activating PVQ, we monitored PVQ neural activity of mutants defective in thermo-sensitivity of ASJ, ADL and ASG thermosensory neurons. We found that the Ca^{2+} levels of PVQ in *tax-4*(CNG channel) mutant defect in thermo-sensation of ASJ were higher than those in wild-type. Conversely, changes in Ca^{2+} levels of PVQ were decreased in *deg-1* mutant impairing DEG/ENaC thermoreceptor of ASG thermosensory neuron. These results suggest that PVQ interneuron integrates temperature signaling from two upstream sensory neurons, ASG and ASJ. We hypothesized that ASG positively regulates the PVQ via the ASK sensory neuron, because this is the shortest pathway from ASG to PVQ. To examine whether ASK bridges between ASG and PVQ, we used reconstituted-caspase that can occur selective ablation of targeted cells. We found that the Ca^{2+} levels of PVQ in both ASG-ablated animals and ASK-ablated animals were decreased, suggesting that ASK bridges neural signaling between ASG and PVQ. Altogether, the ASJ and ASG which transmits an opposite temperature signaling to the tail interneuron PVQ. PVQ in turn releases glutamate to the RMG interneuron located in the head. We propose this body-wide circuit as the modulatory mechanism of temperature acclimation.

943A Quantitative prediction of neuromodulator-programmed behaviors

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By perturbing the activities of subsets of cells within a circuit, neuromodulators such as neuropeptides and biogenic amines drive neural networks to states that generate particular behaviors. We have developed an algorithm for quantitatively predicting the behavioral effects of simultaneous arbitrary higher order neural perturbations from experimentally observed pair perturbation data. This pairwise decomposition algorithm reduces the exponentially scaling problem of predicting behavioral outputs of arbitrary network states to an experimentally tractable and logarithmically smaller quadratically scaling one. We have tested this algorithm *in silico* using neural networks evolved to simulate C elegans chemotaxis behaviors, and found that the approximation agrees well with exact values. We are testing this algorithm experimentally *in vivo* using histamine-based chemical genetic technologies we have developed for controlling neural activity in C elegans.

944B Functional photon-based neurotransmission in a nociceptive avoidance circuit

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Communication between cells is essential during development, homeostasis and nervous system function. The distribution of information is carried out by secreted chemicals, which are released and perceived by different cells. Defects in neurotransmission can lead to neurological disorders and lack of adaptation of the surrounding environment.

Here, we have developed a synthetic, photon-based signaling system that we used to overcome synaptic transmission defects in *C. elegans*, restoring the communication between nervous cells. In particular, we have replaced a conditional defect for glutamate release in the nociceptor neuron ASH with a calcium sensitive light emitting enzyme (enhanced Nanolantern). To prove functionality, we built an optimized microscope capable of detecting how cellular calcium increase triggers light production *in vivo*.

Subsequently, light emission is coupled to downstream interneurons (AVA, AIB) expressing an ultrasensitive light-gated ion channel (Channelrhodopsin-XXL). Finally, we developed a new microfluidic device, which we call Trap'N'Slap, with the aim to deliver mechanical stimuli directly to ASH, while recording calcium transients (by means of Genetically Encoded Calcium Indicators) directly from AVA and AIB. This, together with behavioral nose touch assays, proved that quantum release from ASH, is able to recover functional communication between neurons in an endogenous circuit.

945C Newly-discovered neural branches may release the excitatory signal potentiated by serotonin to activate the *C. elegans* egg-laying circuit

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Serotonin plays a central role in human mood disorders and is thought to function by modulating response to neurotransmitters. We are using the *C. elegans* egg-laying circuit to provide the first detailed example of how serotonin regulates a neural circuit at a cellular and molecular level. Egg laying occurs when the Hermaphrodite Specific Neurons (HSNs) release serotonin to cause two vulval muscle types, the vm1s and vm2s, to contract and open the vulva. Past studies[1] suggest that during every body bend, the vm1s receive an excitatory signal from cell(s) other than the HSNs, but typically this is a subthreshold excitatory signal that does not evoke egg laying. If that were the case, the role of serotonin would be to make the vulval muscles more excitable so that the vulval muscles do contract in response to this excitatory signal[2]. Even so, no major synapses onto the vm1s have been identified, so the source of the hypothesized vm1 excitatory signal has remained unknown. In the course of our studies of two G protein-coupled receptor fosmid-based reporters (DOP-5::GFP and DOP-6::GFP), we observed a set of neural branches emerge from the right ventral nerve cord just anterior and posterior to the vulva and terminate in varicosities that lie over the anterior and posterior vm1s. These neural branches, which have never been described previously, form starting at the late L4 stage at the same time as the rest of the egg-laying system differentiates. We hypothesize that these neural branches form synapses onto the vm1s and provide the excitatory signal that is potentiated by serotonin to trigger vulval muscle contraction and egg laying. Our work to identify the specific neuron(s) that make the branches so far suggests they are cholinergic neuron(s) other than the ventral cord motor neurons. Future experiments will confirm the identity of the neurons, test if they indeed form a synapse onto vm1s, and utilize optogenetic tools to examine how manipulating their activity alters vm1 muscle activity. This study will enable us to study how serotonin modulates responses to the excitatory signal in the neural circuit.

[1]Collins et al. (2016) Elife 5:e21126.

[2]Brewer et al. (2019) PloS Genet 15:e1007896.

946A Dopaminergic neurons are critical for encoding and retrieval of adaptive memory in *Caenorhabditis elegans*

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The neuronal basis of learning and memory has not been well elucidated. Alterations in neurotransmitter release and synaptic plasticity allow connectome alterations to play a critical role in learning and memory formation. Dopamine (DA) is one of the neurotransmitters involved in motivation and motor function. In the present study, we used *Caenorhabditis elegans* to verify the role of DA and dopamine neurons in olfactory adaptive learning. Both wild-type (N2) and DA deficient (cat-2) mutant showed learning and memory formation when worms were trained towards butanone (conditional stimulus) in association with food (unconditional stimulus). However, the transgenic worms (dat-1::ICE) with loss of dopamine neurons due to interleukin-1 beta converting enzyme (ICE) expressing under dat1 promoter showed significant loss of memory retention. A similar loss of memory retention was observed in UA44 worms expressing human alpha-synuclein, a protein involved in dopaminergic neurodegeneration. The worms treated with exogenous DA could partially rescue memory retention. When the dat-1::ICE worms were rescued from dopamine neuron degeneration with siRNA targeting ICE, the memory retention was regained comparable to that of control worms. Transgenic worms expressing GCaMP in dopaminergic neurons (CEP and ADE) were used to measure the calcium dynamics during memory recall. An increased firing pattern of dopamine neurons was observed during memory recall, confirming these neuronal connectomes are crucial in olfactory adaptive learning in worms. Furthermore, worms with dopamine neuron degeneration have been found to have low acetylcholine release. These results suggest the critical role of DA in memory recall and might be playing a significant role in cognitive function.

Key words: Dopamine; Learning and Memory; *C.elegans*; Neurodegeneration; Calcium imaging.

947B Nonequilibrium response functions for functional connectivity in the brain

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Despite the power of the *C. elegans* connectome, the community currently lacks systematic direct measures of the strength, sign (excitatory vs. inhibitory), and temporal properties of the connections between neurons. We refer to these properties as functional connectivity, and they are crucial to understand circuit function. Functional connectivity can now be measured on larger scales thanks to the combination of optogenetics and neural activity imaging. These experiments, however, do not directly measure the direct (monosynaptic) connections between neurons, but only the effective connections, which are a combination of the direct and the indirect (polysynaptic) paths connecting neurons in the network.

We sought a mathematical framework to describe the results of those experiments and obtain the direct contributions from the effective connections observed in experiments. Conventional models start from a description of the direct connections and then consider network effects through numerical simulations. Response functions, which give the activity of a neuron through convolutions with the inputs to that neuron, allow us instead to obtain explicit relations between the properties of the direct and effective connections between neurons. Traditionally, however, this is possible only for linear networks that do not have synaptic adaptation or neuromodulation. We will present a framework based on nonequilibrium response functions, that extends the application of response functions also to nonlinear and time-dependent networks and allows for the description of time-varying functional connectivity.

948C Role of Connectome in Concentration-dependent Odor Adaptation in Caenorhabditis elegans

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Odor is perceived when the molecular signatures in the form of their chemical structures are translated into a specific odor quality and intensity. Animals respond differently to different concentrations of the same odor. We tried to understand the logic of this olfactory information processing at receptor levels. We used Caenorhabditis elegans to analyze the mechanisms of sensory recognition and discrimination by the nervous system. In C. elegans, sensory neurons AWC and AWA mediate attractive behavior whereas AWB, ASH and ADL are involved in repulsive behavior in response to odorants. We tested the behavior of C. elegans towards different volatile odorants isoamyl alcohol, butanone and benzaldehyde with their attractive and repulsive concentrations.

GLR-1 is expressed in motorneurons and interneurons, including four of the five pairs of command interneurons that are required for locomotory control. It is found to have a role in sensing the gradient of odors. Also, food associated appetitive learning of C. elegans with a particular solvent of a low concentration showed an adaptation but interestingly, the same became repulsive at higher concentrations. Our results indicate that the interneuron AIY has a mediatory role in sensing changes in odorant concentrations. We found that SRA-11 which is an odor receptor present in AIY interneuron is essential in the detection of differences in odor concentration. These results suggest that there is a possible pathway downstream of AWC neuron most probably involving AIY interneuron in odor gradient detection.

949A Developing a single-synapse functional imaging assay in *C. elegans*

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Individual synapses in a neuronal circuit—or even within a single neuron—often exhibit dramatically different levels of activity from one another. To better understand the mechanisms regulating functional heterogeneity within neuronal circuits we are developing a single-synapse functional imaging assay in *C. elegans.* A membrane-bound version of the genetically encoded calcium indicator GCaMP5 was targeted postsynaptically at neuromuscular junctions, revealing fluctuating fluorescent transients opposed to individual presynaptic release sites and dependent on the synaptic vesicle release protein UNC-13. Spontaneous calcium transients are analyzed using a custom and automated Matlab suite we have developed. Our analysis pipeline enables us to identify waves of synaptic transmission propagating along an axon via *en passant* synapses. Combining this assay with cell-specific presynaptic markers will enable us to understand how synaptic activity differs between synapses in the same neuron and between neighboring motor neurons of different classes. In addition to spontaneous activity, we will use optogenetic tools to assess evoked synaptic activity and plasticity. Finally, we will assess how synaptic heterogeneity or single-synapse functional properties are affected by mutations in presynaptic proteins including neurexin, a synaptic cell-adhesion molecule associated with functional maturation of synapses and implicated in neurodevelopmental disorders such as Autism.

950B Sexually dimorphic neuronal circuitry drives distinct mechanosensory responses

Hagar Setty, Meital Oren. Weizmann Institute of Science

The recent publication of the anatomical maps of the nervous system of both sexes in *C. elegans* revealed an abundance of dimorphic connectivity between sex-shared neurons. However, the molecular mechanisms that underlie the development of sexually dimorphic neuronal circuits are poorly understood. We focus on the sex-shared interneuron AVG, which in hermaphrodites receives very little inputs, but in males receives many inputs from both male-specific and sex-shared sensory neurons.

By combining behavioral assays with optogenetic manipulation and tracking of freely moving animals, we discovered a novel dimorphic role for AVG in tail mechanosensation and locomotion. Specific silencing of AVG using histamine-gated chloride channels revealed it is required only in males for tail-touch response (a forward movement of the worm in response to a touch applied to the tail (*Li et al, 2011*)). Optogenetic inhibition of AVG inhibited the locomotive behavior of males, but not hermaphrodites. Calcium imaging experiments using a microfluidic device for mechanical stimulation (*Fehlauer et al, 2018*) further support a role for AVG in tail mechanosensation.

To discover molecular candidates that mediate tail mechanosensation we carried out a reverse genetic screen. We found that the AMPA glutamate receptor *glr-1* is required for tail mechanosensation only in hermaphrodites, while the NMDA glutamate receptor *nmr-1* is required in both sexes, with a stronger effect in males. To test whether *nmr-1* and *glr-1* function cell autonomously in AVG, we analyzed the tail-touch responses of mutant animals with a masculinized AVG. Masculinizing AVG in *nmr-1* mutants reduced the response of hermaphrodites to that of the males, while masculinizing AVG in *glr-1* mutants did not alter the phenotype of the tail-touch response. Our preliminary results suggest that *nmr-1,* but not *glr-1,* mediate tail mechanosensation in males through AVG.

To investigate the sensory input to AVG, we focused on the sex-shared sensory neurons PHA, PHB and PHC, which generate sexually dimorphic connections with AVG *(Cook et al, 2019).* Silencing PHA and PHB revealed that PHA is required for tail mechanosensation only in males, while PHB is required in both sexes. PHC silencing affected only hermaphrodites, corroborating previous results by *Serrano-Saiz et al, 2017*.

To summarize, our results show that the circuit for tail mechanosensation is sexually dimorphic in all aspects - molecular, cellular and behavioral.

951C Synapsin is required for dense core vesicle capture and cAMP-dependent neuropeptide release

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Release of neuropeptides from dense core vesicles (DCVs) is essential for neuromodulation. Compared to the release of small neurotransmitters, much less is known about the mechanisms and proteins contributing to neuropeptide release. By optogenetics, behavioral analysis, electrophysiology, electron microscopy, and live imaging, we show that synapsin SNN-1 is required for cAMP-dependent neuropeptide release in *Caenorhabditis elegans* hermaphrodite cholinergic motor neurons. In synapsin mutants, behaviors induced by the photoactivated adenylyl cyclase bPAC, which we previously showed to depend on acetylcholine and neuropeptides (Steuer Costa et al., 2017), are altered like in animals with reduced cAMP. Synapsin mutants have slight alterations in synaptic vesicle (SV) distribution, however, a defect in SV mobilization was apparent after channelrhodopsin-based photostimulation. DCVs were largely affected in *snn-1* mutants: DCVs were ~30% reduced in synaptic terminals, and not released following bPAC stimulation. Imaging axonal DCV trafficking, also in genome-engineered mutants in the serine-9 protein kinase A phosphorylation site, showed that synapsin captures DCVs at synapses, making them available for release. SNN-1 co-localized with immobile, captured DCVs. In synapsin deletion mutants, DCVs were more mobile and less likely to be caught at release sites, and in non-phosphorylatable SNN-1B(S9A) mutants, DCVs traffic less and accumulate, likely by enhanced SNN-1 dependent tethering. Our work establishes synapsin as a key mediator of neuropeptide release.

952A Investigating the role of complexin-1 function in dopamine signaling

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Dopamine signaling plays a role in regulating motor control across the animal kingdom and has been implicated in many motor movement-related diseases, but the full set of regulatory molecules controlling dopamine release is still not fully described. Through RNA-sequencing, we identified *cpx-1* as a gene highly expressed in dopamine neurons. Previous work has suggested that a loss of the complexin protein, CPX-1, is linked to motor movement defects in *C. elegans*. We hypothesize that disruption of *cpx-1* function in dopamine neurons will lead to an excess of extrasynaptic dopamine due to premature synaptic vesicle fusion. In *C. elegans*, mutations leading to an excess of extrasynaptic dopamine result in a Swimming-Induced Paralysis (SWIP) phenotype, when the nematode paralyzes within minutes compared to tens of minutes in the wild-type worm. We have observed that *cpx-1* mutants show a dopamine-related SWIP phenotype similar to *dat-1* mutants. To further investigate the specificity of the role of *cpx-1* in dopamine neurons we are generating *cpx-1;dop-3* double mutants and using a *dat-1p::cpx-1* recovery construct to rescue the *cpx-1* mutant phenotype. If we observe a rescue of the SWIP phenotype in the *cpx-1;dop-3*

double mutants and dat-1p::cpx-1 transgenic worms, then this will suggest *cpx-1* function is crucial for proper dopamine neuron function. This work is supported by NSF CREST Center for Biological Signatures & Sensing (#HRD1547757) and NIH R25 Bridges to the Biomedical Doctorate (#2R25GM107754-06A1).

953B CYLD-1, a lysine 63 deubiquitinase, regulates synaptic transmission and preserves neuronal homeostasis during ageing

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Protein ubiquitination is a central coordinator of cellular physiology, regulating both protein turnover and signal transduction. Ubiquitin monomers are assembled into polymeric chains through conjugation of one ubiquitin molecule to an internal lysine or the N-terminal methionine of another ubiquitin molecule, giving rise to eight types of homotypic polyubiquitin chains and a plethora of heterotypic, mixed and branched chains, collectively referred to as the ubiquitin code. Distinct signals emanate from different ubiquitin chain topologies, eliciting unique biological outcomes for their protein substrates. Lysine 63 (K63) polyubiquitination orchestrates proteasome-independent protein degradation, targeting substrates to the autophagy-lysosome pathway. Cylindromatosis (CYLD), a tumor suppressor, functions as a deubiquitinating (DUB) enzyme with specificity towards K63-linked polyubiquitin chains. CYLD is highly expressed in neurons, where it determines the K63 polyubiquitination status of synaptic proteins in response to neuronal activity, coordinating remodeling of the post-synaptic density and synaptic plasticity. Our previous work has shown that autophagy contributes to synaptic plasticity via direct degradation of synaptic proteins, thereby regulating cognitive behaviors, such as learning and memory. Here, we investigate the role of CYLD and K63 polyubiquitination in neuronal physiology in *Caenorhabditis elegans*. CYLD-1, the nematode CYLD homologue, is expressed in the *C. elegans* nervous system and regulates autophagy. Neuronal depletion of CYLD-1 perturbs neurotransmission, impairs learning and shortens lifespan. Notably, CYLD-1 preserves motor neuron integrity and motility during ageing. We aim to elucidate neuronal functions that are compromised in CYLD-1 deficient worms and characterize the role K63 polyubiquitination in neuronal physiology. Our findings highlight DUB enzymes as potential therapeutic targets to ameliorate age-associated neurodegeneration and cognitive decline.

954C A PP1 holoenzyme regulates synaptic neurotransmission

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Protein Phosphatase 1 (PP1) are holoenzymes made of a catalytic subunit and of regulatory subunits, which specify holoenzyme's substrates, location and function. Although PP1 is known for long to control many aspects of the synaptic function, the PP1 holoenzymes active at synapse are largely unknown, as well as their substrates. PhospHatase and ACTin Regulator (PHACTR) are a family of PP1 regulatory proteins. Structurally, they contain a highly conserved C-terminal which include three RPEL motifs for G-actin (monomeric actin) binding and one PP1 binding motif. PHACTR1 is highly expressed in human brain and mutations in PHACTR1 are associated with the epileptic West syndrome in humans, although the molecular/cellular mechanisms are unknown. Biochemical characterization of PHACTR showed PP1 and G-actin compete for binding to PHACTR C-terminal end.

Caenorhabditis elegans bears a unique ortholog for PHACTRs (we named it *pprp-1*). Our results indicate a presynaptic role for *pprp-1* at Neuromuscular Junctions(NMJ): *pprp-1(null)* mutants are sensitive to cholinesterase inhibitor and this phenotype is rescued by expression of *pprp-1* in cholinergic neurons. Interestingly, mutations in the RPEL motifs generated a constitutively active *pprp-1(gof)* allele that is resistant to cholinesterase inhibitor. We quantified the distribution of multiple synaptic markers at the cholinergic NMJ. The amount and the localization of SNN-1-GFP and ITSN-1-GFP are modified in *pprp-1 (null)* and *(gof)* in opposite direction. Also, genetic interactions with *pprp-1(null)* and *(gof)* suggest *snn-1* and *itsn-1* contributes to PPRP-1 signaling. Synapsin (SNN-1) and Intersectin (ITSN-1) are two phosphoregulated proteins involved in synaptic vesicle cycle. Their dephosphorylation/phosphorylation cycles affect formation of complexes with other proteins, modulating their functions in inactive or active synapse. We propose PPRP-1-PP1 regulates the synaptic function of those key synaptic factors directly or indirectly.

955A Redefining the GABAergic neuron

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The balance between excitation and inhibition is critical to the proper function of neural circuits. GABA is the main inhibitory amino-acid neurotransmitter in mature neurons.

The GABAergic phenotype in vertebrates and invertebrates has been defined classically by the presence of three key players in the presynaptic neurons: the enzyme needed to synthetize GABA from glutamate GAD/UNC-25, the vesicular GABA transporter VGAT/UNC-47 that packages GABA in synaptic vesicles and the plasma membrane GABA transporter GAT/SNF-11 that recaptures GABA from the synaptic cleft. For over 20 years, the *C. elegans* GABAergic nervous system was thought to be composed of only 26 out of the total 302 neurons. However, we have discovered 15 new GABA-positive neurons that do not conform to this classical scheme of GABAergic neurons (Gendrel *et al*, 2016). Indeed, some of these neurons, such as AVBR/L, AVAL/R and AVJL/R, do not express GAD/UNC-25, VGAT/UNC-47 and/or GAT/SNF-11 even though synaptic vesicles are detected at their pre-synaptic terminals and their post-synaptic partners express GABA receptors, showing evidence of alternative modes of GABA transport. We aim to identify and characterize the plasma membrane and vesicular GABA transporters used by these neurons. In the *C. elegans* genome, 55 genes encode putative amino acid transporters. As a first step, we undertook the expression pattern analysis of these candidate genes using a fosmid-based reporter strategy (Tursun *et al*, 2009). Initial results show expression in the nervous system for two out of four studied genes. We are currently mapping to see if they are expressed in our neurons of interest. As a second step, anti-GABA immunostaining will be performed on KO strains for the genes expressed in our neurons of interest. Indeed, an increase in GABA staining would indicate the inability of neurons to transport the neurotransmitter out of the cell, demonstrating that the mutated gene encodes a vesicular GABA transporter. On the other hand, the absence of the plasma membrane GABA transporter in neurons unable to synthetize GABA would be indicated by an absence or a decrease of the signal.

956B Monoaminergic molecular pathways in modulating memory and behaviour

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To exercise information about its immediate surroundings *C.elegans* receive a variety of external stimuli, which establishes behavioral modifications in animals. Monoamines seem to mediate a wide range such functions along with physiological and homeostatic functions in animals. Recent evidences suggest Tyramine (TA), one of the precursors for monoamines like dopamine and serotonin, could act as a neurotransmitter. However, the molecular mechanisms underlying the tyramine pathway is poorly understood. Tyraminergic neurons, expressing SER-2 receptors, could act as neuromodulator and facilitate escape response in *C. elegans*. Yet another receptor TYRA-2 acts as determining factor in shut down of aversive behaviours in unharmonious situations for Risk-Reward pathway.Tyramine secretion being extrasynaptic from Motor interneuron RIM is likely to affect the head neurons, presenting receptors towards tyramine.This mechanism might be credited in establishing essential behavioral modifications and short lived neuronal alterations in the animals. In this study, we verified the role of monoamines by *c.elegans* behaviour using olfactory learning and memory generation. A significant increase in memory was found in worms on providing tyramine extra-synaptically. Mechano-stimulation assay in presence of tyramine also correlates with behavioral alterations via omega turns and reversal of worm, suggesting escape response activity coordinated with motor neuronal stimulation (RIM). These results suggest that the monoaminergic molecular modulation in associated neurons facilitates alterations in memory formations. The top down approach of multisensory decision involving ASH and AWA is advocated by tyramine, this can also possibly alter preferences based on learning and memory in worms.

Key words

- Tyramine, SER-2, TYRA-2, Mechano-stimulation, Associative memory

957C Comparison of electrophysiological and motility assays to study drug effects in *C. elegans*

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Two behavioral phenotypes commonly measured in *C. elegans* research are worm motility and pharyngeal pumping frequency. Anthelmintic drugs developed against parasitic worms impair these behaviors, typically by perturbating ion channels or neurotransmitter receptors involved in neuromuscular transmission. Motility assays can provide high-throughput screening tools for drug effects, whereas electrophysiological recordings can provide a more direct readout of drug action on ion channels and neurotransmitter receptors, at the cost of lower throughput. In the past decade, microfluidic platforms for electrophysiological recordings from intact worms have emerged that pose fewer technical challenges and higher throughput than traditional micropipette recordings. Here we compared three platforms head-to-head to evaluate their respective

strengths and limitations for characterizing drug effects on *C. elegans*. We tested two microfluidic devices that record electropharyngeograms (EPGs) from the nematode pharynx – the ScreenChip™ and the 8-channel EPG platform – and one whole-worm motility platform, the wMicroTracker. The two EPG platforms non-invasively detect pharyngeal neuromuscular activity via recording electrodes positioned near a worm's body, with customized software for data display and analysis. The wMicroTracker uses a multi-well plate format, with worm movements detected by interruption of an infrared laser microbeam, coupled to software that displays and analyzes the results. To compare these platforms, we tested levamisole, which is an agonist of L-type acetylcholine receptors (L-AChRs), and three macrocyclic lactone drugs that activate glutamate-gated chloride channels (GluCls; ivermectin, moxidectin, and milbemycin oxime). These anthelmintic drugs are used against parasitic nematodes of humans and animals and are valuable probes for investigating synaptic function. Drug potencies (IC_{50} values) from concentration-response curves, and the time-course of drug effects, were compared across platforms and across drugs. As predicted by the synaptic locations of GluCls and L-AChRs, EPG recordings were more sensitive than motility assays in detecting macrocyclic lactones, whereas the opposite was found for levamisole. Drug effects on pharyngeal pump patterning (i.e., regular vs. irregular) and EPG waveform shapes were also compared. These experiments revealed clear differences in drug effects within and between drug classes and identified potential drug-class-defining phenotypes. Having determined relative strengths and limitations of these three assay systems, investigators can better select which platform or combination of platforms best meets their research needs. The authors thank Carolin Gojny, Sven Zymny and Kristin Robinson for excellent technical assistance, and the Bayer Life Sciences Collaboration for funding SRH.

958A Understanding how neuroendocrine cells are mechanically activated in *C. elegans*

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Neuroendocrine cells release neurotransmitters and hormones to control body functions often in response to chemical or mechanical activation. However, we still lack the knowledge of how neuroendocrine cells develop, how they are activated, and the functional importance of their extrasynaptic signaling. In *C. elegans*, there are four uterine-vulval (uv1) neuroendocrine cells at the ventral surface of the vulval canal which release tyramine and neuropeptides that inhibit egg-laying behavior. Our previous studies have shown these cells are mechanically activated following egg-laying events, but whether the uv1 cells respond to vulval opening or passage of eggs through the uterus was not clear. We developed an optogenetics approach to stimulate vulval muscle contraction while recording uv1 cell Ca^{2+} activity. These studies indicate that vulval opening, not egg release, drives uv1 activity. We also discovered that mechanical prodding of the uv1 cells was sufficient to induce calcium activity, confirming the uv1 cells respond directly to mechanical activation. We are presently manipulating the time and speed of the mechanical stimulus in juvenile and adult animals to determine how these parameters affect uv1 activation. [KMC1] To determine if prodding of the vulva triggers vulval muscle contraction which then drives uv1 activation, we will analyze uv1 activation in *unc-54* muscle myosin mutants unable to contract the vulval muscles. We have recently shown that vulval muscle contraction stimulates Ca^{2+} activity in the cholinergic VC neurons that innervate the vulval muscles. The uv1 cells express muscarinic acetylcholine receptors that might modulate uv1 activity following VC signaling. We will block VC synaptic transmission with Tetanus Toxin to test whether acetylcholine signaling from the VCs affects uv1 activation. To identify molecules required for uv1 activation, we identified new mutants that do not show uv1 Ca^{2+} activity in response to egg laying. Some mutants affect uv1 cell development while others affect activation. We are presently using a Hawaiian single nucleotide polymorphism mapping strategy followed by whole-genome sequencing to identify the mutant genes. We predict the genes will encode conserved factors that function generally to regulate the development and activation of neuroendocrine cells like uv1 that regulate core body functions including reproduction and metabolism.

959B A gut neuroendocrine signal regulates synaptic assembly in the brain

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The gut-brain axis plays an essential role in regulating neural development in response to internal environmental stimuli, such as microbes or nutrients. Defects in gut-brain communication can lead to various neurological disorders. However, it remains unknown whether gut plays an intrinsic role in regulating neuronal development. Through a genetic screen in *C. elegans*, we uncovered that an intrinsic Wnt-endocrine pathway in gut regulates synaptic development and neuronal activity in brain. Specifically, the Wnt signaling upregulates the expression of the neuropeptide NLP-40 in the gut and facilitates presynaptic assembly through the neuronal expressed GPCR AEX-2 receptor during development. The NLP-40 acts most likely through modulating neuronal activity and promoting synaptic protein trafficking. Our study reveals a novel role of gut in synaptic development in the brain and provides additional molecular basis for gut-brain interactions.

Key words: Gut-brain axis; Synaptic development; Wnts; Endocrine; Neuropeptide/NLP-40; GPCR/AEX-2; Neuronal activity

960C Systematic screening of autism-associated genes for roles in GABAergic neuronal morphologic plasticity

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Neuronal plasticity is crucial to mammalian brain development, function, and the generation of behaviors, including learning and memory. Deficits in neuroplasticity and in the balance of excitation and inhibition have been hypothesized to underlie a number of neurodevelopmental conditions, including autism spectrum (AS). AS encompasses heterogenous conditions and disorders characterized by core behavioral changes. Despite the association of hundreds of genes with AS, how each one contributes to behavioral changes at the molecular and neuronal levels remains relatively unknown. In *C. elegans*, the GABAergic neuron DVB undergoes experience-dependent structural plasticity characterized by branching and neurite outgrowth in adult males, impacting excitatory and inhibitory balance and behavioral output. We are using this simple model of neuroplasticity to screen 50 conserved autism-associated genes for roles in GABAergic neuronal plasticity and maintenance of excitatory and inhibitory balance. Among the genes we have tested, males mutant for *rbr-2/KDM5B*, *snf-11/SLC6A1*, *set-4/KMT5B,* or *unc-10/RIMS1* did not show significant changes in DVB morphology, while mutations in *chd-1/CHD1*, *daf-18/PTEN*, *gap-2/SYNGAP1*, and *unc-44* resulted in increased DVB neurite outgrowth in adult males. *unc-44* is the worm ortholog of ankyrin (*ANK2*), a high confidence autism-associated gene that is important for cytoskeleton organization. In *unc-44* loss of function mutant males, we found branching and neurite outgrowth to be nearly doubled at days 1 and 3 of adulthood. We are currently testing *fkh-7/FOXP1*, *slt-1/SLIT1*, *lin-59/ASH1L*, *nmr-2/GRIN2B, kqt-1/KCNQ3*, and *cca-1/CACNA1H*. We will analyze the role of these genes on DVB-dependent behavioral plasticity and determine molecular mechanisms and potential interactions for genes with identified phenotypes.

961A Novel patient-derived mutation in the presynaptic calcium channel UNC-2 reduces synaptic expression yet increases presynaptic release

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Channelopathies cause a wide variety of disorders, but understanding how specific mutations lead to disease has been complicated by gene redundancy and compensatory effects in various classical and complex vertebrate models. We use C. elegans to evaluate the functional and behavioral effect of a rare and novel human patient-derived point mutation (D1634N) in the P/Q-type voltage-gated calcium channel (CACNA1). Mutations in this channel are typically associated with autosomal dominant neurological disorders like familial hemiplegic migraine type 1 (FHM1), episodic ataxia type 2 (EA-2), and Spinocerebellar ataxia type 6 (SCA6); however, patients with the D1634N mutation have not been classified into any of these disorders, suggesting a novel mechanism of action. The worm has only one ortholog of the channel (UNC-2) expressed in its nervous system and shares high homology in the region of the single point mutation D1634N. Our CRISPR-generated D1634N worms show decreased channel expression at synapses using an endogenously-tagged protein. Surprisingly, acetylcholine release (as assessed by aldicarb sensitivity), miniature EPSC frequency, and behavioral readouts of hyperactivity are all increased, suggesting that this point mutation may affect channel voltage sensitivity and/or sub-synaptic localization at presynapses. Additional interactions with other presynaptic proteins and calcium channel auxiliary subunits are being evaluated to address the impact of this mutation on presynaptic morphology and synaptic function.

962B *Pseudognaphalium obtusifolium* Extract Improves Lifespan and Thermotolerance in *C. elegans*

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For centuries, Lumbee Indians of North Carolina have used indigenous plants like *Pseudognaphalium obtusifolium*, rabbit tobacco, to make medicinal teas. Other tribes, like Cherokees and Creeks, used rabbit tobacco teas to combat a host of illnesses and maladies from muscle pain to colds. These teas were believed to have anti-inflammatory properties, though the biology has not been explored. We harvested and dried local rabbit tobacco and prepared aqueous extracts. The extract improved the lifespan, thermotolerance and motility of young adult nematodes, compared to vehicle control. Animals were age-synchronized and exposed to either the aqueous extract or control media. Then, worms were tested for thermotolerance or motility. A lifespan analysis was run in parallel. Nematodes that were treated with rabbit tobacco extract lived longer and had improved thermotolerance and motility. Work is ongoing to determine the molecular mechanism for this phenotype, as well as testing other Lumbee plants. This work helps pave the way for a biological basis for indigenous medicine.

963C Investigating the basis of severe stress resistance in ageing

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Key words

Severe stress resistance, ageing, CeMbio, IIS, oxidative, heat.

Abstract

Stress resistance and longevity are robustly correlated across heat stress paradigms but not oxidative stress paradigms. In particular, severe stress resistance in ageing *C. elegans* markedly differs between exposure to oxidative and heat stress that kill wild type worms within a couple of hours (Benedetto et al. Aging Cell 2019). Severe stress resistance may engage multiple mechanisms such as basal stress handling vs adaptive stress response pathways, different stress transduction pathways, the ability to execute the organismal death program (Galimov et al. 2019), or protection/sensitisation conferred by the gut microbiota. To disentangle these and evaluate their relative roles in ageing worms, we are screening *C. elegans* mutant in combination with strains from CeMBio collection for resistance to 7% tert-butyl hydroperoxide (t-BHP) and 42°C heat-shock as severe oxidative stress and thermal stress paradigms, respectively, before contrasting these results with aged animals. We are performing these screens on young adults at first, before moving into aged worms. So far, we have found that the capacity to execute the organismal death pathway does not significantly impact severe stress resistance in young adults fed an OP50 diet. However, young adults fed on 48 different bacterial isolates displayed varying levels of severe heat and oxidative stress resistance, some of which were insulin/IGF1-signalling (IIS) pathway-dependent. We are currently investigating the basis of these differences. This poster will provide an overview of the methods used, current results and planned future work.

964A Role of the conserved cholinesterase family member CEST-1.1 and its modular metabolite products in life span control

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Although the role of the *C. elegans* FoxO transcription factor DAF-16 in promoting longevity is well established, how DAF-16/FoxO extends life span remains poorly understood. While the identities of thousands of DAF-16/FoxO target genes are known, mechanistic links between DAF-16/FoxO-dependent regulation of specific genes and DAF-16/FoxO-dependent life span extension are lacking. We have discovered that a conserved cholinesterase family member encoded by the DAF-16/FoxO target gene *cest-1.1* is required for full life span extension in the context of reduced DAF-2 insulin-like signaling and sufficient to extend life span when expressed in a wild-type background. A functional CEST-1.1::GFP fusion protein is expressed specifically in the intestine and localizes to the apical plasma membrane. Comparative metabolomic analysis using HPLC-high resolution mass spectrometry revealed that CEST-1.1 is required for the biosynthesis of two structurally novel nucleoside-like ascarosides termed *uglas#1* and *uglas#11.* These surprising findings support a role for an unprecedented class of metabolites in life span control and orthogonally expand the landscape of biogenic small molecules that may influence aging.

965B The impact of calorie restriction mimetics on cellular phenotypes triggered by an Alzheimer disease-related presenilin-1 protein splice variant in *Caenorhabditis elegans*

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As we age, our brain undergoes a number of changes at the cellular and molecular levels. These changes, such as those associated with protein removal through autophagy, lead to the aggregation of misfolded proteins and eventually to the disruption of the nuclear membrane. Age-related disruption of these cellular events is strongly associated with diseases, such as Alzheimer's disease (AD), the most common form of dementia. Much of the brain malfunction during AD is related with the

beta-amyloid peptide that is generated because of incorrect cleavage of the amyloid precursor protein (APP). This incorrect cleavage of APP has been associated with dysfunction of the presenilin-1 (PS-1) protein. Alterations in the *PS-1* gene have been associated 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. The role of PS-1(SV) during AD and aging is unknown.

Since current AD pharmacological therapies are not effective, there is critical need to find treatment strategies. One promising alternative is caloric restriction (CR), the reduction in dietary caloric intake without inducing malnutrition, which has been shown to increase autophagy, reduce risk of AD and extend lifespan in various species. A new class of compounds called CR mimetics (CRms), which mimic the effects of CR without the need for dietary intervention, have been identified.

The *C. elegans* model is widely used to study aging and neurodegeneration. The *sel-12(or131)* strain, which carries a loss-of-function sel-12, the worm homologue of PS-1, exhibits an egg-laying defect (which is often associated with aging), a reduction in lifespan and a loss of nuclear membrane integrity. Our preliminary results, based on transient expression of *PS-1* in the *sel-12(or131)* background, confirms that *PS-1(WT)* can rescue the egg-laying defect, but that *PS-1(SV)* cannot, suggesting that the PS-1(SV) is non-functional, which might underlie its influence on age-related phenotypes.

Because the defects displayed by the *sel-12* strain can be, in part, attributed to the damaged autophagy, it represents an ideal model to test if the CRms, metformin, resveratrol and everolimus, which have already shown to rescue experimental autophagy impairment, can alleviate age-related phenotypes found in *C. elegans PS-1(SV)*::*sel-12*.

966C Protective roles of imidazolium salts in *C. elegans* models of stress and neurodegeneration

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In this study, we aim to evaluate the role of imidazolium salts as antioxidant and anti-aging agents. We synthesized imidazolium salts and use the nematode *C. elegans* to perform a screening and analyze their ability to improve oxidative stress resistance. We identified a derivate, 1-Mesithyl-3-(3-sulfonatopropyl)imidazolium (MSI), that enhances animal resistance to oxidative stress.

As a first approach to delineate its mechanism of action, we evaluated MSI ability to activate transcription factors involved in cytoprotective stress responses, such as the DAF-16/FOXO and SKN-1/Nrf2 pathways. We found that MSI stress protection was not dependent on DAF-16. Nevertheless, we discovered that GST-4 detoxifying enzyme, a downstream effector of SKN-1 transcription factor, is involved in MSI-mediated oxidative stress resistance.

Oxidative stress has been largely related with aging and neurodegeneration. To gain further insight into MSI role in proteostasis, we evaluated mobility as an indicator of healthspan in Huntington's, Parkinson's and Alzheimer's disease models. We found that MSI ameliorates mobility rate decline in these proteotoxic models of neurodegenerative diseases. Surprisingly, our results show that MSI did not improve mean lifespan neither in wild-type worms nor in Alzheimer's disease animal models. Overall, our results show a scenario where healthspan seems to be uncoupled to lifespan. Additional research is needed to underpin the mechanism responsible for MSI's protective role.

967A The role of FGF signaling in C. elegans' aging

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Fibroblast growth factors are conserved signaling peptides that are important for proper development, as well as regulating metabolic homeostasis. In C. elegans, there are two FGF orthologs, namely egl-17 and let-756. They both act on the one FGFR, egl-15, which is expressed in different tissues such as the hypodermis, muscle, neurons, and intestine. To investigate factors involved in healthy aging, we utilized the swimming assay, an established exercise paradigm for C. elegans. We looked at factors expressed in the muscle that may be affected by swimming exercise. During our initial investigation, we found that let-756 mRNA is increased after swimming. Surprisingly, while trying to investigate the role of let-756 in swimming exercise, we found that feeding RNAi in L4 worms actually increases maximum velocity of aging worms, an indicator of health. In addition, we found that let-756 RNAi treatment starting at L4 worms increases lifespan. Our study aims to investigate the role of FGF

signaling and its downstream signaling pathway that is regulating the aging process. In answering this question, we want to investigate if this effect requires egl-15 in specific C. elegans tissues and what specific egl-15 subtype plays a role in the aging process.

968B Role of the RNA binding protein, NCL-1, on ribosome biogenesis and stress response

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Ribosome production determines the capability of the cell to produce proteins, divide, grow and adapt to new environments. Ribosome biogenesis is controlled at multiple levels in response to both environmental and developmental cues. In *C. elegans*,the RNA binding protein NCL-1has been shown to suppress ribosome production by reducing the levels of the ribosomal RNA processing factor, *fib-1*. NCL-1 is required for lifespan extension induced in stressful conditions such as reduction in mTOR, insulin or mitochondrial activity, as well as caloric restriction, placing it at the center of the stress response. However, the molecular mechanisms of *ncl-1* regulation and its exact role in these pathways are still not well defined. We have found that NCL-1 suppresses the levels not only of *fib-1*, but also of multiple rRNA/tRNA processing factors. Many of the mRNAs coding for these factors contain an UUGUU sequence motif, which is bound by NCL-1 *in vitro*and likely mediates regulation of these transcripts by NCL-1 *in vivo*. Animals deficient for *ncl-1* activity show increased nucleolar size in both germline and somatic cells, which is associated with increased ribosome biogenesis. Interestingly, we found that *ncl-1* mutants show a strong reduction in fertility when grown at high temperatures (27°C) and this phenotype seems to be associated with defects in the germline cell stem pool. We have observed that the influence of different bacterial diets on fertility also depends on the function of *ncl-1*.These results point to an essential role of *ncl-1* in the regulation of fertility in response to environmental cues. We are currently studying the RNA targets of NCL-1 as well as the co-factors that could form complexes with NCL-1 to regulate its function. We believe this study will advance our understanding of the regulation of the stress response and its connection with ribosome biogenesis, fertility and lifespan.

969C Ubiquitin-Dependent Dimer-Monomer Switch Defines Substrate Specificity and Processivity of the E3 Ligase CHIP

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Proteostasis is achieved by quality control pathways that support the generation of correctly folded proteins, prevent protein misfolding and remove toxic proteins. The quality control E3 ligase CHIP ubiquitylates damaged proteins consigned by chaperone partners for disposal through the endo-lysosomal pathway, proteasomal degradation, or autophagy. Additionally, CHIP has been reported to modulate essential signaling pathways by specifically delivering a myriad of native proteins to destined fates homeostasis (Paul I and Ghosh M.K., 2015). We aimed at understanding the substrate specificity and processivity through a “structure to function” approach, by examining the modeled 3D structure of the *C. elegans* ortholog of CHIP, CHN-1, based on the reported structure of murine CHIP (Zhang M et al., 2005). Using *C. elegans* and mammalian cells as model system and with various genetic and biochemical analyses, we demonstrate that monomeric CHN-1/CHIP has preserved ubiquitylation activity and promotes longevity via the DAF-2 Insulin-like signaling pathway (Tawo R et al., 2017). Our data reveal that the CHN-1/CHIP autoubiquitylation and its chaperone binding interplay modulates the alteration between monomer and dimer (Balaji V and Hoppe T., 2020). Together, the conserved dimer-monomer transition provides a molecular switch regulating CHN-1/CHIP activity in response to proteotoxic stress and aging.

970A All trans retinoic acid extends *C. elegans* lifespan in an *aak-2* and *hsf-1* dependent manner by modulating metabolism.

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Aging is a pan-metazoan phenotype that significantly impacts human health and society. Across metazoans, the rate of aging is influenced by a zero-sum allocation of resources between maintenance and growth. We therefore sought to manipulate signaling components from conserved resource allocation systems to identify potential anti-aging interventions. Here we present the exploration of the endogenous vitamin A derivative all trans Retinoic Acid (atRA), and its role promoting gluconeogenesis, as a modulator of aging in *Caenorhabditis elegans*. We found that supplementation with atRA extends

lifespan in a concentration dependent manner. As predicted based on the conserved pathway, atRA acts independently of *daf-16*, but it does require the conserved AMP sensor *aak-2*, consistent with a change in metabolism and energy storage. The observed shift in metabolism comes at a reproductive cost, consistent with a redirection of energy resources to somatic maintenance. This approach shows that the manipulation of a conserved metabolic regulatory circuit by co-opting endogenous signaling molecules can extend lifespan.

971B Characterization of downstream steps in the Intracellular Pathogen Response-mediated thermotolerance in *C. elegans*

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Infection by intracellular pathogens can induce proteotoxic stress in host organisms. In research to characterize the host response to intracellular infection in *C. elegans*, our lab found that a common transcriptional response to diverse natural pathogens called the Intracellular Pathogen Response (IPR) is associated with increased tolerance of proteotoxic stress. The IPR is inhibited by *pals-22*, a gene of unknown biochemical function, and *pals-22* loss-of-function mutants have constitutively induced IPR gene expression and improved tolerance of heat shock. The IPR includes upregulated expression of several ubiquitin ligase components, and through a combination of genetic and biochemical approaches, we found that several of these components comprise a novel multi-subunit ubiquitin ligase complex that is required for increased proteostasis of *pals-22* mutants. However, the steps downstream of this ubiquitin ligase complex that lead to increased tolerance of proteotoxic stress remain unclear.

In our proposed model for the IPR, survival after heat shock (thermotolerance) in *C. elegans* is conferred by increased ubiquitylation of proteins, perhaps because of their increased degradation. As ubiquitylated proteins can be degraded either by the proteasome or the lysosome, we tested pharmaceutical inhibition of both of these systems. When thermotolerance testing is performed on WT and *pals-22* mutants after inhibition of the proteosome with bortezomib, we see a decrease in survival in both strains. However, when treated with bafilomycinA, a macrolide antibiotic V-ATPase inhibitor that prevents the acidification of the lysosome, the increased survival in response to heat shock of *pals-22* mutants is rescued back to wildtype levels, while wildtype animals remain unaffected. This *pals-22*-specific effect is a promising indication that *pals-22* increased thermotolerance depends on the lysosome. Ongoing studies include genetic approaches to analyze a role for the lysosome, as well as efforts to identify ubiquitylated targets downstream of *pals-22* that might be degraded by the lysosome.

972C CBP-1/p300 acetyltransferase regulates the heat shock response in *C. elegans*

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The decline of proteostasis is a hallmark of aging that is, in part, affected by the dysregulation of the heat shock response (HSR), a highly conserved cellular response to proteotoxic stress in the cell. The heat shock transcription factor HSF-1 is well-studied as a key regulator of proteostasis, but mechanisms that could be used to modulate HSF-1 function to enhance proteostasis during aging are largely unknown. The activation and attenuation of HSF-1 during the HSR is fine-tuned via post-translational modifications, including the recently emerging regulation through acetylation. In this study, we examined histone acetyltransferase regulation of the heat shock response and HSF-1 in *C. elegans*. We performed an RNA interference screen of histone acetyltransferases and examined mRNA expression of the heat-shock inducible gene *hsp-16.2*, a widely used marker for HSR activation. From this screen, we identified one histone acetyltransferase, CBP-1, as a negative regulator of the HSR. We found that while knockdown of CBP-1 decreases overall lifespan of the worm, it also enhances heat shock protein production upon heat shock and increases thermotolerance of the worm in an HSF-1 dependent manner. Similarly, we examined a hallmark of HSF-1 activation, the formation of nuclear stress bodies (nSBs). We examined the recovery rate of nSBs and found that knockdown of CBP-1 enhanced the recovery and resolution of nSBs after stress. Collectively, our studies enhance our knowledge of the regulation of HSF-1 by CBP-1 and its physiological effects at the organismal level upon stress.

973A Exploring muscarinic regulation of oxidative homeostasis during neuromuscular transmission

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The heightened metabolic demands of excitable cells, such as neurons and muscles, have the potential to produce elevated levels of reactive oxygen species (ROS) and oxidative stress. ROS, such as singlet oxygen ions and peroxides, are generated as by-products of ATP synthesis during aerobic metabolism. High levels of ROS produce cellular damage by reacting with proteins

and lipids, and this oxidative damage may accumulate over the lifetime of non-dividing neurons and muscle cells. It is therefore critical for neurons and muscles to have mechanisms for controlling oxidative stress. *Caenorhabditis elegans* provides a simple genetic system to explore molecular links between neuronal activity and oxidative stress. Our preliminary studies of cholinergic synapses at the *C. elegans* neuromuscular junction suggest an interesting potential mechanism for homeostatic regulation of oxidative stress at synapses. Our findings suggest that synaptic activation of ligand-gated nicotinic acetylcholine receptors (nAChR) heightens susceptibility to oxidative stress, while activation of muscarinic G protein-coupled acetylcholine receptors (mAChR) reduces oxidative stress. Specifically, we found that deletion of muscle nAChRs extends survival in the presence of the chemical oxidative stressor paraquat, suggesting that synaptic activation of muscle nAChR increases vulnerability to oxidative stress. In contrast, deletion of the mAChR *gar-3*, enhances the toxic effects of paraquat. Consistent with prior work [1], we observed *gar-3* expression in pharyngeal muscles, head neurons, body wall muscles, and ventral nerve cord motor neurons. Notably, we found that body wall muscle-specific expression of wild type *gar-3* in *gar-3* mutants reverses the heightened sensitivity of *gar-3* mutants to paraquat, indicating that body wall muscle expression of *gar-3* may be critical for organismal oxidative stress regulation. Oxidative damage has been linked with several neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease[2], yet the underlying mechanisms remain unclear. Gaining an improved understanding of how excitable cells minimize oxidative damage will be important for developing new therapeutics to combat degenerative disorders and other conditions associated with oxidative stress.

Keywords: Cholinergic neurotransmission, ROS, oxidative homeostasis

1.Chan, J. P. *et al. J. Neurosci.* (2013)

2.Manoharan, S. *et al. Oxidative Medicine and Cellular Longevity* (2016)

974B Mitochondrial fusion and fission balance is required for exercise-induced benefits in *Caenorhabditis elegans*

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Exercise has been used as a valuable tool in the diagnostics of age-related diseases, as well as a nonpharmacological intervention capable of improving healthspan. Overall, exercise benefits are triggered by transient changes in mitochondrial connectivity and functionality, thereby rewiring cellular metabolism. Mitochondrial fusion and fission are critical effectors of mitochondrial dynamics, which allows a fine-tuned regulation of organelle connectiveness, content, distribution, size, turnover and bioenergetics. The question arises as to whether there is any inter-dependence between mitochondrial fusion-fission balance and exercise capacity during aging, and whether this synergism drives health aging. Here, we set out to genetically dissect the role of mitochondrial fusion-fission balance to exercise-induced benefits in *C. elegans*. Using acute (4h) and long-term (1h/day for 10 days) swimming protocols, we determined the contribution of mitochondrial dynamics to physical capacity and responsiveness to exercise. While one bout of 4h-swim induces mitochondrial fission in muscle followed by a fusion state after a 24h-period of recovery, daily exercise sessions delay the mitochondrial fragmentation, as well as the decline in physical capacity with aging in WT worms. These benefits are abrogated in mutants with compromised fusion (*fzo-1*) and fission (*drp-1*), and a complete lack of mitochondrial plasticity (*drp-1;fzo-1* and *drp-1;eat-3*). These mutants also display reduced physical capacity with aging when compared to WT. Knowing that many anti-aging mechanisms act, at least in part, by influencing mitochondrial fusion and fission events, we next submitted long-lived mutants to exercise. Exercise fails to induce benefits in long-lived mutants *isp-1*, *nuo-6*, *daf-2*,*eat-2* and *CA-AAK-2*, some of which reduced mitochondrial plasticity seems to be required for longevity. Finally, we explored molecular mechanisms that orchestrate mitochondrial dynamics with aging. Interestingly, constitutive AMPK activation (*CA-AAK-2*) also does not allow exercise adaptation but uniquely preserves muscle function during aging. As seen with exercise, this benefit is abolished upon fusion-fission impairment. Together, our study provides evidence that perturbing mitochondrial morphology and connectivity, as well as impairing organelle plasticity are critical to exercise-induced benefits. Therefore, targeting mechanisms to optimize mitochondrial plasticity may represent a novel strategy to promote healthy aging, and also possibly for recapitulating exercise benefits.

975C AGE-1/PI3K Signaling-Independent Effects of DAF-18/PTEN on Starvation Resistance During L1 Arrest in *Caenorhabditis elegans*

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Caenorhabditis elegans has a variety of signaling pathways that sense nutrient availability and regulate gene expression and metabolism to support homeostasis. For instance, Insulin/Insulin-like Growth Factor-1 Signaling (IIS) is transduced by the AGE-1/phosphoinositide 3-kinase (PI3K) signaling cascade to antagonize DAF-16/FoxO and promote growth and development, but during starvation, when IIS is reduced, DAF-16 activates expression of genes that promote developmental arrest and survival. AGE-1/PI3K signaling is opposed by DAF-18/PTEN's lipid-phosphatase activity at the level of phosphatidylinositol 3,4,5-trisphosphate (PIP_3). However, it is unclear whether DAF-18/PTEN's understudied protein-phosphatase activity plays a role and mediates AGE-1/PI3K signaling-independent effects. We assayed three facets of L1 starvation resistance (survival as well as growth rate and fecundity upon recovery) in wild type and mutants affecting AGE-1/PI3K signaling and DAF-18. We observed non-additive effects of *daf-16* and *daf-18* on L1 starvation resistance, suggesting DAF-16/FoxO-independent effects of DAF-18/PTEN. In agreement, transcriptome-wide epistasis analysis of *daf-18* and *daf-16* suggests a branched pathway with *daf-18* acting through and in parallel to *daf-16*. We show that gain-of-function mutants of *akt-1* and *pdk-1*, the major AGE-1/PI3K signaling effectors, alone and combined, are less starvation-sensitive than *daf-18*(null), suggesting DAF-16/FoxO-independent effects of DAF-18/PTEN arise from outside of AGE-1/PI3K signaling instead of other PI3K signaling targets. We report that an *age-1* null mutant with no PIP_3 present is not epistatic to *daf-18*(null), which strongly supports that DAF-18/PTEN regulates starvation resistance through mechanisms besides counteracting AGE-1/PI3K. We generated *daf-18* point mutants disrupting each phosphatase activity individually by CRISPR-Cas9. The protein phosphatase-dead mutant is starvation-sensitive, suggesting the protein-phosphatase activity mediates AGE-1/PI3K signaling-independent effects of DAF-18/PTEN. Overall, this study reveals a novel mechanism of the tumor suppressor DAF-18/PTEN in maintaining homeostasis during starvation in *C. elegans*.

976A A novel proteostasis adaptation in the long-lived *Caenorhabditis elegans rpn-10* proteasome subunit mutant

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The loss of proteostasis due to reduced efficiency of protein degradation pathways has been highlighted in several aging processes and age-related disorders. Paradoxically, we have reported that the *Caenorhabditis elegans rpn-10(ok1865)* proteasomal subunit mutant exhibits enhanced proteostasis, elevated stress resistance and extended lifespan. The RPN-10/PSMD4 subunit is a ubiquitin receptor on the 26S proteasome that targets polyubiquitinated substrates to its catalytic core for degradation. The *rpn-10* mutant possesses mild proteasome dysfunction and a distinct proteasomal peptidase activity profile. Notably, compensatory activation of autophagy and SKN-1/Nrf-regulated responses only partially underlie the robust *rpn-10* mutant phenotype, thus prompting our further investigation into its novel protective processes. To this end, we observed that several endoplasmic reticulum protein quality control (ERQC) genes were transcriptionally upregulated in the *rpn-10* mutant. This is functionally relevant in the *rpn-10* mutant which exhibits higher ER stress resistance and altered ER homeostasis compared to the wild-type. Moreover, as a significant subset of the upregulated ERQC genes was enriched for ER-associated proteasome-mediated degradation (ERAD), we sought to determine the turnover of ER substrates in the *rpn-10* mutant. As expected, the attenuated accumulation of the ER-localized aggregation-prone mutant α-1 antitrypsin (ATZ) reporter indicated that ER proteostasis is augmented in the *rpn-10* mutant. Via a forward genetics screen for suppressors of decreased ATZ aggregation in the *rpn-10* mutant, we identified an unexpected player, *ecps-2*, which is a homolog of the proteasome-associated adaptor protein ECM29. While we observed that *ecps-2* did not regulate proteasomal subunit expression or the ER stress response, we found that the loss of *ecps-2* in the *rpn-10* mutant strongly reduces its proteasomal chymotrypsin-like activity. Altogether, this suggests that the modified proteasomal assembly of the *rpn-10* mutant contributes to its cellular proteostasis. Furthermore, the increased *rpn-10* mutant lifespan appears to depend partially on *ecps-2* but more strongly on its ERQC status. Therefore, we propose that the *ecps-2*-proteasome interaction induces a unique ERQC adaptation which supports the superior proteostasis and longevity of the *rpn-10* mutant.

977B Age-dependent changes in *C. elegans* gut microbiome composition and their consequences

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Aging involves a multi-tissue deterioration. Among other things, it affects the gut microbiome. Changes in gut microbiome composition, in turn, could contribute to age-related pathologies, as imbalances in microbial composition (dysbiosis) are associated with pathology. Identifying central themes in age-dependent microbiome changes is lagging, as is our understanding of their consequences. To examine the bidirectional relationships between host aging and the gut microbiome, we followed age-dependent changes in microbiome composition in worms raised on either natural-like compost microcosms or on well-defined synthetic microbiotas consisting of natural worm gut colonizers. Microbiome composition was analyzed using 16S next generation sequencing or with CFU counts of bacteria cultured on selective media. Additionally, gut colonization at different ages was evaluated using fluorescent microscopy of an RFP-expressing derivative of an *Enterobacter* commensal.

Experiments in both compost microcosms and synthetic microbiotas revealed an expansion of the *Enterobacteriaceae* family in aging worms, a trend similarly observed in humans. Worms sampled during continuous growth on complex microbiotas showed similar age-dependent changes in their gut microbiomes as worms shifted to complex bacterial communities for a fixed time at advancing ages, suggesting that the aging intestinal niche is more important for determining changes in microbiome composition than the duration of exposure to bacterial communities. Further supporting this expansion, experiments with the RFP-expressing *Enterobacter* demonstrated increased age-dependent gut colonization. This particular *Enterobacter* commensal was previously shown to protect young worms from *Enterococcus* infection, but was found to bloom in immune mutants, reversing its contributions and causing increased pathogen susceptibility (Berg *et al.* 2019). Indeed, increased commensal colonization in aging worms abolished the commensal's beneficial effects and increased worm susceptibility to infection, as compared to age-matched worms raised on *Escherichia coli.* Current experiments are underway to examine whether rebalancing of the gut microbiota in aging worms could ameliorate host survival.

978C Genetic regulators of stress-induced RNA mis-splicing in *Caenorhabditis elegans*

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Splicing of pre-mRNA is an essential process for all eukaryotic dividing cells. Pre-mRNA splicing defects are implicated in numerous human diseases, including Alzheimer's disease and cancer, however, its cause is poorly understood. Using the nematode *Caenorhabditis elegans* as a model, we have recently shown that exposure to the environmental heavy metal cadmium can cause RNA splicing disruption, implicating loss of RNA metabolism regulation as a potential mechanism of cadmium toxicity. To understand the genetic mechanism of RNA splicing regulation under environmental stress, we sought to identify and characterize genes that, when knocked down, can protect against cadmium-induced RNA splicing errors. Using a *C. elegans in vivo* splicing reporter, we found that majority of the gene knock-downs that improved RNA splicing under stress encode various components of the protein synthesis machinery, including *ifg-1*, which encodes the human eIF4G gene previously shown to regulate aging in worms. Knockdown of various protein translation related genes not only increase *C. elegans* lifespan but also enhance resistance to cadmium survival. Using RNA-seq, we found that *ifg-1* mutants show increases in expression of >80 genes that regulate RNA splicing, importantly, *ifg-1* mutants exposed to cadmium show a 50% decrease in cadmium-induced alternative splicing events observed in N2. Downstream of *ifg-1*, we have identified RNA splicing regulators that do not affect N2 lifespan when knocked-down but abolishes *ifg-1*'s long-lived phenotype. Suppression of protein synthesis has been shown to be beneficial in promoting longevity and stress resistance in various organisms including *C. elegans*, and our study may have implicated a potential mechanism through which these physiological benefits are achieved in part by improvements to RNA splicing fidelity.

979A UNC-45 has a crucial role in preventing sarcopenia in *C. elegans*

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As longevity increases, age-related diseases will become a greater public health concern. Sarcopenia, the age-related decline in muscle mass and function without any underlying disease, is estimated to affect 40-50% of people over the age of 80 and is a major contributor to physical disability and mortality among the elderly. The molecular mechanisms responsible for this pathology remain unknown. Muscle function is dependent on having properly organized and functioning thick filaments, which are primarily composed of myosin. UNC-45, the first myosin head chaperone discovered, is required for the folding of the myosin head initially after translation and likely re-folds the myosin head to regain functionality after thermal or chemical stress causes unfolding. Here we show that myosin, UNC-45, and its co-chaperone HSP-90 are decreased during aging in *C. elegans*. HSP-90 transcript declines at day 2 of adulthood, directly before its decline in protein at day 3 of adulthood. The loss of HSP-90 protein directly precedes a loss of UNC-45 protein at day 4 of adulthood, and precedes the loss of the major client of UNC-45, myosin MHC B, at day 8 of adulthood. Myosin and UNC-45 protein decline appear to be independent of steady state

mRNA levels. This decrease in chaperone protein correlates to decreased assembled thick filaments and a decline in mobility. We also see a decrease in UNC-45 protein, but not transcript, in an *hsp-90* loss of function temperature sensitive mutant, suggesting a role for HSP-90 in UNC-45 protein stabilization or regulation. Using a temperature sensitive *unc-45* mutant, we can observe early onset of sarcopenia when UNC-45 is lost during young adulthood. Additionally, there is an increase in UNC-45 phosphorylation with age that may be related to its increased degradation. This leads us to investigate the possibility that during aging a loss of HSP-90 leads to UNC-45 phosphorylation (and other post-translational modifications) and degradation, which then leads to a loss of muscle mass and function. A better understanding of how myosin and its chaperone proteins are regulated and affected by aging will lead to better preventative care and treatment of sarcopenia and, possibly, the age-related decline of heart muscle function.

980B Material states of protein cargo in neuronal exophers

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Neurodegenerative diseases are devastating disorders that affect millions of Americans and cause hundreds of thousands of deaths each year. Amyotrophic lateral sclerosis (ALS), commonly referred to as Lou Gherig's disease, is caused by the degeneration of upper and lower motor neurons that results in progressive paralysis, whereby cognitively healthy patients are "trapped" within paralyzed bodies and experience respiratory failure and death within 3-5 years. Protein aggregation is a hallmark of pathology in neurodegenerative diseases. FUS and TDP43 are two RNA-binding proteins that are commonly found to aggregate in the neurons of patients with ALS. Aggregation pathology spreads through the brain along neuronal circuits suggesting a mechanism of neuronal transfer of aggregates that may be exploited for novel interventions against these devastating neurodegenerative diseases. Our lab has recently observed that *C. elegans* neurons can expel aggregated mCherry or PolyQ protein from the soma in large membrane-bound vesicles (called exophers) to promote proteostasis within the neuron. It is interesting that proteins such as FUS and TDP43 can form a variety of material states including liquid droplets, hydrogels, and insoluble aggregates. The material state phenomenon is also observed for the P granule proteins PGL-3, which forms a liquid, and MEG-3, which forms a hydrogel. It is unknown whether liquid droplets or hydrogels (or both or neither) are expelled in exophers. We are expressing FUS, TDP43, PGL-3, and MEG-3 in *C. elegans* touch neurons to determine their capacity to be recruited to exophers. The results I will present will further our understanding of exopher cargo selection and the potential for exopher biology to be a target for intervening in neurodegenerative diseases.

981C Putative role of *Caenorhabditis elegans huntingtin* in stress response

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CAG repeat expansion mutation in exon 1 of *huntingtin* (*HTT*) causes neurodegenerative disorder called Huntington's disease (HD). HD is both gain of function of the mutant protein and a loss of function of the wild type protein. *Huntingtin* functional study is important for the pathology and for the therapeutic studies. *Huntingtin* studies using different research models indicate that the gene is necessary for survival and required for the normal development in many different eukaryotes. However, the normal function of *huntingtin* remains elusive. Null mutation of *Caenorhabditis elegans huntingtin* is a non-lethal mutation, and this makes *C. elegans* a strong research model to study the normal function of *huntingtin*. There's no CAG repeat sequence near the N-terminus in *Ce_huntingtin.* However, according to the sequence alignment analysis, *Ce_huntingtin* and human *HTT* have very similar sequences especially near the C termini. Expression pattern analysis shows that *Ce_huntingtin* is expressed in multiple tissues and enriched in the neurons including the serotonergic neurons that are closely related to stress responses in *C. elegans*. We observe that *huntingtin* in *C. elegans* has roles in stress response pathway. Decreased survival to bacterial pathogen and thermal stress is caused by a deletion mutation of *Ce_huntingtin*. Our research evaluating the function of *Ce_huntingtin* in stress response will help to find the unknown normal function of *huntingtin* in human as well.

982A Balancing aging, proteostasis and nervous system function: differential effects of multiple lifespan-extending genetic manipulations in MJD/SCA3.

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Over the past few years evidence that contradicted aging as an inevitable phenomenon has surged, leading the scientific community to concentrate efforts to test drugs that effectively tackle aging. In parallel, this approach aimed to decrease the prevalence of a number of different disorders, such as neurodegenerative diseases, for which aging is a key risk factor.

Here, the hypothesis that delaying aging is neuroprotective was assessed in a *C. elegans* model of Spinocerebellar Ataxia (SCA) Type 3, also known as Machado-Joseph disease (SCA3/MJD), the most common SCA worldwide. This neurodegenerative disease has a clear genetic cause, the abnormal expansion of a CAG triplet in the ataxin-3 gene. However, the contribution of additional genetic/environmental factors have been proposed to explain the variable disease phenotype.

Lifespan-increasing mutations that are representative of well-known and conserved aging regulator mechanisms (insulin/IGF-1 signaling, dietary restriction, germline ablation and mitochondrial dysfunction) were introduced in the genetic background of the SCA3 nematode model. Their impact in key aspects of the disease was then assessed.

Lifespan-extension improved the SCA3 motor phenotype if induced by altered nutrient sensing pathways, as is the case of the insulin/IGF-1 and mTOR signaling, but not when associated with other pathways, such as mitochondrial dysfunction and germline ablation. This challenges the idea that delaying aging is by itself beneficial and regarded a guaranteed therapy for these diseases. Additional experiments pointed to significant transcriptomic alterations in the proteostasis network caused by the downregulation of IGF-1/insulin signaling. However, not all insulin/IGF-1-dependent transcriptional responses seemed disease-modifying, suggesting that neuroprotective effects of aging can be restricted to more specific aging factors. Finally, chronic treatment of the *C. elegans* SCA3 model with insulin/IGF-1 signaling inhibitors also improved the motor phenotype, further demonstrating the therapeutic value of insulin/IGF-1 downregulation for the disease, increasing prospects for additional drug repurposing centered in this pathway.

These results provide key insights to guide future therapeutic strategies for neurodegenerative diseases based on the manipulation of the aging process.

984C Local regulation of mRNA fate governs mitochondrial biogenesis during ageing in *C. elegans*

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Mitochondria are important regulators of healthspan and lifespan, and their perturbation has been correlated with various pathological conditions. Mitochondrial abundance and function are controlled by the opposing processes of mitochondrial biogenesis and mitophagy. While the mechanisms underlying mitophagy have been extensively studied, mitochondrial biogenesis is not well understood. We find that the mRNA decapping and the CCR-4/NOT complexes physically associate with mitochondria and oppositely regulate mitochondrial abundance during ageing. Components of the two complexes tightly control the fate of specific nuclear encoded mitochondrial transcripts, including those for ETC components and mitochondrial biogenesis regulators. Our findings indicate that post-transcriptional regulation of select mitochondrial transcripts modulates mitochondrial abundance and function, as well as, lifespan and stress resistance in *C. elegans*. The tight evolutionary conservation of the decapping and the CCR-4/NOT complex components suggests that similar mechanisms contribute to mitochondrial homeostasis during ageing across diverse organisms.

985A Persistent DNA repair complex binding in the absence of DNA damage excision impairs neuron functionality

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Nucleotide excision repair (NER) is a major DNA repair pathway that removes a large variety of helix-distorting DNA damage. Hereditary mutations in NER genes that encode proteins that make up the core NER machinery, such in endonucleases XPF and XPG, can give rise to the cancer prone disorder xeroderma pigmentosum (XP) or to more severe disorders in which XP is combined with progressive neurodegeneration or progeroid Cockayne syndrome (CS) features, called XP-CS. Intriguingly, mutations in other factors of the core NER complex give rise to a milder phenotype. It is not properly understood why mutations in genes that act in the same DNA repair pathway cause different types of disease.

Here, we use *C. elegans* as a model to study the phenotypic impact of different mutations in the evolutionary conserved NER pathway. We found that XPF-1 or XPG-1 deletion mutants show severe developmental arrest and neuronal dysfunction upon

induction of UV damage. In the absence of XPF-1 or XPG-1, core NER-intermediates accumulate at DNA damage and stay stably bound to DNA. We hypothesized that this could shield the lesion from other DNA repair pathways and block transcription, which could cause this severe phenotype. Intriguingly, we found that inhibiting the binding of the core NER-intermediates to DNA in XPF-1 or XPG-1 worms alleviated the severe phenotype, confirming that the accumulation of repair intermediates can indeed be more toxic for cells than the damage itself. Together, these results strongly suggest that the persistence of NER intermediates adversely affects cell functionality, which may be a plausible explanation for why mutations in XPF or XPG can lead to more severe disease features than mutations in other core subunits.

986B Heterochromatin protein 1 regulates longevity and the mitochondrial unfolded protein response

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Prohibitins (PHB-1 and PHB-2) form a large macromolecular structure at the mitochondrial inner membrane. Prohibitin deficiency shortens the lifespan of wild type animals, but dramatically extends the lifespan of a variety of metabolically compromised animals such as insulin/IGF-1 receptor (*daf-2*) mutants. This phenotype is accompanied by a differential induction of the mitochondrial Unfolded Protein Response (UPR^{mt}), a stress protective mechanism that is attenuated in *daf-2* mutants.

Through a genome wide RNAi screen we identified Heterochromatin Protein 1 (HP1) as a new regulator of the UPR^{mt}. HP1 proteins (HPL-1 and HPL-2 in *C. elegans*) bind histone H3 methylated on lysine 9 to maintain chromatin in a repressed state during development. We observed induction of the UPR^{mt} in HP1 single and double mutants, as well as decreased basal and maximal respiration compared to wild type animals, suggesting a mitochondrial dysfunction. This is supported by an increased sensitivity of HP1 mutants to the antibiotic doxycycline, which specifically blocks mitochondrial translation. Furthermore, HP1 protein levels increased in response to *phb-1* depletion, both, in otherwise wild type animals and in *daf-2* mutants. Interestingly, *hpl-1* mutants reduced the UPR^{mt} triggered by *phb-1* depletion, but suppressed the attenuated UPR^{mt} in PHB-depleted *daf-2* mutants.

HPL-1 protein levels increase as animals age, while HPL-2 remains steady. Interestingly, *hpl-1* deletion extended lifespan independently of *phb-1*. However, in *daf-2* mutants, *hpl-1* was required for the enhanced longevity conferred by PHB depletion. We are currently investigating which genes are targeted by HP1 proteins under conditions leading to opposing ageing phenotypes. Given the evolutionary conservation of ageing mechanisms and the proteins under study, research in short-lived model systems amenable to genetic dissection, such as *C. elegans*, is likely to be beneficial to human health.

987C Prohibitin depletion extends lifespan of a TORC2/SGK-1 mutant through autophagy and the mitochondrial UPR

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Mitochondrial prohibitins (PHB) are highly evolutionarily conserved proteins with a peculiar effect on lifespan. While PHB depletion shortens lifespan of wild type animals, it enhances longevity of a plethora of metabolically compromised mutants, including target of rapamycin complex 2 (TORC2) mutants *sgk-1* and *rict-1*. SGK-1 belongs to the AGC kinase family and is the sole *C. elegans* homologue of the mammalian Serum- and Glucocorticoid-inducible Kinase. SGK-1 regulates aging and mitochondrial homeostasis as part of TORC2 downstream of RICT-1. Intriguingly, TORC2 mutants induce the mitochondrial unfolded protein response (UPR^{mt}), while reducing the strong UPR^{mt} elicited by PHB depletion.

Here we show that *sgk-1* mutants have increased mitochondrial size, mitochondrial interconnectivity, respiration rate and ROS production. Remarkably, all these features are suppressed by PHB depletion. A transcription factor RNAi screen identified lipid and sterol homeostasis as UPR^{mt} modulators in *sgk-1* mutants. Further, we show that *sgk-1* mutants have impaired lipogenesis and lipoprotein/yolk formation, plausibly due to alterations in membrane lipid and sterol homeostasis. Surprisingly, both defects are suppressed by PHB depletion. Lifespan analysis shows the requirement of the sterol binding protein SRBP1/SBP-1 for the extended lifespan of *sgk-1* mutants and the further increase conferred by PHB depletion.

Additionally, we show that while autophagy is similarly induced in *sgk-1* mutants, PHB-depleted and *sgk-1*;PHB-depleted animals, lysosomal function is particularly enhanced in *sgk-1*;PHB-depleted worms. Interestingly, autophagy and the UPR^{mt} are dispensable for the lifespan of *sgk-1* mutants and PHB-depleted animals. However, the enhanced longevity caused by PHB depletion in *sgk-1* mutants requires both, the UPR^{mt} and autophagy, but not mitophagy. We hypothesize that UPR^{mt} induction upon PHB depletion extends lifespan of *sgk-1* mutants through autophagy and probably modulation of lipid metabolism.

988A Stress discrimination by body-wide, stochastic DAF-16/FoxO nuclear translocation pulses

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In *C. elegans*, the insulin/IGF-1 pathway is responsible for mounting tailored responses to a broad range of external stresses. DAF-16/FOXO, the primary output of this pathway, translocates to the nucleus upon stress to induce gene expression. 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. Pulses were present both in DAF-16::GFP integrated transgenes and CRISPR knock-ins. The pulses were strikingly synchronized across the worm body, with cells from different tissues switching within minutes. Quantitative analysis revealed that DAF-16 pulse dynamics was specific to different types of stress: starvation resulted in pulses that resembled stochastic oscillations, osmotic shock yielded random pulses whose average durations increased with salt concentration while their amplitude remained constant, and temperature shock gave rise to a single high-amplitude pulse, followed by persistent DAF-16 nuclear localization. These results suggest that the worm could use differences in DAF-16 nuclear translocation dynamics to determine both magnitude and type of stress. We use mathematical modelling to provide a potential mechanism for DAF-16 pulse generation and their body-wide synchronization. Finally, we also observed pulsatile dynamics of the DAF-16 homolog FoxO in mammalian cells at low nutrient levels that activate insulin signaling. Overall, this indicates that DAF-16/FoxO translocation pulses are a general feature of insulin signaling.

989B Nuclear Hormone Receptor NHR-49 controls a HIF-1-independent hypoxia adaptation pathway in *Caenorhabditis elegans*

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Cells encounter many harmful stresses, and their ability to mount responses specific to each stress is critical for survival. The response to insufficient oxygen (hypoxia) is orchestrated by the conserved Hypoxia-Inducible Factor (HIF). However, HIF-independent hypoxia response pathways exist that act in parallel to HIF to mediate the physiological hypoxia response. We have found a HIF-independent hypoxia response pathway controlled by *Caenorhabditis elegans* Nuclear Hormone Receptor NHR-49, an orthologue of mammalian lipid metabolism regulator Peroxisome Proliferator-Activated Receptor alpha (PPARα). We show that *nhr-49* is required for worm survival in hypoxia and is synthetic lethal with *hif-1* in this context, demonstrating that these factors act independently. Our RNA-seq analysis shows that in hypoxia *nhr-49* regulates a set of genes that are *hif-1*-independent, including autophagy genes that promote hypoxia survival. We further show that Nuclear Hormone Receptor *nhr-67* and Homeodomain-interacting Protein Kinase *hpk-1* act in the NHR-49 pathway. The former acts during normoxia to repress NHR-49; however, during hypoxia, an increase in NHR-49 protein levels in turn represses *nhr-67* levels, forming a feedback loop that may serve to reinforce NHR-49 activity. In contrast to *nhr-67*, the upstream kinase HPK-1 positively regulates the NHR-49 hypoxia response, as it is required to activate the NHR-49 regulated hypoxia response genes and to survive hypoxia. Together, our experiments define a new, essential hypoxia response pathway that acts in parallel to the well-known HIF-mediated hypoxia response.

990C Effect of individual members of gut microbiome on *C. elegans* stress resistance, lifespan and healthspan

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Dysregulation of the human gut microbiome has been linked to the development of many human diseases, including inflammatory disorders such as irritable bowel syndrome and ulcerative colitis. Moreover, studies have shown that the microorganisms colonizing the GI tract interact extensively with host signaling pathways, including the highly conserved

ageing-related Insulin/IGF signaling pathway. The mechanisms by which the gut microbiota influences host gene expression and physiology remains unclear. With a rapid lifespan of less than one month, *C. elegans* have been used to study the relationship between host phenotypes and gene expression for decades. *C. elegans* are typically grown on a non-native singular food source, *E. coli* OP50, selected for its accessibility and ease of growth in the laboratory environment. The versatility of the soil dwelling nematode offers a unique platform to study complex microbial communities in a well-defined gnotobiotic environment, however very little is known about the effects of non-*E.coli* bacteria on *C. elegans* health and survival.

Here we conduct comprehensive screen of select individual bacteria isolated from natural *C. elegans* microbiome. Sterile *C. elegans* can be housed in pillared microfluidic chambers where bacterial membership can be precisely controlled and readily delivered to control quality and quantity of the bacteria. Using this platform, we show that individual members of the natural microbiome colonize the *C. elegans* gut and exert variable effects on host physiology including delayed development and growth, stress resistance and survival. Specifically, we find that two bacterial isolates, which dominate the guts of *C. elegans*, *Ochrobactrum* BH3 and *Myroides BIGb*0244, extend lifespan and healthspan in our semi-liquid microfluidic environment. Our results lay the foundation for future, high-throughput screens of larger communities and panels of microbes, such as the BIGbiome and CeMbio model microbiomes. This robust system will allow for simultaneous and comprehensive assessment of the effects of both individual isolates and multi-member communities on host gene expression and aging related phenotypes.

991A Tissue-Specific Roles of microRNA Argonaute Proteins in Aging

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MicroRNAs (miRNAs) are short, non-coding RNA molecules that negatively regulate gene expression post-transcriptionally. When bound with Argonautes (AGOs) and other companion proteins, miRNAs can target mRNAs for degradation or translational repression. The miRNA pathway is conserved through many organisms and is involved in development, longevity, and stress responses. Two integral *Caenorhabditis elegans* AGOs (ALG-1 and ALG-2) are 81% identical at the amino acid level and have similar spatiotemporal expression patterns in developing *C. elegans*, but recent work shows that these developmentally redundant AGOs take on opposing roles in adults. There is a global reduction of ALG-1 expression in adults, while ALG-2 levels remain constant. Loss of function (LOF) *alg-1* mutants have shortened lifespans, whereas LOF *alg-2* mutants live longer than wildtype organisms. Through transcriptional profiling of these mutants, we've identified unique sets of misregulated miRNAs and protein-coding genes. Interestingly, there is an enrichment of up-regulated neuronal-specific genes in the *alg-1* adult mutant, and previous work has shown that individual tissues play pivotal roles in regulating longevity. We have begun to test well-characterized aging phenotypes at the tissue level, which reveal that ALG-1 is important in the maintenance of pharyngeal, muscular, and intestinal integrity in adulthood. Additionally, we have generated tissue-specific ALG-1- or ALG-2-depleted strains to begin testing the hypothesis that ALG-1 and ALG-2 regulate miRNA targets in a tissue-specific manner, leading to opposing roles in the lifespan of *C. elegans*.

992B Insulin-like signaling and starvation resistance via *daf-16/FoxO*-dependent and independent gene regulatory pathways

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In the absence of food, *C. elegans* are able to arrest development in order to survive extended periods of starvation. This period of quiescence is reversible upon feeding, and the ability to start and stop development is dependent on the transcriptional effector of insulin-like signaling, *daf-16/FoxO*. *daf-16* is responsible for inhibiting pathways that promote development and activating stress-response genes, and it is regulated by the insulin receptor *daf-2/*InsR via the PI3K pathway. We are interested in exploring the gene regulatory mechanisms of *daf-2* that promote starvation resistance in *daf-16*-dependent and independent manners. Gene expression data revealed that the histone variant *hil-1/H1-0* is activated by *daf-16* during starvation, and that its expression decreases upon feeding. Assays for survival, growth and reproduction following recovery from starvation using *hil-1* mutant alleles *tm1442* and *gk229* suggest that *hil-1* plays a critical role in *C. elegans'* ability to survive and recover from starvation. We hypothesize that HIL-1 alters chromatin structure, regulating expression of nutrient-responsive genes. In addition, RNA-seq-based epistasis analysis suggests a function of *daf-2* independent of *daf-16*. Phenotypic assays of *daf-2* and *daf-16* single and double mutants revealed that simultaneous disruption of *daf-2* and *daf-16* leads to increased starvation resistance over the *daf-16* single mutant. Together these results suggest DAF-2 regulates starvation resistance through one or more effectors in addition to DAF-16. Identifying *daf-2* signaling effector mechanisms as well as the

DAF-16-dependent function of *hil-1* in cellular quiescence will be valuable for understanding the genetic basis of physiological adaptation to nutrient availability with implications for aging and the pathological consequences of nutrient stress in humans.

993C 14-3-3 and its interacting proteins in aging and neurodegeneration

Akshatha Ganne[1,2], Meenakshisundaram Balasubramaniam[1,4], Robert Shmookler Reis[1,3,4], Srinivas Ayyadevara[1,3,4] 1) UAMS; 2) UALR; 3) Central Arkansas Veterans Healthcare Service, Little Rock; 4) Department of Geriatrics. The 14-3-3 proteins are an evolutionarily conserved family of proteins that are ubiquitous from nematodes to humans. They are intrinsically unstructured proteins that bind to a diverse array of key regulatory-protein targets, modulating their functions. They were shown to bind to DAF-16 and SIR-2.1 proteins, with substantial effects on C.elegans lifespan. In mammals, these regulatory proteins are most highly expressed in brain/cerebral tissue, predominantly in neurons. Their presence in cerebrospinal fluid may serve as biomarkers of neuronal damage associated with Alzheimer's disease (AD), Creutzfeldt–Jakob disease (CJD), spongiform encephalitis, brain cancers, and stroke. We also observed a significant enrichment of specific 14-3-3 isoforms among the proteins we identified in neuropathy-associated protein aggregates. Intriguingly, the interacting partners of 14-3-3 isoforms are altered in AD. We pursued these observations in the present study, by immunoprecipitation to isolate specific aggregate types, followed by mass spectrometry to identify interacting protein partners of 14-3-3, and thus to gain insights into their roles in C.elegans aging and models of age-associated neurodegeneration.

994A Rationing yolk affects offspring quality, not quantity, in *C. elegans*

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Embryonic development demands energy to drive biological processes like chromosome segregation and cell division, as well as molecular building blocks such as nucleotides and amino acids to meet the needs of DNA replication and protein synthesis. To conform to these requirements, egg-laying species rely on a finite maternal energy supply incorporated into the egg, known as yolk. Throughout evolution, however, it has not been uncommon for parental generations to experience hardships to the extent that they cannot provide sufficient resources to their offspring. Wild animals still regularly face such challenges, and people often associate these situations with them having to decide to only feed some of their children, sealing the fate of others. This is in line with many observations done in egg-laying species, where resources limitation due to maternal yolk deprivation will result in a lower fecundity. Nevertheless, for some oviparous species, including *Caenorhabditis elegans*, yolk availability does not seem to correlate with fecundity, which challenges this paradigm.

Via differential proteomics and physiological experiments using multiple yolk-deprived mutants, we found that, unlike species where egg production correlates with yolk protein levels, *C. elegans* appears to invest in massive yolk production to gain a small temporal advantage during embryonic development. Moreover, yolk provisioning also allowed offspring to be more resistant to post-embryonic larval starvation. Under conditions of limited food availability, as are common in nature, these characteristics grant well-provisioned offspring a competitive edge over yolk-deprived animals. These results suggest that, in contrast to other oviparous species, *C. elegans* resorts to prioritizing offspring numbers over quality when confronted with yolk deprivation.

995B Three programmatic mechanisms of aging in *C. elegans*

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One of the main objectives of research on aging in *C. elegans* is to develop an understanding of the fundamental principles that govern the process of senescence (aging) including the development of diseases of aging. Attaining this understanding has proven to be difficult, despite major advances in terms of knowledge of genes and pathways which affect lifespan. Work in the Gems lab has investigated two different ideas about aging in *C. elegans*. First, and initially, that it results mainly from the accumulation of stochastic molecular damage. Second, and more recently, that it results mainly from non-stochastic programmatic mechanisms driven by wild-type gene action. Tests of the damage theory yielded largely negative effects [1-6]. By contrast, tests of programmatic theories have both supported such theories [7-11] and led to elaborations of them [12-16].

Our recent work suggests a new picture of *C. elegans* aging as driven by three distinct forms of programmatic mechanism. First, programs optimized for fitness in early life that later run on in futile fashion, causing pathology. This is exemplified by

uterine tumor formation which results from run-on of embryogenetic programs [10, 11], consistent with recent programmatic theories [17-19]. Second, costly programs that promote aging as part of a suicidal reproductive effort (semelparous reproductive death, c.f. Pacific salmon). For example, consumption of intestinal biomass to support production of yolk that is vented to support larval growth leads to gut atrophy (see presentation by C.C. Kern)[8, 16, 20]. Third, programmed adaptive death, that can evolve in organisms that exist in clonal, high-density populations and, particularly, where reproductive death occurs [12-14]. We suggest that the unusually high degree of plasticity in aging in *C. elegans* reflects suppression of genetically determined reproductive death and adaptive death.

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996C Uncovering protective mechanisms of the probiotic *Bacillus subtilis* against α-synuclein aggregation

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Recent discoveries have implicated the gut microbiome in the progression and severity of Parkinson's disease. However, how gut bacteria affect neurodegenerative disorders remains unclear. We previously showed that a probiotic *Bacillus subtilis* strain inhibits α-synuclein aggregation and clears preformed aggregates in an established *Caenorhabditis elegans* model of synucleinopathy (Goya et al, 2020). The reduction in aggregates can be triggered by multiple *B. subtilis* strains and correlates with improved locomotion in α-synuclein-expressing worms. We provide evidence for distinct contributions of spores and vegetative cells in inhibiting α-synuclein aggregation, and a role of biofilm formation in the gut for maintaining low levels of aggregation during aging. We are taking genetics and metabolomics approaches to uncover bacterial metabolic pathways that mediate the protective effect, and host response mechanisms triggered by the *B. subtilis* diet. Our findings provide a basis for exploring the disease-modifying potential of *B. subtilis* and its metabolic products in synucleinopathies.

998B Cryptic transcription and deregulation of alternative 3' splice site selection are associated with physiological aging in *C. elegans*

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Aging is associated with changes in diverse biological processes, including gene expression at transcriptomic levels. The age-associated changes scale with two types of aging, chronological and physiological aging. Understanding these two aspects is important for delaying or even reversing physiological aging processes. However, dissecting the difference between chronological and physiological ages at the transcriptomic level has been a challenge due to its complexity. Here we analyzed transcriptomic features that scaled with physiological or chronological aging using various ages of wild-type *C. elegans* and long-lived *daf-2*/insulin/IGF-1 receptor mutants, which display delayed physiological aging in various aspects. We found that while changes in the abundance of protein-coding transcripts were independent of ages, upregulation of non-exonic (intronic and intergenic) or non-coding transcripts generally scaled with aging in wild-type worms. This result raises the possibility of impaired transcriptional repression and increased diversity of expressed transcripts in aged worms. Next, we analyzed transcriptomic features associated with physiological ages. We showed that *daf-2* mutations delayed age-dependent increases in cryptic transcription and the use of rarely used distal 3' splice sites in transcripts. Thus, cryptic transcription and the usage of distal 3' splice sites appear to scale with physiological ages, which may contribute to the increased diversity of expressed transcripts in aged worms. We then sought to identify factors that mediated the physiological, age-dependent increase in transcriptomic deregulation by analyzing the expression changes in genes associated with RNA processing. While the expression of most genes that encode RNA processing components generally decreased and scaled with chronological ages, eleven of them exhibited downregulation scaling with physiological ages. Currently, we are functionally characterizing these eleven genes by performing an RNAi-based lifespan screen, and will test whether any of these genes underlie physiological age-dependent transcriptomic changes. Together our results suggest that cryptic transcription and deregulation of alternative 3' splice site selection are transcriptomic features scaling with physiological ages. We believe our work will provide novel insights into dissecting chronological and physiological aging at the transcriptomic level.

999C Homolog of ELAC2 is responsive to mitochondrial stress and activates the mitochondrial unfolded protein response

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Proper mitochondrial function is imperative for cell health and homeostasis. Defects in mitochondrial function have profound impacts on aging and have been associated with a growing list of diseases and disorders. To maintain mitochondrial function, cellular mechanisms have evolved that are responsive to mitochondrial stress. However, the molecular underpinnings of these mechanisms are poorly understood. Using a targeted screening approach, we have discovered that the tRNA processing enzyme HOE-1 (homolog of ELAC2) is responsive to mitochondrial stress. Upon mitochondrial stress, nuclear levels of HOE-1 are elevated. We find that HOE-1 is integral for the activation of an important stress mechanism called the mitochondrial unfolded protein response (UPR^{mt}). Loss of HOE-1 attenuates UPR^{mt} induction, while forcing its nuclear accumulation is sufficient to robustly induce UPR^{mt}, even in the absence of mitochondrial stress. Blocking tRNA export from the nucleus is sufficient to suppress HOE-1 dependent UPR^{mt} induction. These data suggest that HOE-1 generates tRNA species required in the cytosol to trigger UPR^{mt}. Taken together we have identified a novel and unusual pathway of UPR^{mt} activation. Our work provides a better understanding of how cells sense and respond to mitochondrial stress.

1000A LEA motifs promote desiccation tolerance *in vivo*

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Cells and organisms typically cannot survive in the absence of water. However, there are some notable exceptions, including animals such as nematodes, tardigrades, rotifers, and some arthropods. One class of proteins known to play a role in desiccation resistance 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. To study the endogenous function of an LEA protein in an animal, we created a true null mutant of *C. elegans* LEA-1, as well as endogenous fluorescent reporters of the protein. We confirmed that *C. elegans* lacking LEA-1 are sensitive to desiccation. LEA-1 mutant animals 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. We identified minimal motifs within *C. elegans* LEA-1 that are 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* survived mild desiccation and osmotic stress 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 suggests the possibility of engineering LEA-1-derived peptides for optimized desiccation protection.

1001B Identifying the mechanisms of NLP-14/Orcokinin signaling during sleep.

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Despite sleep being essential and conserved, an understanding of its mechanisms are lacking. We recently described a role for NLP-14/orcokinin neuropeptides during the regulation of sleep, most notably during stress-induced sleep (SIS). Orcokinins are found in ecdysozoan animals and have been shown to regulate circadian rhythms and molting in insects. Despite their conservation, their downstream mechanisms are not well understood. Taking advantage of the observation that over-expression of *nlp-14* induces a strong sleep phenotype, we conducted a forward genetic screen for suppression of the sleep phenotype. Our experimental approach uses 3D-printed chambers which allow for the sorting of animals using gravity. Mutagenized animals are induced to fall asleep by *nlp-14* over-expression. Those who fell asleep were trapped in the lower chamber, while the rare suppressors swam against gravity to the upper collection platform. We have isolated multiple mutants, which we call orcokinin suppressors (*orcs*), and have sequenced their genomes. Currently, we are identifying the causative alleles. Two of these unmapped mutants *orcs*(*327*) and *orcs*(330) display drastically reduced levels of SIS. These new mutants may represent novel sleep genes essential for SIS regulation.

1002C Crosstalk between HSR and mTOR regulates hibernation and longevity

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Population aging accelerates in the world and the anti-aging study becomes more and more important. During these decades, anti-aging studies have discovered several regulatory mechanisms of aging, such as mTOR and insulin signals. And clinical studies on anti-aging have launched recently. However, it is still unclear “why we age“. To answer the fundamental question, we are now studying slowed aging at low temperatures, as a new anti-aging model.

In *C. elegans*, lifespan and developmental speed are inversely correlated with living temperature even in both long-lived and short-lived mutants. It suggests that aging speed in whole-life, from development to aging, is slowed down at low temperatures. But, the regulatory mechanism of aging at low temperatures is poorly understood. In previous studies, we found components of HSP90 complex, HSP90/*hsp-90* and co-chaperone p23/*daf-41*, controlled lifespan in response to temperature (PLoS Genet 2015). A mutation of *daf-41* caused short-lived and a gain-of-function mutation of *hsp-90* extremely extended the lifespan at 15°C. The maximum lifespan of the *hsp-90* mutant was longer than 100 days. According to the result, we thought some chaperones are involved in cold adaptation and investigated the functions of chaperones at low temperatures. And, we found the *hsf-1(sy441)* mutants, heat shock response (HSR), stopped development at 9°C and they restarted growing when the mutants were transferred to 20°C. Interestingly, the *hsf-1* mutants could survive at least for 60 days in the diapause at 9°C. Therefore, we named this cold-inducible diapause phenotype «hibernation».

Next, we explored mutants that show the hibernation phenotype from known aging mutants and found the *rict-1(mg451)* mutants, in mTORC2 pathway, also stopped growth at 9°C. Although the *rict-1* mutants were mostly killed by severe vulval bursting, approximately 10% of total worms could enter into hibernation and survived longer than 60 days as larvae. We also tested crosstalk between HSR and mTORC2 pathways and found that the gain-of-function mutation of *hsp-90* inhibited the hibernation-entry of the *rict-1* mutants. We found another crosstalk that the overexpression of *skn-1* extending lifespan only at 15°C (H Miller, Aging Cell 2017) also prevented the hibernation-entry of both the *rict-1* and *hsf-1* mutants.

With these findings, we hypothesized that there is a crosstalk between the regulation of hibernation-entry and longevity, and it can be used for the screening of novel longevity genes. By EMS mutagenesis, we have already isolated more than 50 hibernation-exit mutants in *hsf-1* and *rict-1* mutants background and now measure the lifespan of the worms.

1003A Are levels of autophagy increased or decreased in *daf-2* insulin/IGF-1 mutants?

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According to one theory, molecular damage is the principal cause of ageing, while somatic maintenance mechanisms act against damage accumulation and ageing. One such maintenance mechanism is autophagy, which can remove damaged cellular constituents. Autophagy is considered a likely mechanism protecting against ageing. 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 evidence supports the view that gut atrophy is the result of the intestine consuming its own biomass to support yolk synthesis in sperm-depleted hermaphrodites [1], an example of a programmatic mechanism of ageing [2]. This yolk is vented via the vulva after reproduction and can support larval growth [3] (see presentation by C.C. Kern). Such intestinal biomass repurposing is facilitated by autophagy, and greatly reduced in *daf-2* mutants [1,4]. This suggests that autophagy is decreased in *daf-2* mutants. Consistent with this, it has been reported that protein turnover is also reduced in *daf-2* mutants.

To try to resolve the conflicting conclusions about the relationship between IIS, autophagy and ageing in *C. elegans* we are re-examining effects of knockdown of genes encoding the machinery of autophagy on pathology and ageing, taking into consideration issues of timing and severity of knockdown, *daf-2* allele class, temperature, bacterial status and other issues. Our interim data will be described.

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1004B Quantitative Analysis of DAF-16 Lifelong Spatiotemporal Activity under Dietary Restriction as a Predictor of *C. elegans* lifespan

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Several environmental factors affect longevity in *C. elegans*. Most of these function through the DAF-16 transcription factor, which regulates expression of genes involved in aging and stress response. Current methods to track DAF-16 activity have a destructive nature, and use strains with multiple copies of *daf-16*. Thus, we created a single copy GFT-tagged strain at the endogenous locus of *daf-16*. This strain, along with quantitative analysis of fluorescence imaging enables lifelong tracking of DAF-16 activity in response to environmental perturbations *in vivo*. Thus, the relationships between environmental perturbations, longitudinal gene activity, and lifespan can be explored. We aim to elucidate how the lifelong molecular activity of *daf-16*, driven by environmental interventions, determines lifespan in *C. elegans*.

Using a custom image-processing algorithm, we tracked DAF-16 under various dietary restriction (DR) regimes. In particular, the image processing approach enabled evaluation of complex patterns of DAF-16 nuclear migration at the tissue and cellular levels. To characterize DAF-16 activity, we developed a strain that labels the endogenous DAF-16 protein with GFP using the CRISPR/Cas9 system. We observed migration of DAF-16 to cell nuclei of nematodes under DR conditions. This pattern increased the longer the animals were under this condition, reaching a peak after 12 hours of DR and decreasing thereafter. Moreover, under repeated and intermittent exposure of the same *C. elegans* population to DR, we identified a decreasing activity of DAF-16 in subsequent days. Additionally, we have observed migration of DAF-16 to nucleoli, a phenomena not described previously, and an increased response of DAF-16 in neurons when compared to other tissues. We aim to determine how tissue specific activity of DAF-16 contributes to longevity in this nematode. Lifespan measurements were performed for animals under the same DR regimes, enabling assessing how lifelong DAF-16 activity, , measured as cumulative intensity at the cellular level, correlates with lifespan.

We characterized lifespan in *C. elegans,* while correlating this metric to quantifiable endogenous activity of DAF-16 under various DR regimes. Furthermore, our findings show that DAF-16 activity is tissue specific and its lifelong activity is correlated with longevity. These results will help understand the fundamental mechanisms by which this transcription factor regulates the aging process.

1005C Defining a functional role for splicing factors in modulating longevity in *C. elegans*

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Aging is the decline in proliferative processes and organismal metabolism that promotes deterioration of cellular and physiological function and eventually leads to mortality. Biological aging can be uncoupled from chronological age and slowed by restricting food intake without malnutrition through dietary restriction (DR) and altering nutrient-sensing pathways, such as the mechanistic target of rapamycin (mTORC) pathway. The Mair lab has identified a conserved splicing factor, SFA-1/SF1, that modulates the longevity phenotype usually attained through dietary restriction (DR) and suppressed-mTORC1 activity in *C. elegans*. However, the mechanism that links DR and mTORC1 longevity to the regulation of SFA-1/SF1 is unknown. SFA-1 is the *C. elegans* homolog of mammalian splicing factor SF1, which coordinates with other splicing factors to initiate splicing of pre-mRNA transcripts. SF1 splicing efficiency is altered by phosphorylation events at conserved residues, and we have found that altering SFA-1 by inhibiting phosphorylation events in two distinct residues abolishes the suppressed-mTORC1 longevity phenotype in *C. elegans*. In future studies, I will characterize the functional role of SFA-1 and its downstream targets and interaction partners to identify their contribution to aging. Uncovering biological processes that are regulated by SFA-1 and drive lifespan extension could define novel RNA homeostatic modulators of longevity that may contribute to age-related diseases.

1006A Tubular lysosome induction links starvation to animal longevity

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Lysosomes are digestive organelles that govern cellular metabolism and homeostasis. Despite their importance to animal health and disease, the current model of lysosome structure and function is quite simplistic; autophagic lysosomes are thought to exist mainly as discrete vesicles with uniform activity. Here, we report that vesicular lysosomes in the *C. elegans* gut transform into an extensive, dynamic, tubular network in response to animal starvation. These tubular lysosomes (TLs) are acidic, serve as preferential sites of starvation-triggered autophagy, and are stimulated via inhibition of the mTOR pathway. Consistent with our past studies in flies, we identify *spin-1*, a *C. elegans* Spinster ortholog, as a critical TL gene. Using an endogenously-tagged SPIN-1::Cherry transgenic line, we find that genetic models of caloric restriction show atypically high *spin-1* expression levels, which correlates with the constitutive presence of TLs throughout the gut. TLs also appear to contribute to the transgenerational effects of starvation; well-fed descendants of starved worms can show gut TLs for several generations. Notably, the presence of gut TLs in well-fed progeny is predictive of enhanced adult lifespan. Further, we find that experimental expression of the *Drosophila* TL stimulator SVIP in *C. elegans* is sufficient to induce gut TLs in well-fed worms and improve *C. elegans* health during aging. These findings highlight a new class of degradative lysosomes that act at the center of the cellular response to starvation, potentially coordinating its positive effects on animal health and longevity.

1007B *pqm-1/SALL2* promotes oncogenic eicosanoid metabolism following early-life starvation

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C. elegans develop tumor-like gonad abnormalities in early adulthood after extended L1 starvation followed by unlimited feeding. We determined that increased maternal oocyte provisioning of vitellogenin protects progeny from starvation-induced pathology by reducing insulin/IGF signaling (IIS) during larval development. Here we show L1 starvation and IIS interact to affect phosphatidylcholine (PC) metabolism and adult pathology. PQM-1/SALL2 activity is increased in fed worms following L1 starvation. Knock-down of the sole known IIS receptor *daf-2/InsR* activates DAF-16/FoxO and inhibits PQM-1, and *pqm-1* RNAi during larval development reduces starvation-induced pathology. PQM-1 promotes fatty acid synthetase *fasn-1/FASN* expression following L1 starvation, and mutation of *fasn-1* reduces starvation-induced pathology. Lipid profiling revealed L1 starvation increases PC abundance in early adulthood, and *daf-2* RNAi during development suppresses this increase in a *daf-16*-dependent fashion. Powerful signaling molecules called oxylipins are derived from the oxidation of polyunsaturated fatty acid tails of PC. Oxylipins derived from arachidonic acid (AA), known as eicosanoids, are best studied. *fat-4/FADS2/FADS3* mutants, which cannot desaturate fatty acids to produce AA, are resistant to starvation-induced pathology. Mutation of the omega-3 fatty acid desaturase *fat-1* increases starvation-induced pathology, and *fat-4* is epistatic to *fat-1*, suggesting elevated levels of AA or AA-containing phospholipids is oncogenic following early-life starvation. Indeed, lipid profiling revealed that PC species containing AA and other long polyunsaturated tails are particularly increased following L1 starvation. Furthermore, oxylipin profiling showed that *fat-4* mutants, which do not develop starvation-induced tumors, have reduced levels of eicosanoids. In addition, gene expression profiling suggests *fat-4* affects cell-cell adhesion and the innate immune system, suggesting consequences of eicosanoid signaling. Finally, treatment with an antioxidant during larval development suppressed starvation-induced pathology. Together, these findings provide insight into the metabolic consequences of early-life starvation, which can result in adult disease. Moreover, this study implicates IIS in regulation of PC metabolism, which plays an understudied role in regulation of critical factors governing growth and homeostasis including TOR, RAS, and PPAR.

1008C A Golgi protein MON-2/MON2 mediates longevity via upregulating autophagy

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The Golgi apparatus is a crucial organelle for post-translational modification and transport of macromolecules, including proteins and lipids. Impaired functions of the Golgi complex cause various diseases but its role in organismal longevity remains elusive. Here, we show that an evolutionarily conserved Golgi protein MON-2, a mediator of trafficking between endosome and the Golgi, promotes longevity caused by inhibition of mitochondrial respiration. By performing quantitative proteomics

followed by RNAi-based lifespan screen, we identified factors that are required for the extended lifespan of the respiration *isp-1(qm150)* and *clk-1(qm30)* mutants. Among them, we found that genetic inhibition of *mon-2* by RNAi and mutation suppressed the longevity of the respiration mutants. We showed that other factors that mediate trafficking between the Golgi and the endosome, such as *snx-3*/sorting nexin 3, *tbc-3*/TBC1D22A and PAD-1/DOP1, were also required for the longevity of respiration mutants. Next, we asked how two organelles, the Golgi and mitochondria, communicated with each other for the regulation of longevity. We hypothesized that MON-2 and established pro-longevity factors coordinately mediate the longevity of mitochondrial mutants. We demonstrated that MON-2 was required for enhancing autophagy, a process essential for long lifespan of respiratory mutants. We found that the number of LGG-1 puncta, a reporter of autophagy, was increased by *isp-1(qm150)* and *clk-1(qm30)* but decreased by *mon-2(xh22)* mutation. We further showed that MON-2 was required for the long lifespan caused by several regimens that upregulate autophagy, such as mutations in insulin/IGF-1 receptor, *daf-2(e1370)*, dietary restriction mimetic *eat-2(ad1116)* and food deprivation. We found that HLH-30/TFEB and BEC-1/Atg6, key regulators of autophagy, promoted longevity by acting together with MON-2. We then showed that mammalian MON2 also upregulated autophagy under starvation conditions by binding to GABARAP L2, a member of LC3 family proteins. Interestingly, we found that starvation induced the translocation of MON2 from the Golgi to recycling endosome, which may contribute to autophagosome formation in cultured mammalian cells. Thus, evolutionarily conserved MON-2/MON2 appears to promote longevity via upregulating autophagy. Altogether our study highlights the crucial roles of inter-organelle communications between mitochondria, the Golgi and autophagosome in the regulation of longevity.

1009A Antagonistic pleiotropy in the function of stress-activated kinase KGB-1 is mediated by *mir-71*

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The Antagonistic Pleiotropy theory for the evolution of aging proposes that a gene may have pleiotropic effects at different life stages, and these effects could be under varying, and in fact opposite, evolutionary pressures. Because the strength of natural selection declines with age, deleterious effects of genes late in life may be outweighed by early-life beneficial qualities. This would culminate in positive selection of gene variants that are beneficial early in life, for development or reproduction, in spite of their detrimental contributions late in life, i.e. to aging and to lifespan. Despite the elegance and simplicity of the theory, a mechanistic understanding of how this principle manifests is still lacking.

Here we investigate antagonistic pleiotropy as manifested in the function of the *Caenorhabditis elegans* JNK homolog KGB-1, which provides stress protection in developing larvae, but sensitizes adults to stress and shortens their lifespan. Previously we had shown that KGB-1's antagonistic contributions depended on age-dependent and opposing regulation of the stress transcription factor DAF-16 (Twumasi-Boateng et al., 2012) and that contributions were mostly cell-nonautonmous (Liu et al., 2018), but the underlying mechanism remains unknown. Here, we describe a role for the microRNA *mir-71*in mediating the effects of KGB-1 on DAF-16 and its downstream antagonistic phenotypes.

Fluorescent imaging along with genetic and survival analyses revealed age-dependent regulation of *mir-71* expression by KGB-1 - upregulation in larvae, but downregulation in adults. Furthermore, *mir-71* was required both for early life effects of KGB-1 - resistance to cadmium - as well as for KGB-1-dependent infection sensitivity late in life and shortened lifespan. In addition, KGB-1 activation in long-lived *mir-71* over-expressors reduced their lifespan to the same level as in wildtype animals, suggesting that KGB-1 gauged the effects of *mir-71*.

Not all of the effects of KGB-1 depended on *mir-71.* KGB-1 activation increases larval resistance to protein folding stress, but *mir-71* disruption had no effect on that. At the same time, disruption of the argonaute gene *alg-1*, a central component of the microRNA machinery, did. These results suggest that microRNAs play a role in mediating age-dependent antagonistic contributions of KGB-1 to survival. *mir-71* plays a central role, but additional microRNAs likely contribute redundantly.

1010B Adult longevity of late-generation Piwi/*prg-1* mutants

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The *C. elegans* Piwi Argonaute protein PRG-1 associated with piRNAs to preventing germ cells from expressing foreign genetic elements such as transposons. PRG-1 promotes germ cell immortality and lines of *prg-1* mutants are initially fertile but become sterile if they are grown for many generations. *prg-1* mutant sterility is a form of reproductive arrest (1), which may resemble reproductive arrest in response to environmental stresses such as starvation (2). We found that early-generation *prg-1* mutants have normal lifespans and fertility, while later generations had reduced fertility yet longer lifespans. Mutation

of the stress response transcription factor Daf-16/FOXO, caused the longevity phenotype of late-generation *prg-1* mutants to disappear, leading to the conclusion that *prg-1* mutant longevity may be a hormetic stress response. Phenotypes that arise from epigenetic defects can be inherited for at least 3 generations (3,4), and we found that longevity of late-generation *prg-1* mutants is inherited by F1 but not F2 cross progeny. We are currently asking if the *daf-12* germline signaling pathway that regulates aging is required of *prg-1* mutant longevity. Overall, our results imply that the longevity of late-generation *prg-1* mutant is distinct from the longevity that is transmitted for several generations by germ cells of mutants deficient for the ASH-2/SET-2/trithorax transcriptional activation complex (3,4).

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1011C Disrupting Polyunsaturated Fatty Acid Biosynthesis Modulates Lifespan and Healthspan

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Specific omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) have protective effects against aging-related conditions such as cardiovascular disease, inflammation, and neurodegenerative diseases. However, it is unclear which PUFAs are required in the diet and how they affect human health and disease. This study will investigate the physiological roles of individual PUFAs in order to pharmacologically and dietarily promote healthy aging. We will use fatty acid desaturase enzyme knockout transgenic *C. elegans* strains to assess the *in vivo* effects of PUFAs on the aging process. This investigation will create a dataset that includes lifespan and healthspan data (as determined by thrashing and/or egg laying) for every available fatty acid desaturase enzyme genetic knockout in the worm. Additionally, lipidomic or metabolic analysis will be used to assess the lipidome of key strains. We hypothesize that specific PUFAs modulate physiological processes through their corresponding downstream metabolites' role in lipid signaling. Our data showed that mutants with genetically disrupted PUFA biosynthesis displayed a decreased median lifespan, poor physical fitness, and altered egg laying patterns. Interestingly, our results from several mutants are different from published data involving the use of FuDR, a chemical that prevents progeny. It is expected that lipidomic analysis of the knockout worms will reveal that our *in vivo* observations are associated with an altered lipid panel, especially downstream metabolites. Our data suggest that genetically altering endogenous levels of PUFAs modulates lifespan and healthspan. However, specifically limiting omega-3 PUFA biosynthesis has a rescuing effect on healthspan. Investigating these metabolic pathways may elucidate novel drug targets that could revolutionize chronic disease treatment and prevention.

1012A Air pollution triggers protein misfolding in C. elegans

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Environmental degradation caused by human behavior is of great concern for human health and the health of Earth's ecosystems. Air pollution, and, especially, traffic-associated particulate matter (TRAP) is a significant health concern. Epidemiological studies have shown that exposure to TRAP triggers Alzheimer's disease (AD) symptoms. Likewise, controlled laboratory studies in which AD mice were continuously exposed to TRAP revealed an increase in amyloid beta (Aβ) plaques. Aβ plaques are composed of misfolded protein. Their increase could be a direct consequence of the disruption of the proteostasis

network or an indirect consequence of neuroinflammation. To determine whether TRAP directly impairs the folding of disease-associated proteins, we utilized *C. elegans* as a model that lacks a canonical inflammatory response. To this end, we exposed animals expressing Aβ or Huntington's disease-associated polyQ protein to nano-sized traffic-derived particulate matter. We found that TRAP triggered polyQ protein misfolding and increased Aβ toxicity. Together, our data suggest that TRAP may act, at least in part, to trigger disease by disrupting the proteostasis balance.

1013B Spaceflight effects on muscle size in *C. elegans*

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Many countries are planning on space colonization, researching space habitation and even privatizing space travel. However, if we travel to space in the future, our bodies will be exposed to space environment during long-term space flight. Previous studies have revealed that space microgravity induces muscle atrophy. To understand how microgravity affects muscle and motor neuron that traverses body wall muscle, we focused on the GABAergic motor neurons in *C. elegans*, the DD/VD neurons. We sent *C. elegans* to the ISS in space to find any changes in DD/VD neurons and muscles. The worms who were born in microgravity environment were frozen after six days from birth and parental worms who experienced both 1G and microgravity condition were frozen together, and we received these samples. While we were analyzing the neurons as well as muscle size and shape by immunostaining, we were faced with a difficulty seeing GFP of DD/VD neurons because of their weak signal. Thus, we could check only muscle size of *C. elegans* using phalloidin staining method. Preliminary results show that space microgravity-exposed worms aboard the ISS may show differences in size compared to ground-control samples. We are currently analyzing muscle size in other space samples, as well as DD/VD motor neuron development.

Keywords : space flight, microgravity, muscle size

1014C ALGN-2, asparagine-linked glycosylation protein, is critical for longevity conferred by enhanced nonsense-mediated mRNA decay

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Orchestrated protein and DNA quality control plays a major role in preventing premature aging and promoting longevity in many species. Several recent studies have shown that RNA quality control is also critical for longevity. Nonsense-mediated mRNA decay (NMD) is a biological surveillance mechanism that eliminates mRNA transcripts with premature termination codons. We previously showed that NMD in *Caenorhabditis elegans* contributes to longevity by enhancing RNA quality (Son et al., 2017, *Nat. Comm.*). Here, we aimed at identifying NMD-modulating factors that affect longevity in *C. elegans* by performing RNAi and mutagenesis screens. From a genome-wide RNAi screen using a sensitized NMD-responsive GFP reporter strain, *smg-1(tm849); sec-23p::gfp::lacZ(PTC)* (Longman et al., 2007, *Genes Dev.*), we identified several NMD-modulating factors: *algn-2*/asparagine-linked glycosylation protein, *zip-1*/bZIP transcription factor, and *C44B11.1*/FAS apoptotic inhibitory molecule. We further showed that knocking down each of *algn-2*, *zip-1*, and *C44B11.1* increased the levels of the *rpl-7A* transcript, an established endogenous NMD target. We found that two of the mutants isolated from our mutagenesis screen displayed decreased *rpl-7A* transcript levels. We further characterized *algn-2*, which exhibited an age-dependent decrease in its expression and was required for maintaining normal lifespan. We showed that knocking down *algn-2* significantly reduced longevity conferred by various genetic interventions, including *daf-2*/insulin/IGF-1 receptor mutations, dietary restriction mimetic *eat-2* mutations, and mitochondrial respiration-defective *isp-1* mutations. We further demonstrated that genetic inhibition of *daf-2*/insulin/IGF-1 receptor upregulated ALGN-2, which contributed to long lifespan in an NMD dependent manner. Thus, upregulation of ALGN-2 conferred by reduced insulin/IGF-1 signaling enhances NMD in *C. elegans*. Overall, our study identified novel modulators of NMD, including *algn-2*, which plays key roles in RNA quality control and organismal longevity. Our study will help understand how NMD-mediated mRNA quality control extends animal lifespan.

1015A An Alzheimer disease-related phenotype in *C. elegans* is exacerbated by serotonin uptake inhibitor antidepressants

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An association is seen between depression early in life and the risk for developing Alzheimer disease (AD). Population studies suggest that some of this risk for AD might be more associated with the type of antidepressant drug being used rather than the depression itself. The Selective Serotonin Reuptake Inhibitors (SSRIs), which include drugs such as fluoxetine (Prozac®), are associated with the greatest risk. The pathology of AD is thought to relate to the accumulation of the β-amyloid (Aβ) peptide which aggregates in the form of plaques in the brains of patients with AD. Preliminary studies reveal that human cell cultures overexpressing the human *APP* gene (encodes the precursor molecule that yields the Aβ peptide) and treatment with fluoxetine secrete less Aβ into the culture medium. This is the first evidence that the serotonin transporter, the target for SSRIs, might influence Aβ clearance. We took advantage of a *C. elegans* model for ectopic Aβ expression and mutants with disrupted serotonin signaling to examine *in vivo* whether fluoxetine or its major metabolite, norfluoxetine, could exacerbate phenotypes associated with accumulation of Aβ peptides. Treatment with either drug resulted in an increased paralysis compared to treatment with vehicle. Furthermore, worms that express the human β-amyloid$_{1-42}$ but that did not express MOD-5, the worm analogue of the human serotonin transporter, showed more paralysis than the backgrounds strain. In addition, a worm that expresses human Aβ$_{1-42}$ and does not express TPH-1, an enzyme required for serotonin biosynthesis, confirmed that the increased paralysis seen with SSRIs is likely not due to the altered levels of serotonin. These data have major clinical relevance as fluoxetine has been increasingly prescribed for off-label purposes, which might be exposing many individuals to an unexpected increased risk of developing AD.

1016B Cold survival driven by ferritin-mediated iron regulation

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Hibernation is employed by animals to withstand periods of low energy supply associated with cold temperatures. When adapted to cold, *C. elegans* can survive near-freezing temperatures for several days. Among factors promoting *C. elegans* cold survival is the conserved ribonuclease REGE-1/Regnase-1. The main target of REGE-1 is mRNA encoding a transcription factor, ETS-4, previously implicated in insulin signaling and the regulation of body fat. We found that the abnormal accumulation of ETS-4 is also responsible for the reduced cold resistance of *rege-1* mutants. Conversely, *ets-4* mutants survive cold much better than wild type. Through genetics and functional genomics, we found that, in the cold, the loss of ETS-4 leads to the activation of two transcription factors, DAF-16/FOXO and PQM-1. In contrast to standard cultivation temperatures, where these transcription factors play antagonistic functions, our analysis suggests that, in the cold, these transcription factors cooperate to induce transcription of specific genes. We show that one of their targets, FTN-1/ferritin, facilitates cold survival, and propose that it does so by detoxifying harmful iron species.

1017C Understanding the Molecular Basis of Aging of Sensory Neurons in *C. elegans*

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Though aging is inevitable, an average human life expectancy has substantially increased for the last 150 years, but the extension of lifespan does not necessarily equate with improved healthspan. The nervous system and neuronal cells gradually deteriorate with aging, but the molecular basis of neuronal aging, especially the aging of sensory neurons and sensory functions, remains a mystery. We employ *C. elegans* to address the following questions: (1) When does aging in the sensory neurons and sensory neurons related functions start? (2) Does aging in the neurons always occur at the same time or do different neuronal cells display distinct patterns? (3) Can we slow down neuronal aging? To this end, we generated several transgenic strains with various neuronal markers (endogenous and fosmid-based) labeled with various fluorescent tags. Then, using our fluorescence-based system, we examined whether a variety of mutant strains could reverse the neuronal aging. Our initial analysis has started to answer some of these questions. We will present the initial results in the meeting.

1018A Allele-specific effects of mitochondrial dysfunction: A *C. elegans* model of Multiple Mitochondrial Dysfunctions Syndrome 1

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Multiple mitochondrial dysfunctions syndrome 1 (MMDS1) is a neurodegenerative and pediatric fatal disease of the Iron-Sulphur cluster (ISC) biogenesis pathway. ISCs are essential cofactors of many proteins with a broad range of cellular functions. MMDS1 is caused by mutations the factor *NFU1* that is responsible for trafficking ISCs to the appropriate mitochondrial target proteins. I hypothesized that in depth analyses of patient-specific *NFU1* variations would reveal previously unappreciated roles for this factor in ISC delivery and a clearer connection between the genetics of *NFU1* variants and clinical presentations of MMDS1.

Using the nematode *Caenorhabditis* (*C.*) *elegans,* I have recreated five MMDS1 patient-specific variants of *NFU1* in its ortholog *nfu-1*. By using *C. elegans* I have been able to investigate complex metabolic and multicellular phenotypes that are highly relevant to MMDS1-individuals. Analyses of these variants includes molecular, biochemical, cellular, and physiological approaches which reveal an allelic series of phenotypic severity. There are significant decreases in mitochondrial function and extensive, albeit insufficient, compensatory changes in alternative metabolic pathways. A balance between up-regulation of glycolysis and fatty acid oxidation is regulated by the transcription factors DAF-16 and its downstream effector NHR-49. Additionally, oxidative stress caused by the apparent mishandling of ISCs appears to have a significant role in the phenotypic progression. Most notably, each of the five patient-specific alleles demonstrates unique differences in the causes of metabolic and oxidative stress due to impaired ISC mishandling. Therefore, by modeling MMDS1 in *C. elegans*, I have been able to distinguish differences between pathogenic variants in *nfu-1* which lead to complex and multicellular responses.

1019B Nucleolar size is modulated by autophagy protein LGG-1/GABARAP

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Active nuclear-cytoplasmic transport of several proteins and RNAs is facilitated by nuclear import and export receptors. Exportin 1 (XPO-1/XPO1/CRM1) is one such conserved receptor that aids nuclear export of a few mRNA and over 200 proteins including pre-ribosomal subunits, transcription factors, tumor suppressors, and oncoproteins. Previously, we had shown that genetic and pharmacological inhibition of XPO-1 conferred proteostatic benefits and enhanced lifespan in nematodes by transcriptional upregulation of autophagy via the transcription factor HLH-30/TFEB. Since XPO-1 is a major export receptor, we sought to characterize the nucleo-cytoplasmic distribution of proteins upon *xpo-1* silencing. Proteomic analysis of nuclear and non-nuclear (cytoplasmic) fractions of worms upon *xpo-1* RNAi by TMT-MS revealed repartitioning of several proteins involved in ribosome biogenesis, translation initiation, mRNA functions, and proteostasis. We also found transcriptional downregulation of ribosomal genes and a global reduction of protein synthesis by RNA-sequencing and polysome profiling, respectively. Additionally, the size of nucleoli, the hubs of rRNA synthesis inside the nucleus, and levels of nucleolar protein fibrillarin were significantly decreased, in line with recent reports on long-lived nematodes. Probing altered nucleolar dynamics further, we uncovered RPL-11.1, a ribosomal large subunit protein that has a role in nucleolar stress response, to be repartitioned to the nucleus in *xpo-1* RNAi nematodes. We found that RPL-11.1 modulates nucleolar size in an LGG-1-dependent fashion, i.e., nuclear RPL-11.1 levels and nucleolar size increase when the autophagosome protein, LGG-1/GABARAP, is silenced. Our results also indicate an interaction between RPL-11.1 and LGG-1, highlighting a novel link between two convergent hallmarks of aging, nucleolar size and proteostasis. Furthermore, silencing *rpl-11.1* in nematodes expressing Alzheimer's Disease protein, Aß, in muscles, significantly reduced paralysis suggesting proteostatic benefits of reduced RPL-11.1 levels. Altogether, our data uncover that autophagy protein LGG-1/GABARAP is a key modulator of nucleolar function and lifespan by surveilling ribosomal subunits in the nucleus.

1020C Dietary vitamin B_{12} impacts amyloid-β proteotoxicity by alleviating oxidative stress and mitochondrial dysfunction

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Alzheimer's Disease (AD), the most common cause of dementia, is a multifactorial neurodegenerative disorder characterized by accumulation of amyloid-beta (Aβ) plaques, hyperphosphorylated tau, oxidative stress, and mitochondrial dysfunction. Some AD risk factors including genetic predisposition and aging cannot be changed, but diet, as a modifiable risk factor for AD, could potentially be targeted to slow disease onset and progression. However, it is challenging to determine which individual nutrients are neuroprotective in humans due to organismal complexity, genetic diversity, consumption of complex diets, and indirect dietary effects of gut microbiota. *C. elegans* eat a simple diet of *E. coli* and have been used extensively to identify and characterize factors that influence Aβ toxicity. Transgenic expression of toxic human Aβ in *C. elegans* body wall muscles generates robust time-dependent paralysis as well as AD-like pathological features including defects in mitochondrial function and morphology. We discovered that Aβ-expressing *C. elegans* fed OP50 *E. coli* exhibited faster paralysis, lower ATP levels,

more mitochondrial fragmentation, and increased reactive oxygen species compared to those raised on HB101 *E. coli*. This was not due to differences in macronutrient content, but rather on the availability of vitamin B_{12}. We found the B_{12} supplementation alleviated mitochondrial fragmentation, bioenergetic defects, and oxidative stress, delaying Aβ-induced paralysis without affecting Aβ accumulation in animals fed OP50. Vitamin B_{12} did not have an additive effect on animals raised on HB101 indicating that supplementation was only beneficial for animals with mild B_{12} deficiency. Vitamin B_{12} is an essential cofactor for methionine synthase (METR-1) and methylmalonyl coenzyme A (CoA) mutase (MMCM-1). We discovered that the protective effects of vitamin B_{12} required METR-1, suggesting that B_{12} is functioning as an enzyme cofactor rather than as an antioxidant. Methionine supplementation also eliminated the dietary shift in paralysis, consistent with our model in which the amount of vitamin B_{12} dependent methionine synthase activity impacts Aβ proteotoxicity. Introducing vitamin B_{12} at adulthood was beneficial for B_{12} deficient Aβ animals. In conclusion, our results demonstrate the potential for vitamin B_{12} as a therapy to target pathogenic features of AD triggered by both aging and proteotoxic stress.

1021A Regulation of the hypertonic stress response by the 3' mRNA cleavage and polyadenylation complex

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Maintenance of osmotic homeostasis is one of the most aggressively defended homeostatic setpoints in physiology. Challenges to osmotic can occur under both physiological and pathophysiological conditions. Increased extracellular osmolarity (hyperosmolarity) causes a rapid decrease in cell volume leading to increased intracellular ionic strength, macromolecular damage, and cell-ECM mechanical strain. To counteract these stressors, cells activate several physiological pathways. One major pathway involves the upregulation of proteins that catalyze the accumulation of solutes called organic osmolytes. Organic osmolytes restore cell volume, reduce cell-ECM mechanical strain, and prevent macromolecular damage. However, the mechanisms by which multicellular animals detect osmotic dyshomeostasis and activate osmosensitive gene expression remain poorly understood. *C. elegans* responds to hypertonic stress by upregulating the glycerol-3-phosphate dehydrogenase enzyme (GPDH-1) to accumulate the osmolyte glycerol. To understand how the osmotic stress response is coordinated in multicellular animals, we conducted an ENU-based genetic screen with a *gpdh-1::GFP* reporter to identify mutants with no induction of osmolyte biosynthesis gene expression (Nio mutants). Through whole genome-resequencing, we discovered that *nio-3* was caused by a missense mutation in the *cpf-2* gene and *nio-7* was caused by a missense mutation in *symk-1*. Both *cpf-2* and *symk-1* are interacting components of the highly conserved 3' mRNA cleavage and polyadenylation complex. *cpf-2* and *symk-1* block the hypertonic induction of *gpdh-1* and other osmotically induced mRNAs, suggesting they act at the transcriptional level. Although null mutations in both *cpf-2* and *symk-1* are lethal, the *nio* alleles are viable under control conditions. However, under hypertonic stress conditions, *nio-3* and *nio-7* are unable to adapt and grow. Both *nio-3* and and *nio-7* suppress phenotypes known to be dependent on 3' mRNA processing and cleavage, suggesting the missense alleles identified in our Nio screen cause partial loss of function in this complex. SYMK-1::GFP and CPF-2::RFP proteins are expressed ubiquitously and co-localize within the nucleus. Interestingly, hypertonic stress causes CPF-2::RFP to rapidly reolocalize into subnuclear puncta. The cell biological properties of SYMK-1::GFP are under investigation. Our data show that *cpf-2* and *symk-1* are essential for physiological activation of the hypertonic stress response through a transcriptional mechanism. We are currently investigating how *cpf-2* and *symk-1* shape the mRNA polyadenylation landscape to control the hypertonic stress response pathway, as well as other stress response pathways, using RNAseq approaches

1022B Role of Stress Granules in Stress Responses and Ageing

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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. Much of our understanding of SG function and behaviour is derived from cell-based studies, so it is important to address their role in an animal model. Our group has shown that key pathways regulating translation are important for SG assembly and function in human cell lines and we are now determining the *in vivo*significance of this regulation in *C. elegans*. For example, we have found that the mTOR-S6 kinase pathway regulates the assembly and maintenance of SGs in both human cells and in *C. elegans* in response to heat shock. We have also found that specific translation initiation factors play key regulatory roles in SG assembly and aim to uncover the relationship between stress-induced translation inhibition, SG

dynamics and organism adaption over the lifespan of *C. elegans*. This research will provide new insights into the role SGs play in the integrated organismal response to both acute environmental insult and longer-term stress.

1023C Tissue-specific DNA repair activity of ERCC-1/XPF-1

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Hereditary DNA repair defects affect tissues differently, suggesting that *in vivo* cells respond differently to DNA damage. However, knowledge of the DNA damage response is largely based on *in vitro* and cell culture studies. A prime example of a DNA repair defect leading to pleiotropic and tissue-specific symptoms, including neurodegeneration, developmental defects, cancer, bone marrow failure and accelerated aging, is deficiency of the ERCC1/XPF complex. ERCC1/XPF is a structure specific endonuclease that is involved in several DNA repair pathways and has a critical role in nucleotide excision repair (NER). This major DDR pathway is responsible for removing bulky DNA lesions, including those formed by UV light.

Here, we use *in vivo* imaging of ERCC-1/XPF-1 in *C. elegans* to demonstrate tissue-specific NER activity. In oocytes, XPF-1 functions as part of global genome NER to ensure extremely rapid removal of DNA-helix distorting lesions throughout the genome. In contrast, in post-mitotic neurons and muscles, XPF-1 participates in NER of transcribed genes only. Strikingly, muscle cells appear more resistant to the effects of DNA damage than neurons. These results suggest a tissue-specific organization of the DNA damage response and may help to better understand pleiotropic and tissue-specific consequences of accumulating DNA damage.

1024A NGLY1 deficiency suppressors reveal connections between nucleotide metabolism, the proteasome, and longevity.

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NGLY1 deficiency is a rare autosomal recessive disorder caused by loss of function mutations in NGLY1. NGLY1 (*png-1* in *C. elegans*) encodes an enzyme that removes N-linked glycans from glycoproteins delivered to the cytoplasm by the endoplasmic reticulum associated degradation (ERAD) pathway. NGLY1 deficiency causes global developmental delay, movement disorder, peripheral neuropathy, hypotonia, abnormal liver function, and alacrima. How defective protein deglycosylation causes these symptoms is poorly understood. We recently found that PNG-1/NGLY1 regulates the proteasome via deglycosylation of the SKN-1A/Nrf1 transcription factor. In animals lacking PNG-1, SKN-1A is inactive, leading to impaired proteasome function, reduced lifespan, and increased sensitivity to proteotoxic stress. These findings raise the possibility that the symptoms of NGLY1 deficiency are caused by a failure of proteasome regulation. To uncover new mechanisms of proteasome regulation and identify targets for therapeutic activation of the proteasome in NGLY1 deficiency, we isolated genetic suppressors of *png-1* mutants' proteasome dysfunction. The suppressor mutations do not reverse the defect in proteasome subunit gene expression caused by loss of PNG-1. Instead, the suppressors appear to cause enhanced proteasome function via distinct mechanism(s) that are SKN-1A/Nrf1-independent. The suppressor mutations also have positive effects on proteostasis and lifespan in the wild type, suggesting these benefits are not limited to situations in which SKN-1A/Nrf1 is inactive. The suppressor mutations include loss of function alleles of *tald-1* and *ent-4*. *tald-1* encodes transaldolase, an enzyme in the pentose phosphate pathway, which generates ribose for *de novo* nucleotide biosynthesis. *ent-4* encodes a putative member of the SLC29 family of nucleoside transporters. ENT-4 is localized to the apical surface of intestinal cells and may mediate uptake of dietary nucleosides. In support of a connection between nucleotide availability and the proteasome, mutants defective in nucleotide biosynthesis display enhanced proteasome function, whereas a nucleotide-rich diet can antagonize degradation of a model proteasome substrate. Our findings suggest that changes in nucleotide availability can profoundly alter proteasome function and proteostasis. Understanding the mechanistic basis for this connection may lead to new therapies for NGLY1 deficiency and diseases associated with impaired protein degradation.

1025B Increased DNA content in the *C. elegans* intestine promotes body size and lifespan

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Polyploidy, the condition in which cells obtain additional sets of chromosomes, is a normal feature of cells in many diploid organisms, yet little is known about why an increase in ploidy may be advantageous. Polyploidy can protect liver cells from becoming cancerous after the loss of a tumor suppressor gene, but this cannot be the only purpose as many cell types become polyploid through post-mitotic DNA replication. A second hypothesis is that the increased DNA content in polyploid

cells supports greater cell size. For example, the growth of the nematode *C. elegans* is driven primarily through cell growth as opposed to cell proliferation, and its overall body size is thought to be regulated by the nuclear ploidy of its syncytial epidermis. The *C. elegans* intestine is also composed of large, polyploid cells, but it is unknown whether any physiological benefits result from this increased DNA content. Using a tissue- and temporally-specific protein degradation system, we degraded a *C. elegans* G1/S cyclin dependent kinase, CDK-2, to reduce the ploidy of the intestine and the epidermis. Preliminary results show that body size is not reduced upon reduction of epidermal polyploidy. In contrast, ploidy reduction in the intestine leads to slow growth, small body size, and reduced lifespan. Finally, low ploidy intestines may not have wild-type capacity for nutrient absorption, as these worms show signs of starvation even with abundantly available food. These results implicate polyploidy as essential for the function of the intestine and challenge existing models of *C. elegans* growth regulation. Future studies will investigate whether polyploidy is involved in other intestinal processes such as yolk production and innate immunity.

1026C Food additives target the gut-neural axis: Impaired peptide trafficking and amyloid protein aggregation lead to premature aging phenotypes

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Nanoparticles like nano silica (E551) are commonly used as food additives although bio-interactions with the gut are not well understood. Nutrients, like oligopeptides, together with nano silica are taken up via the same route via the pharynx to the intestinal tract. At the apical domain of intestinal cells, oligopeptides are transferred by the OPT-2/PEP-2 transporter to the cytoplasm and degraded downstream by hydrolases to provide amino acids for protein biosynthesis.

For analyses of the peptide transport, the fluorophore-conjugated dipeptide ß-Ala-Lys (AMCA) was used which normally distributes diffusely throughout the cytoplasm. Exposure with nano silica lead to an altered morphology of the intestine and disturbed peptide trafficking. Dipeptides accumulated in spherical subdomains that grow over time to a diameter of ≥6 µm. The peptide subdomains did not colocalize with known organelles such as gut granules and formed independently of the insulin/IGF-1 receptor (DAF-2) signaling pathway. Mutation of the daf-2 tyrosine kinase domain showed distinct patterns of peptide subdomains along the apical membrane of intestinal cells which indicated specific trafficking defects (Piechulek et al., 2019). We suggest that nano silica-induced peptide subdomains represent new compartments of dipeptide storage leading to the inhibition of hydrolysis and peptide metabolism which in turn results in a decline of translation. Aberrant segregation of dipeptides by phase separation leads to a petite phenotype resembling OPT-2/PEP-2 transporter deficient mutants.

In addition, nano silica induce global amyloid protein aggregation that segregates components of the protein homeostasis to an insoluble aggregome. Candidate proteins include components of the translational machinery and ribosomal RNA processing (Scharf et al., 2016). Taken together, the results suggest that impairment of translation occurred by a lack of amino acid supply and amyloid segregation of proteins critically involved in protein synthesis. As translation was shown to be involved in the generation of both, premature aging and neurodegenerative diseases, further investigation of its role in the organ-cross talk between intestine and the neural system in xenobiotic-exposed *C. elegans* is required.

1027A *De novo* serine biosynthesis couples mitochondria to longevity

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Sustained mitochondrial fitness is intimately linked to healthy aging and longevity while it relies on coordinated mitochondrial biogenesis, maintenance, and clearance. These processes are fine-tuned by the constant targeting of proteins into the organelle. Mitochondrial protein import is a complex procedure mediated by conserved protein translocases in the outer and inner mitochondrial membranes. We are investigating how modulation of mitochondrial protein import translocases affects mitochondrial abundance, morphology, and function, ultimately impacting organismal physiology. We find that reduction in cellular mitochondrial load through mitochondrial protein import system suppression, referred to as MitoMISS, triggers a discrete longevity paradigm. MitoMISS longevity depends on ATFS-1, the transcription factor driving mitochondrial unfolded

protein response. However, mitochondrial chaperones are not causatively linked to longevity. Mechanistically, we show that MitoMISS initiates an ATFS-1-dependent unconventional UPR^{mt}, leading to metabolic rewiring. Metabolic profiling of MitoMISS animals points to an adaptive metabolic response, encompassing fat mobilization, glycolysis and *de novo* serine biosynthesis. Genetic epistasis indicates that both glycolysis and *de novo* serine biosynthesis are prerequisites for MitoMISS associated longevity. Our findings extent the pro-longevity role of UPR^{mt} and reveal a homeostatic mechanism that engages ATFS-1 to coordinate an adaptive metabolic shift that drives lifespan extension.

1028B Meiotic mutations impact lifespan and healthspan in *C. elegans*

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Meiotic chromosomal defects increase with age and are a major cause of miscarriages and age-related fertility loss in women. The link between maternal age and fertility has been well studied, however it is unknown if, and, how germline integrity impacts the rate of aging of the whole organism. We are utilizing the nematode model *Caenorhabditis elegans* to address the causative role of germline health on somatic aging. Specifically, we have examined genes that govern meiotic fidelity in the germline and addressed their role in somatic aging. *C. elegans* is uniquely suited to address this question as the somatic cells in the adult organism are post mitotic, allowing us to selectively disrupt germline integrity through meiotic gene mutations. We found that 14 of the 38 mutations we examined, in genes that govern different steps of meiosis, significantly reduced the lifespan of the animal. We also found that germline-specific RNAi knockdown of selected meiotic gene candidates, *spo-11, dsb-2,* and *htp-3,* also reduced lifespan. These genes have roles throughout meiosis including double strand break formation during the process of meiotic recombination (*spo-11* and *dsb-2)* and the formation of the synaptonemal complex (*htp-3)*. We also examined how meiotic gene disruption impacts the rate of aging by measuring healthpsan parameters. We found mutations in all of the selected candidate meiotic genes, exhibited deficits in at least one, and often more than one, healthspan feature, including loss of mobility, diminished pharyngeal muscle pumping, tissue integrity and neurological function. Overall, our data demonstrate that genes that govern meiotic fidelity in the *C. elegans* germline impact the physiological health of the somatic tissues and aging of the whole organism.

1029C A *C. elegans* model to study the molecular pathogenesis of Cockayne Syndrome progeria

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Cockayne Syndrome (CS) is a rare congenital disease that causes photosensitivity, growth and mental retardation, impaired nervous system development and premature ageing. The syndrome displays a complex array of symptoms that are caused by mutations in the CSB, or CSA gene, respectively, which facilitate DNA damage recognition in transcription-coupled nucleotide excision repair (TC-NER). While CS mouse models are photosensitive, and have been important for understanding some of the clinical features observed in humans, they lack concrete evidence for neurodegeneration. Here, we present evidence of neuronal and mitochondrial aberrations in *csb-1(ok2335)* mutant worms, which can be rescued by transgenic expression of p_{csb-1}CSB-1::GFP. Neurodegeneration manifests progressively and is paralleled by neuro-muscular functional decline substantiated by reduced pharyngeal pumping, locomotion, touch sensation and chemosensation abilities, which are further enhanced upon UVB-induced DNA damage or transcription-blocking lesions caused by the cytotoxin Illudin M. The *csb-1* mutant shows the accumulation of dysfunctional mitochondria, and increased hyperfusion, which is exacerbated upon exposure to UVB, resulting in reduced respiratory activity. Our data support the causal role of endogenous DNA damage for neurodegeneration and mitochondrial dysfunction in CS, and warrants the use of the model for identifying pharmacological interventions to improve CS and ageing.

1030A A super-long lived mutant and a multi-omics discovery platform for new regulators of lifespan.

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The Insulin like signaling (ILS) pathway and the peroxisomal beta oxidation (PBO) are highly conserved central 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 modifications but our and other laboratories work showed that small molecular products of *C. elegans* PBO can trigger pro-health and anti-ageing effects (ascarosides #2, #3, #8, ncas#3 (2, unpublished)).

Crossing *daf-22(ok693)* worms lacking the CoA C-acetyltransferase DAF-22 and *daf-2(e1370)* mutants in which the function of the insulin receptor tyrosine kinase domain is compromised, we generated a *d2d22* double mutant, which exhibits an extremely high long lifespan, almost double the lifespan of the already long-lived *daf-2(e1370)* worms. *d2d22* double mutants further exhibit slow development, an extended fertility period, reduced number of progeny, and formation of large triglyceride containing droplets during adulthood.

Comparative metabolomic and transcriptomic analyses of wild type, *daf-2*, *daf-22* and *d2d22* mutants at different ages reveal small molecules and transcripts with starkly altered abundances in the long-lived strains. Accompanying proteomic analysis will provide further insight into the biosynthesis and regulatory aspects of those compounds. Selected compounds and mutants are tested in bioassays for developmental pace and lifespan. Integration of the all-omics data will facilitate the creation of a *C. elegans* pathway map of age-related small molecules.

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1031B Axin-Mediated Regulation of Lifespan and Muscle Health in *C. elegans* Requires AMPK-FOXO Signaling

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Aging is a significant risk factor for several diseases. Studies have uncovered multiple signaling pathways that modulate aging, including the insulin/insulin-like growth factor-1 signaling, and dietary restriction. In *C. elegans*, lifespan extension of *daf-2/InR* mutants depends on the transcription factor DAF-16/FOXO and the catalytic subunit homolog of the energy sensor AMPK, AAK-2. DAF-16 and AAK-2 also mediate low diet and mitohormesis induced longer lifespan of animals. One of the kinases that affect DAF-16 function is AAK-2.

We have found that the Axin scaffolding protein homolog PRY-1 plays an essential role in aging and muscle health maintenance. Our genetic experiments show that *pry-1* interacts with both *aak-2* and *daf-16* to regulate these two processes. Earlier, a genome-wide transcriptome profiling of *pry-1* mutants revealed differentially expressed genes, many of which are associated with aging and muscle function. Consistent with this, PRY-1 is strongly expressed in muscles, and muscle-specific overexpression of PRY-1 extends life span, delays muscle aging, and improves mitochondrial morphology in an AAK-2-DAF-16-dependent manner. Additionally, our data shows that PRY-1 is necessary for AAK-2 phosphorylation. Taken together, these findings demonstrate that PRY-1 functions cell-autonomously in muscles to promote the life span of animals. Current and future studies are aimed at further investigating the mechanism of PRY-1 function in regulating muscle health and aging.

Keywords:

PRY-1, Axin, Aging, Muscle, AMPK, AAK-2, DAF-16, FOXO, *C. elegans*

1032C Neuroprotective effects of rutin on ASH neurons in *Caenorhabditis elegans* model of Huntington's disease

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Huntington's disease (HD) is an autosomal dominant, progressive neurodegenerative disease. It occurs due to a mutation in the huntingtin gene with an abnormal CAG repeat, leading to a variable length N-terminal polyglutamine chain (poly-Q) which confers toxic functions to mutant Htt leading to neurodegeneration. Rutin is a flavonoid found in plants, buckwheat, some teas and also in apples. Although our previous studies have already indicated that rutin has protective effects in HD's models, more studies are needed to unravel its effects on protein homeostasis and the underlying mechanisms. In our study, we investigated the effects of chronic treatment with rutin in *Caenorhabditis elegans* model of HD focusing on ASH neurons

and antioxidant defense. The synchronized L1 worms were placed on rutin-NGM plates and kept at 20°C. Rutin was added every 24 hours at concentrations of 15, 30, 60 and 120 µM. We assessed octanol response, neuronal polyQ aggregates and dye filling assay. In addition, we analyzed the downstream heat-shock protein-16.2 (HSP-16.2) and superoxide dismutase-3 (SOD-3). Overall, our data demonstrate that chronic rutin treatment maintains the function of ASH neurons in addition to decrease the degeneration of their sensory terminations. The mechanism proposed is antioxidant activity, through the overexpression of antioxidant enzymes and chaperones regulating proteostasis. Our findings provide new evidences about rutin playing a neuroprotective role in *C elegans* model. In addition to information for treatment strategies for neurodegenerative diseases and other diseases caused by age-related protein aggregation.

1033A MicroRNA cluster 229-66 promotes longevity through interaction with SKN-1 and DAF-16 in *C. elegans*

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Recently, several microRNAs have emerged as potential contributors towards pathways that regulate aging. We have previously shown that miR-228 is required for dietary restriction mediated longevity through interaction with PHA-4 and SKN-1 transcription factors. *mir-229,64,65,66* is a cluster of microRNAs that are in the same family as *mir-228* and share the same human homologs. They are present immediately adjacent to each other on the same chromosome and are thought to share the same promoter and identical seed sequences. Interestingly, we found that in contrast to the anti-longevity role of miR-228, miR-229-66 cluster is required for *C. elegans* lifespan and longevity observed in *mir-228* mutants. Dietary restriction and reduced insulin signaling up-regulates the expression of miR-229-66 cluster which in turn is critical for the complete extension of lifespan observed under these longevity perturbations. We also show that expression of transcription factors SKN-1 and DAF-16, which are known for their vital role in DR and low IIS mediated longevity, is positively regulated by miR-229-66 cluster. Conversely, these TFs also positively regulate expression of this miRNA cluster, indicating a positive feedback loop essential for longevity. Infact, we observed that miR-229-66 is a requisite for lifespan extension conferred by SKN-1 over expression. Given the conservation of these miRNAs and TFs across species, these interactions are likely to be conserved for longevity in more complex organisms as well.

1034B High Throughput Exopher Whole Genome RNAi Screening with Machine Vision and Machine Learning Approaches

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It has recently come to be appreciated that neurodegenerative disease proteins/aggregates can be found outside of mammalian neurons, and when outside can actually be taken up by neighboring cells. Transfer of offending molecules has been suggested to be a mechanism of pathogenesis spread for multiple neurodegenerative diseases, including the prevalent Alzheimer's and Parkinson diseases. We discovered a novel capacity of young adult C. elegans neurons to extrude substantial membrane-bound packages of cellular contents via exohers, which can include aggregated human neurodegenerative disease proteins, mitochondria, or lysosomes, but no nuclear DNA.

We speculate that the mechanism of exopher formation in C. elegans is analogous to that used in the transfer of aggregated proteins in human neurodegenerative disease. If so, it will be absolutely critical for us to identify genes contributing to the recognition/sorting of cellular trash and to the expulsion of this material. Although we have identified some exopher mechanism players in candidate gene RNAi screens, measurement rates are slow and important players are likely hard to predict, necessitating an unbiased candidate approach and faster methods in order to elucidate a genetic mechanism.

To accomplish genome-wide screens for exopher-genesis modifier genes, we have developed a highly automated, high-throughput whole-genome RNAi screening platform that can be implemented at a fraction of the time and cost of manual screens. Our screening protocol employs robotic dispensers and aspirators, coupled with a high content imaging system for animals grown and measured in a 96-well plate format that mimics a standard solid media plate environment. We are developing several machine vision approaches to allow for automated scoring of animals with an exopher to expedite the analysis. Our screen protocol allows for an entire genome to be screened in about two weeks, fast enough to allow for replicated screens and epistasis analysis of hits. We have developed approaches to store a 3D digital library and physical library of prepared samples for later re-imaging at higher resolution and re-analysis. We will present our current genetic approaches to establish a high exopher baseline, and computational approaches to detect exopher events in a crowded well.

1035C Role of Coelomocytes and Immunity in Axenic Dietary Restriction

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Axenic dietary restriction (ADR) is the most potent form of dietary restriction in *C. elegans* known to date. Simply removing bacteria and growing worms in a sterile (semi-)defined culture medium results in more than doubling of lifespan, despite an abundance of calories and nutrients. The processes underlying this lifespan extension have remained enigmatic, as the mechanism seems distinct from other forms of DR and does not rely on known longevity pathways. One of the few molecular players that has been linked to ADR is CUP-4, a putative ligand-gated ion channel which when absent significantly reduces the longevity effect of ADR. CUP-4 is expressed solely in the coelomocytes, endocytic cells that have been suggested to serve scavenging, immune or hepatic functions. We wish to understand the underlying mechanisms of ADR longevity, and how the coelomocytes fit into this picture.

We performed lifespan assays to determine the effect of coelomocyte ablation or disruption of endocytosis upon longevity under ADR. After optimizing a cell-specific RNA-sequencing set-up for adult *C. elegans* coelomocytes using fluorescence-activated cell sorting (FACS), we applied this pipeline to differentially analyze bulk coelomocyte transcriptomes under different dietary conditions and endocytic capacities. Our results revealed many differentially regulated immune genes, prompting us to investigate the potential involvement of diverse immune pathways (within the coelomocytes) in axenic dietary restriction. We hope that this will provide new insights into the lifespan-extending mechanisms of ADR, that can potentially be extrapolated to teach us more about the ageing processes at play in animals in general.

1036A Lipid droplets modulate lifespan and selective autophagy receptor p62/SQST-1 dynamics

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Organismal health and longevity depend largely on the maintenance of proteome stability, and age-associated proteostatic decline contributes to protein aggregation-induced pathologies found in many neurodegenerative diseases. Proteostasis is maintained via cellular mechanisms including the autophagy and the proteosome degradation machineries. A key regulator of the autophagic degradation of specific organelle and aggregating proteins that are prone to damage and ubiquitination is the selective autophagy receptor p62/SQST-1/SQSTM1. We tested whether increasing p62 levels (p62 OE) would improve proteostasis and consequently increase the lifespan of *C. elegans*. Unexpectedly, we found that simply overexpressing p62 is not sufficient to extend lifespan in *C. elegans* at the standard growth temperature of 20C. Instead, we found that p62 OE is detrimental to the lifespan of wild-type (WT) animals under modest heat stress at 25C, which, similar to aging, caused abnormal accumulation of p62 aggregates. To systematically identify modulators of p62 levels under these conditions, we employed p62 OE strains with fluorescent reporters in an unbiased genome-wide RNAi screen and identified several p62 modulators that coded for proteins associated with lipid droplets and those prone to aggregate with age.

A role for lipid droplets (LDs) in lifespan extension has emerged, as several long-lived *C. elegans* strains, including Insulin/IGF-1 receptor *daf-2* mutants, maintain elevated intestinal lipid stores throughout life, but a mechanistic understanding of the LD role in longevity is still lacking. We demonstrate that the detrimental effects of unprocessed p62 is rescued in *daf-2* mutants, underscoring the need for elevated lipid storage for proper p62 dynamics. To further support this point, we show that expansion of the intestinal LDs by silencing the cytosolic triacylglycerol lipase gene *atgl-1*/ATGL was sufficient to extend lifespan in WT animals and in comparatively short-lived animals exhibiting impaired proteostasis or p62 accumulation, as well as mitigate the age-related p62 accumulation and reduced overall ubiquitination of proteins. Conversely, depleting LDs accelerated the age-dependent accumulation of p62 and decreased lifespan at 25C. Altogether, our study supports the notion that LDs serve as a buffer for proteostasis under proteostatic strain, which reduces protein ubiquitination and ultimately unburdens p62-selective autophagy and promotes longevity in *C. elegans.*

1037B A High-Glucose Diet Reduces Male Fertility and Sperm Quality in *C. elegans*

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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 or *fog-2* females. 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 dervied from male sperm, suggesting a reduction in sperm quality. We then measured sperm size, which is known to correlate with sperm competitiveness, and found that male sperm size is significantly decreased on a high-glucose diet. We also find a decrease in male spermatid production on a high-glucose diet. However, a high-glucose diet had no effect on spermatid transfer during mating or in mating behavior.

We are currently testing other known indicators of sperm quality to determine whether glucose affects other facets of male fertility, and whether the fertility defects we observe are separabale phenotypes. 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.

1038C GLA-3/TTP plays an important role in the germline stress response of *Caenorhabditis elegans*

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GLA-3 is the *C. elegans* homolog of the mammalian tristetraprolin (TTP) which encodes a protein that contains two CCCH-like zinc-finger domains that function either as transcription factors or RNA-binding proteins. This gene which encodes two splice variants GLA-3A and GLA3B and the protein is expressed in both germline and soma.

Loss of *gla-3* function present different alterations: protein degradation in muscle leading to a progressive loss of motility, increased germ cell death by apoptosis, severe defects in meiosis progression, reduced brood size and a low frequency of embryonic lethality.

Stress granules (SG) are dynamic cytoplasmic membrane-less organelles that are formed under harsh conditions. SG are composed of mRNAs that are stalled in translation pre-initiation complexes and different types of mRNA binding proteins like TIA-1 and TTP.

It has been probed by immunoprecipitation that GLA-3A-B associates with the MAP kinase protein MPK-1/ERK, which is required for pachytene progression during oogenesis, but this association is poorly understood. Additionally, in mammals was observed that TTP is phosphorilated by ERK and this modification affects SG formation, however this interaction has not been studied in *C. elegans*.

Since TTP has been shown to be associated with SG, the aim of this work is to study the function of GLA-3 and to identify whether this protein forms SG under different adverse conditions and the role of MPK-1 / ERK in SG formation.

We tested different stress conditions: heat shock (31°C for 3 hours), starvation (6 hours without bacteria) and oxidative stress (1 hour in Paraquat 0.2 mM) and evaluated SG formation using the strain tn1734 which had the protein GFP fused whit GLA-3A ([gfp::3xflag::gla-3a]).

It was proved that GLA-3 express in germ line from larval stage L2 to adulthood, and in adult gonads, GFP::GLA-3A form SG under heat shock, starvation and oxidative stress. We also observed that iRNA knock-down by feeding animals with dsRNA for *mpk-1* prevent the formation of GLA-3 SG.

We conclude that GLA-3 is a SG component and MPK-1 participate in SG assembly.

1039A KLF Transcription Factors Regulate SKN-1 Activity in *C. elegans*

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SKN-1/Nrf (NF-E2 related factor) is a transcription factor that regulates redox regulators, such as glutathione S-transferase, and lipid metabolism in *Caenorhabditis elegans* (*C. elegans*). SKN-1 counters damage caused by reactive oxygen species, which is often implicated in age-associated diseases such as Alzheimer's disease, and arteriolosclerosis. Recently, SKN-1 was shown to mediate fat accumulation and oxidative stress resistance in worms missing germline stem cells (GSC(-)). The mechanism by which this occurs is still unknown. A preliminary genome-wide screen identified Kruppel-like family of transcription factors (KLF) as possible mediators of SKN-1, under GSC(-) conditions. KLF proteins have roles in adipogenesis and autophagy, suggesting they may mediate SKN-1 regulated fat accumulation and oxidative stress resistance in GSC(-) worms. To evaluate the role of KLF transcription factors, we assessed the role of *klf-1* and *klf-2* in SKN-1 activation, SKN-1 mediated stress resistance, lipid metabolism, and longevity. We found that the knockdown of *klf-1,* under GSC (-) conditions, decreased SKN-1 activity, specifically the SKN-1c isoform, eliminated stress resistance, reduced lipid accumulation, and eliminated lifespan extension. *klf-2* had less of an effect on SKN-1 however the knockdown of *klf-2* surprisingly increased lipid accumulation under both basal and GSC(-) conditions suggesting that while *klf-1* positively regulates SKN-1c, *klf-2* may negatively regulate SKN-1c, possibly through lipids.

1040B The transcriptional signature of long vs. short life is distinct from that of chronological age

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What determines an individual's lifespan? The intuitive answers of "genetics" or "environment" do not tell the full story—even isogenic populations of *C. elegans* reared in a controlled environment show the same degree of inter-individual variation in lifespan as that seen in outbred human populations. One hypothesis is that individuals are driven toward different fates by stochastic differences in the expression of key regulatory genes. We recently showed that the expression levels of 10 out of the 22 microRNA promoter::GFP constructs we examined were sufficient to predict an individual's future lifespan. At least two of these GFPs predict lifespan independent of the activity of *daf-16*, and at least three report on lifespan redundantly despite being expressed in distinct tissues. We wondered whether these results might be evidence of a global gene expression state that determines (or is determined by) an individual's remaining lifespan. To address this, we set out to understand the global transcriptional states underlying high vs. low expression of these lifespan-predictive GFPs. We performed RNA-seq on populations sorted by the GFP intensity of several of these predictive markers, as well as intestinal autofluorescence, a phenomenological biomarker of aging. We found that, regardless of biomarker, the differentially expressed genes between prospectively long- vs. short-lived populations were extremely similar. However, we also noted that these differences overlapped significantly with genes that change over time during chronological aging. We reasoned that this was due to the "apparent age" of the worms; that is, worms predicted to be long-lived had gene expression reflective of chronologically younger animals, while worms predicted to be short-lived showed premature genetic hallmarks of aging. We then asked whether this accelerated or retarded "apparent age" was the only difference between prospectively long- vs. short-lived populations. By first aligning each population to a high-resolution transcriptional time course, we account for and remove the effects of "apparent age," revealing a signature that specifically reflects long vs. short future lifespan. Surprisingly, we found that, for all the predictive markers we tested, the expression of germline-related genes is highly related to future lifespan, even after controlling for the "apparent age" of the transcriptome.

1041C Glucose-induced developmental delay is modulated by insulin signaling in *C. elegans*

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A significant amount of research has shown that type 1 diabetes in children causes a delay in puberty. However, it is unclear how hyperglycemia contributes to this delay in puberty. Hyperglycemia, which is associated with diabetes, refers to elevated blood glucose levels. To model hyperglycemia at a molecular level, *C. elegans* are fed a glucose-supplemented diet and the physiological and molecular impacts are studied. We found that a glucose-supplemented diet induces a developmental delay in comparison to the control food (OP50 *E. coli* diet). The glucose induced developmental delay was also observed in animals fed the ΔPTS *E. coli* OP50 bacteria strain, which lacks a glucose transporter, indicating the phenotype is not due to metabolites produced by OP50. Developmental delay can be induced by environmental stress or genetic mutations. For example, mutations that impact ceramide biosynthesis, electron transport chain activity, ubiquinone biosynthesis, or insulin

signaling (*hyl-2(tm2031)*, *isp-1(qm150), clk-1(e2519)* and *daf-2(e1370*), respectively) lead to developmental delay. We tested if a glucose diet prolonged developmental progression in these mutants. A glucose-supplemented diet further exacerbates the developmental delay of the *hyl-2(tm2031), isp-1(qm150)* and *clk-1(e2519)* mutants but did not induce a developmental delay in the *daf-2(e1370)* animal. The resistance to glucose-induced developmental delay observed in the *daf-2(e1370)* animal is suppressed by *daf-16(mu86)*. To further understand how glucose impacts insulin signaling and developmental progression we are using RNA sequencing and genetic analysis to identify the DAF-16 regulated genes that have a role in developmental progression. This could provide insight into how hyperglycemia and altered insulin signaling is linked to delayed puberty in children with type 1 diabetes.

1042A Modulation of small RNA pathways suppresses innate immunity

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Procreation is widely known to cause immunosuppression yet the mechanisms by which immunity is suppressed during peak fertility remain unknown. *tcer-1*, which encodes the *Caenorhabditis elegans* homolog of the human transcription elongation and splicing factor, TCERG1, is once such gene involved in coordinating the immunity-fertility relationship. Our lab showed that TCER-1 is essential for healthy reproduction and prevention of age-related reproductive decline, and that it enhances lifespan in response to reproductive signals by ensuring lipid homeostasis. Our preliminary experiments have suggested that TCER-1 suppresses immunity, in part, through regulation of endogenous small RNA (smRNA) pathways. smRNA are short (~18-30nt) RNA molecules known for their ability to silence self and foreign genetic material to regulate host gene expression. Despite the fact endogenous smRNAs are reported to regulate immune-defense genes and microbes target host smRNA pathways during infection, little is known about the molecular mechanisms involved. We have discovered that loss-of-function mutations in biogenesis genes for endogenous small interfering RNAs (siRNAs) induce exceptional resistance against the human opportunistic pathogen *Pseudomonas aeruginosa*. Our epistasis analyses have demonstrated that these genes act in the same genetic pathway as TCER-1 to repress immunity. smRNA sequencing has also shown that *tcer-1* mutation causes a ~10% reduction in total endogenous siRNAs. These observations, and other data, have led us to hypothesize that TCER-1 suppresses innate immunity through modulation of the endogenous siRNA pathway and their targeted immunoresistance genes.

1043B *Lactobacilli* in a clade prevent age-dependent decline of thermotaxis behavior in *Caenorhabditis elegans*

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Diet is proposed to affect brain aging. However, the causality and mechanism of dietary effects on brain aging are still unclear because the long time scales of aging hinder in-depth research. The nematode *Caenorhabditis elegans* (*C. elegans*) has led aging research because of its short lifespan and ease of genetic manipulation. *C. elegans* fed the standard laboratory diet, *Escherichia coli* (*E. coli*), experiences an age-dependent decline in temperature-food associative learning, called thermotaxis. To address if diet affects this decline, we screened 35 different lactic acid bacteria as alternative diets. While *Lactococcus, Streptococcus, Leuconostoc, Bifidobacterium*, and homofermentative *Lactobacillus* species did not affect the age-dependent decline in thermotaxis, *Lactobacilli* in a clade enriched with heterofermentative bacteria prevented it. Among these heterofermentative *Lactobacilli*, *Lactobacillus reuteri* did not alter the lifespan or locomotion. We found that DAF-16, a FOXO transcription factor, acts in neurons to regulate the effect of *Lactobacillus reuteri*. Our results demonstrate that diet can impact brain aging without affecting the lifespan and that bacterial screen using *C. elegans* is a powerful approach to investigate age-dependent behavioral decline.

1044C H3K4me3 modifiers regulate amyloid toxicity in *C. elegans*

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Neurodegenerative diseases such as Alzheimer's disease (AD) and Huntington's disease (HD) are characterized by the pathological deposition of amyloidogenic proteins which results in neurotoxicity and neurodegeneration. Aging is the greatest risk factor for these diseases, and as a result, interventions that delay aging also slow the progression of these age-associated diseases. As the precipitating events in AD and HD are thought to begin well before the onset of clinical

symptoms, identifying processes early in life may be key to significantly alter the trajectory of these diseases. Our lab has recently discovered one such early-life event: naturally-occurring fluctuations in levels of reactive oxygen species (ROS) that are predictive of lifespan and stress resistance. These effects, identified in *C. elegans*, were found to be mediated by redox-dependent changes to the levels of trimethylated H3K4 (H3K4me3), an epigenetic mark associated with gene activation. These data demonstrate that transient changes in the levels of H3K4me3 during development can exert effects on organismal health and longevity that persist into adulthood. On this basis, we hypothesize that changes to H3K4me3 will also influence susceptibility to amyloid toxicity seen in AD and HD. In this study, we find that reduction of H3K4me3 levels by knockdown of *set-2* and *ash-2* (components of the H3K4me3 complex) in a *C. elegans* model strain of AD expressing amyloid-β (Aβ) reduced Aβ-induced paralysis. A similar delay in paralysis was observed following transient exposure of Aβ-expressing animals to mild concentrations of the ROS-generator paraquat during development. Importantly, these protective effects of developmental ROS are lost in animals that lack *set-2*. In addition to models of AD, *C. elegans* strains expressing amyloidogenic polyglutamine repeats (Q40) characteristic of HD were also protected from paralysis by *ash-2* or *set-2* knockdown, indicating that disruption of H3K4me3 levels can protect against proteotoxicity from multiple types of amyloidogenic proteins. Despite having a resistance to paralysis, polyglutamine-expressing animals with lower levels of H3K4me3 appear to have an increase in aggregation of the Q40 protein in both young and aged animals. We hypothesize that disruption of H3K4me3 may heighten the capacity of these animals to mount transcriptional responses to proteotoxic stress, and we are currently investigating the mechanisms that may confer this stress resistance.

1045A Metformin Treatment of Diverse *Caenorhabditis* Species Reveals the Importance of Genetic Background in Longevity and Healthspan Extension Outcomes

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Metformin, the most commonly prescribed anti-diabetes medication, has multiple reported health benefits, including lowering the risks of cardiovascular disease and cancer, improving cognitive function with age, extending survival in diabetic patients, and, in several animal models, promoting youthful physiology and lifespan. Due to its longevity and health effects, metformin is now the focus of the first proposed clinical trial of an anti-aging drug - the Targeting Aging with Metformin (TAME) program. Genetic variation will likely influence outcomes when studying metformin health effects in human populations. To test for metformin impact in diverse genetic backgrounds, we measured lifespan and healthspan effects of metformin treatment in three *Caenorhabditis* species representing genetic variability greater than that between mice and humans. We show that metformin increases median survival in three *C. elegans* strains, but not in *C. briggsae* and *C. tropicalis*strains. In *C. briggsae*, metformin either has no impact on survival or decreases lifespan. In *C. tropicalis*, metformin decreases median survival in a dose-dependent manner. We show that metformin prolongs the period of youthful vigor in all *C. elegans* strains and in two *C. briggsae* strains, but that metformin has a negative impact on the locomotion of *C. tropicalis* strains. Our data demonstrate that metformin can be a robust promoter of healthy aging across different genetic backgrounds, but that genetic variation can determine whether metformin has positive, neutral, or negative lifespan/healthspan impact. These results underscore the importance of tailoring treatment to individuals when testing for metformin health benefits in diverse human populations.

1046B Dietary restriction promotes healthspan via a glucagon-like signaling pathway in *C. elegans*

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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, 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. 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. Similar to candidate glucagon receptor *pdfr-1*, we found that a *C. elegans* adenylate cyclase ortholog, *acy-1*, is required for increased lifespan under DR. 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.

1047C Compensation and Epistasis in the role of RNA polymerase II in *C.elegans* aging

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Aging is a biological phenomenon that involves a gradual decline in physiologic homeostasis. As an essential molecular mechanism, transcription is clearly crucial for this homeostasis and yet the specific causal pathways through which transcription influences lifespan are difficult to identify. Disruption of transcription produces diverse pleiotropic effects and compensatory mechanisms, making it hard to isolate the specific pathways of influence involved.

To understand the role of basic transcriptional machinery in aging, we developed the Auxin Inducible Degradation (AID) system (Zhang, Liangyu, et al, 2015) as a tool to quantitatively tune the steady-state abundance of essential genes in long-term, time-series experiments. Focusing on rpb-2, an RNA Polymerase II (B) subunit, we obtained an rpb-2::AID transgenic line that is viable in the presence of a highly-expressed somatic TIR-1 auxin-dependent E3-ubiquitin ligase. We found that rpb-2::AID animals exhibit a dose-dependent relationship between auxin and lifespan, with high auxin concentrations replicating the adult lethal phenotype of rpb-2 RNAi. The dose dependence of lifespan on RPB-2 highlights the quantitative role RNA Polymerase II plays in *C.elegans* aging.

To investigate the pathways through which rpb-2 influences lifespan, we collected transcriptomic data from populations across an auxin dosage series. Selecting a set of differentially expressed genes for functional validation, we identified profound epistasis in the physiologic response to rpb-2 knockdown. RNAi of a single gene was sufficient to invert the dose-dependence of RPB-2 degradation on lifespan, causing a paradoxical extension of lifespan. This lifespan extension is similar to the previously characterized increased lifespan of the ama-1 mutant which is another RNA Polymerase II subunit (Debès, Cédric, et al, 2019).

We believe that physiologic dosage series provide a novel, quantitative means for mapping out the pleiotropic action of interventions in aging.

Zhang, Liangyu, et al. *Development* 142.24 (2015): 4374-4384.

Debès, Cédric, et al. *bioRxiv* (2019): 719864.

1048A Olfaction regulates organismal proteostasis and longevity via microRNA-dependent signalling

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The maintenance of proteostasis is crucial for any organism to survive and reproduce in an ever-changing environment, but its efficiency declines with age. Post-transcriptional regulators such as microRNAs (miRNAs) control protein translation of target mRNAs, with major consequences for development, physiology and longevity. Here we show that food odour stimulates organismal proteostasis and promotes longevity in *Caenorhabditis elegans* through miR-71-mediated inhibition of *tir-1* mRNA stability in olfactory AWC neurons. Screening a collection of miRNAs that control ageing, we found that the miRNA miR-71 regulates lifespan and promotes ubiquitin-dependent protein turnover, particularly in the intestine. We show that miR-71

directly inhibits the Toll-receptor-domain protein TIR-1 in AWC olfactory neurons and that disruption of miR-71–*tir-1* or loss of AWC olfactory neurons eliminates the influence of food source on proteostasis. miR-71-mediated regulation of TIR-1 controls chemotactic behaviour and is regulated by odour. Thus, odour perception influences cell-type-specific miRNA–target interaction, thereby regulating organismal proteostasis and longevity. We anticipate that the proposed mechanism of food perception will stimulate further research on neuroendocrine brain-to-gut communication and may open the possibility for therapeutic interventions to improve proteostasis and organismal health via the sense of smell, with potential implications for obesity, diabetes and ageing.

1049B Recovery from Heat Shock Requires the miRNA Pathway in *Caenorhabditis elegans*

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The heat shock response (HSR) is a highly conserved cellular process that promotes survival during stress. A hallmark of the HSR is the rapid induction of heat shock proteins (HSPs), such as HSP-70, by transcriptional activation. Once the stress is alleviated, HSPs return to near basal levels through incompletely understood mechanisms. Here, we show that the microRNA pathway acts during heat shock recovery in *Caenorhabditis elegans.* We present evidence that *hsp-70* is repressed by miR-85 and sequences in the *hsp-70* 3'UTR that include target sites for this miRNA. Regulation of *hsp-70* by miR-85 was found to be particularly important during recovery from HS, as animals that lacked miR-85 or its target sites in the *hsp-70* 3'UTR overexpressed HSP-70 and exhibited reduced viability. In summary, our findings show that down-regulation of *hsp-70* by miR-85 after HS promotes survival, highlighting a previously unappreciated role for the miRNA pathway during recovery from an episode of stress.

1050C Regulation of temperature-induced longevity response by neuronal GPCR NPR-8 in *Caenorhabditis elegans*

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Various studies in both poikilotherms and homeotherms have associated lower temperature with longer lifespan and higher temperature with shorter lifespan. These inverse effects of temperature on longevity were traditionally explained by the "rate of living theory", which posits that higher temperatures increase chemical reaction rates, thus speeding up the aging process. Recent studies have identified specific molecules and cells that mediate the longevity response to temperature, suggesting that the temperature effects on aging are not simply thermodynamic but regulated processes. The mechanisms underlying such regulation, however, are not well understood. In the current study, we found that *Caenorhabditis elegans* lacking NPR-8, a neuronal GPCR related to mammalian neuropeptide Y receptors, exhibited extended lifespan at warm temperature. Further investigation uncovered an NPR-8-longevity regulatory circuit that includes the thermosensory neurons AFD and the amphid sensory neurons AWB, AWC, and probably ASJ. Transcriptomic analyses revealed that NPR-8 controls collagen gene expression in the longevity response to temperature. Functions of the NPR-8-regulated collagen genes in this process are currently under investigation. These results suggest the temperature effects on longevity are regulated by the nervous system possibly via controlling collagen gene expression. Our study potentially provides mechanistic insights into understanding the relationship between temperature and longevity in humans, which could prove useful in mitigating negative impacts of increasing temperature due to global warming in a world with an aging population.

1051A Mitochondrial defects manifest an early pathogenic event undermining organismal fitness in a Tauopathy model

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Battling age-associated neurodegenerative pathologies and their pervasive societal impact, is a global enterprise. Alzheimer's disease (AD) is the most common dementia affecting elderly population and there is not any efficient therapeutic strategy until now. Age-dependent impairment of mitochondrial homeostasis is a common feature in evolutionary divergent organisms and is associated with neuronal loss and cognitive decline in AD. However, whether mitochondrial dysfunction is a culprit or bystander of AD pathology remain still elusive. Here, we utilized transgenic nematodes expressing the full length of wild type Tau ($Tau^{wt\text{-}lo}$) in neuronal cells and monitored several aspects of mitochondrial morphology. Although $Tau^{wt\text{-}lo}$ expressing nematodes do not present Tau aggregates during larval stages, they display increased mitochondrial damaged and locomotion

defects. Interestingly, calcium chelating agents restores mitochondrial activity and motility in Tau$^{wt\text{-}lo}$ expressing larvae suggesting that cytoplasmic calcium elevation mediates neuronal impairment. Our findings in their totality suggest that mitochondrial damage is an early pathogenic event of AD that is taking place before Tau aggregation undermining neuronal homeostasis and organismal fitness during aging.

1052B Diacetyl odor shortens food deprivation-induced longevity via downregulating DAF-16

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Dietary restriction extends lifespan in multiple species by reducing nutrient intake and access to non-nutritional food-derived cues. The nutritional effects of dietary restriction on longevity has been extensively studied. However, the roles of food-derived sensory cues in longevity conferred by dietary restriction or the identity of specific food-derived chemical cues that alter lifespan remain unclear. Here, we sought to identify food-derived volatile sensory cues that regulate lifespan in *C. elegans*. We first determined the effects of seven selected bacteria-derived volatile attractants on lifespan under food deprivation, one of life-extending dietary restriction regimens in *C. elegans*. We found that the odor of diacetyl, 2,3-pentanedione, 2,4,5-trimethylthiazole, or benzaldehyde decreased the longevity conferred by food deprivation. We then tested whether the lifespan-reducing chemicals affected the activity of DAF-16, a longevity-promoting transcription factor acting downstream of sensory and insulin/IGF-1 signaling. We found that the odor of diacetyl or 2,3-pentanedione reduced the nuclear localization of DAF-16 under the food-deprived condition. We further showed that the diacetyl odor, which caused the greatest effect on the subcellular localization of DAF-16, reduced the expression of DAF-16-regulated genes, including *sod-3*, *mtl-1*, and *hsp-12.6*. These findings suggest that diacetyl reduces longevity conferred by food deprivation via downregulating DAF-16. Unexpectedly, we found that the odor of diacetyl decreased longevity caused by food deprivation in two established diacetyl receptor mutants, *odr-10(ky225)* and *sri-14(ok2865)* single and double mutants. Thus, diacetyl, a food-derived odorant, may shorten food deprivation-mediated longevity via downregulating DAF-16 by binding to unidentified receptors. We then examined whether diacetyl that is produced under physiological conditions regulated DAF-16 activity or lifespan. We found that the odor of diacetyl-producing lactic acid bacteria reduced the nuclear localization of DAF-16 under food deprivation but did not shorten longevity. These data suggest that the odor of diacetyl-producing lactic acid bacteria downregulates DAF-16 but is not sufficient to decrease lifespan in food-deprived conditions. Together, our study identified diacetyl as a longevity-reducing food-derived volatile sensory cue that downregulates DAF-16. Our study will provide valuable insights into mechanisms by which reduced food odors contribute to dietary restriction-mediated longevity via sensory signaling.

1053C Sulfated steroid hormones regulate longevity and aging-related diseases

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We have recently described that mutations in sul-2 gene, which encodes to the sulfatase of steroid hormone, or its inhibition with STX64 regulates aging and protein aggregation diseases.

Our research has revealed that *sul-2* acts on sulfated steroid hormones in *C. elegans*, the orthologue to STS in humans. *sul-2* mutants have an increased pool of sulfated steroid hormones, increased life-span and ameliorate symptoms of protein aggregation diseases. The *sul-2* longevity depends on germline-mediated factors as *daf-16*, *daf-12*, *kri-1*, *tcer-1* and *daf-36* genes, but fertility is not affected. Interestingly, *sul-2* is only expressed in sensory neurons, suggesting a regulation of sulfated hormones state by environmental cues.

We managed to set up a treatment with the STX64, the specific STS inhibitor. STX64 in *C. elegans* increases longevity and ameliorates Alzheimer's, Parkinson's and Huntington's disease models. Furthermore, testosterone-derived sulfated hormones reproduce the longevity and protein aggregation diseases phenotypes of *sul-2*, supporting that the presence of sulfated steroid hormones is the responsible of the phenotypes.

Remarkably, oral STX64 treatment in acute and chronic Alzheimer's disease mammalian models suppresses the cognitive impairment and reduces the presence of senile plaques in the brain. STX64 is non-toxic in humans and together with our results open the possibility of reallocating steroid sulfatase inhibitors or derivates for the treatment of aging and aging related diseases.

1054A RNA splicing regulation of lipid metabolism and longevity.

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The containment of infectious diseases has catalyzed an increase in life-expectancy and age-related disease. Dietary restriction (DR), a reduced food intake without malnutrition, and reduced TORC1 signaling are highly conserved interventions to promote healthy aging and disease resistance. Our lab defined pre-mRNA splicing as a biomarker of aging and determined it has a causal role in DR and reduced TORC1 lifespan extension through splicing factor 1 (SFA-1). However, the mechanistic link between RNA splicing and longevity remains unknown.

We found lipid metabolic pathways are enriched in genes differentially spliced between subpopulations of *C. elegans* with different life expectancy based on splicing status. Similarly, fat metabolic pathways are enriched among genes with SFA-1 dependent splicing changes. In fact, here we show that SFA-1 modulates fat storage in DR and reduced TORC1 longevity. Upon *sfa-1* RNAi, *eat-2* and *raga-1* mutant *C. elegans* (genetic models of DR and reduced TORC1 longevity) have increased fat storage, while *age-1* mutants (reduced insulin signaling longevity model) do not. Moreover, using Cross Linked Immuno-Precipitation (CLIP) methods in *C. elegans* and Mouse embryonic fibroblasts (MEFs), we identified that SFA-1 binds *pod-2* (mammalian *acaca*), the rate limiting enzyme in fatty acid synthesis. Very excitingly, we found that *pod-2* is a pathway-specific regulator of longevity. *pod-2* RNAi suppresses the lifespan extension of *eat-2* and *raga-1* mutants, but not *age-1* mutant worms.

Here we provide evidence on the interplay between pre-mRNA splicing and lipid metabolism in DR and TORC1 longevity. This brings us closer to the mechanistic understanding of RNA homeostasis as a novel paradigm of aging and physiology.

1055B Sex affects responses to environmental stress in *C. elegans*

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In many species, physiological responses to disease and aging vary by sex. Although environmental stress is a major factor in disease pathogenesis and aging, there is poor understanding of how sex influences responses to stress at the cell and molecular levels. Stress responses are well-understood in *C. elegans*, but almost all studies focus exclusively on self-fertilizing hermaphrodites. Different types of environmental stress induce distinct sets of cytoprotective genes. We used quantitative PCR to measure expression of diverse cytoprotective genes. We found that N2 males have higher basal expression of osmotic, endoplasmic reticulum, detoxification, and heat shock stress response genes than N2 hermaphrodites. There was no difference for mitochondrial and innate immune stress response genes. We also compared stress resistance between sexes to determine if differences in gene expression correlate with physiological performance. Our results suggest that males are generally more resistant to environmental stressors than hermaphrodites. To determine if differences in basal gene expression extend to a broader array of stress response genes, we analyzed previously published RNAseq data for N2 males and hermaphrodites. Out of 92 cytoprotective genes representing osmotic, innate immune, detoxification, heat shock, heavy metal, DNA damage, endoplasmic reticulum, and mitochondrial stress responses, 17 were enriched at least 2-fold in males and 5 were enriched in hermaphrodites. We observe similar patterns of cytoprotective gene expression and stress resistance in natural isolates of *C. elegans* demonstrating that the differences are not unique to the domesticated N2 strain. Feminized mutants and hermaphrodites have similar basal stress response gene expression suggesting that the presence or absence of sperm is not driving the differences we observe. Analysis of previously published RNAseq data also suggests that males have greater cytoprotective gene expression than females or hermaphrodites in *C. remanei* and *C. briggsae*. These differences in

cytoprotective gene expression and stress resistance could serve as a model for defining fundamental mechanisms driving sex-based differences in stress and aging biology. This work was supported by NSF grant IOS-1452948 to KPC.

1056C RBBP-5 regulated methylation at Histone 3 Lysine 4 promotes longevity in *C. elegans*

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Histone 3 lysine 4 methylation (H3K4me) is an epigenetic mark regulating transcription, metabolism, and longevity. H3K4me necessitates, in addition to KMT2 enzymes, the conserved core components WDR5, ASH2, and RBBP5. In *C. elegans*, deficiency in WDR-5 or ASH-2 reduces H3K4me3 levels and extends lifespan. Transgenerational experiments whereby only the parental generation is deficient in WDR-5 or ASH-2 leads to incomplete reprogramming of the next generation resulting in long-lived descendants. Whether RBBP-5 has similar functions remains unclear. Herein, using spike in ChIP-seq, we show that RBBP-5 is required for H3K4 mono- and multi-methylation and adults lacking RBBP-5 are short-lived. In contrast with WDR-5 or ASH-2 deficiencies, our transgenerational experiments show that reprogramming of the next wild type generation is normal. Instead, we revealed that RBBP-5-deprived descendants originating from mothers heterozygous for the *rbbp-5* deficiency inherit a wild type lifespan. However, at the fifth generation the *rbbp-5(-)* short-lived phenotype fully manifest. Using RNA-seq and reporter assays, we found that RBBP-5 is important to maintain mitochondrial bioenergetic and core translational functions. We also show that restoring RBBP-5 expression in the soma is sufficient to recapitulate the wild type lifespan, hence ruling out the germlines as the primary site for H3K4me longevity promoting functions. Collectively, this work shows RBBP-5 regulated H3K4me promotes longevity and wild type lifespan can be epigenetically transmitted to H3K4me-depleted descendants most likely by a mechanism alleviating mitochondrial and translational dysfunctions in somatic cells.

1057A Elucidating Valine's pro-survival role during infection via the mitochondrial UPRmt

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The age-old battle between host and pathogen has driven numerous mechanisms used by each to outcompete the other. The mitochondrial unfolded protein response (UPRmt) is one such pathway used by the host in the defense against infection. The UPRmt not only helps in recovering mitochondria that are damaged during infection but also induces an immune response to thwart pathogen colonization. In the model organism *Caenorhabditis elegans*, pathogens such as *Pseudomonas aeruginosa* activate the UPRmt through the production of damaging toxins but also can downregulate the UPRmt during chronic infection. In our previous work we uncovered that loss of the *P. aeruginosa* acyl-CoA dehydrogenase FadE2 prolongs UPRmt activity and extends host survival. FadE2 showed substrate preferences for the coenzyme A intermediates produced during the breakdown of the branched-chain amino acid valine and to a lesser extent, leucine. Our data suggests that during infection, FadE2 restricts the supply of valine to the host hindering host energy metabolism in addition to the UPRmt. Consistently, valine supplementation re-established the UPRmt and prolonged host survival during infection with *P. aeruginosa*. However, the mechanism by which valine promotes the UPRmt during infection with *P. aeruginosa* is unclear. We are currently using RNA-Seq analysis, gene knockdown and overexpression to unravel the mechanistic basis of UPRmt regulation by valine during infection and will report our findings.

1058B A microfluidics-based chemical screening platform for lifespan and healthspan extension in *Caenorhabditis elegans*

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C. elegans is a premier model organism used to identify pharmacological interventions that enhance lifespan and healthspan. Generally, animals are cultured on agar surface where drugs are either dissolved in agar or spread over solidified agar. The effects of the drug on lifespan are measured by the change of median or mean lifespan. The agar-based screening method has some severe limitations, including (i) limited throughput, (ii) progeny blocking methods can complicate outcomes, (iii) drug molecules may not be accessible to the animal due to transport limitation, (iv) bacteria can metabolize drug molecules, (vi) animals may avoid the bacterial lawn and therefore minimize drug exposure, and (vii) animals may be lost from the assay due to desiccation or burrowing. We present a microfluidic technology named an «Infinity screening system,» where animals are cultured in a confined, micro-structured liquid environment with an integrated fluid processing and imaging hardware. Food and drugs are prepared in a batch (no FuDR) for the entire lifespan experiment for precise control over the consistency of daily dose of food and drug quality. Images of animals moving in the chip pillar environment are acquired each day and processed for live/dead count and locomotion-based cohorts with a software built in-house. This study used seven well-studied anti-

aging drugs to benchmark the infinity platform for the drug effectiveness and consumption for whole-life analysis. We used three concentrations for each drug, concentration identified in plate-based assays, 1/10th, and 1/100th of the plate-based dosage. The entire experiments require approximately 3 man-hr/day and 30 days to complete one biological replicates. We found that infinity chip requires approximately 7.5 -750 times less drugs to achieve similar effects to that of agar plates. In this study, we found Thioflavin T as toxic in the microfluidic environment at the highest concentration. The actual dependency of drug type on the effective concentration is still unclear. In general, we observed significant increase in mean lifespan without appreciable changes in maximum lifespan at an effective concentration. Moreover, α-Ketoglutarate was able to rescue plate like lifespan enhancement in infinity chip contrasting the lifespan outcome from Lifespan Machine. Although, all the drugs extend lifespan, only a few of them helped maintain a larger fraction of animal highly active in the older age.

1059C Whole-animal *in vivo* screening for small molecule inhibitors of the mitochondrial UPR

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Mitochondria are essential for energy production, metabolic signaling, calcium homeostasis, and other roles. Consequently, mitochondrial dysfunction is associated with several human pathologies including neurodegenerative, cardiovascular, and metabolic diseases, as well as cancer. Thus, cells use recovery mechanisms such as the mitochondrial unfolded protein response (UPR^{mt}) to mediate mitochondrial recovery. Importantly, the UPR^{mt} may have clinical relevance as it has been shown to play a role in cancer survival. Hence, inhibition of the UPR^{mt} may be leveraged as an anticancer strategy with great therapeutic promise. We employed a whole-animal chemical screening approach to identify small molecule inhibitors of the UPR^{mt} using the *C. elegans* UPR^{mt} transcriptional GFP reporter strain *hsp-6*::GFP. We screened a library of 1280 FDA-approved drugs for their ability to inhibit *hsp-6*::GFP reporter expression during stress. Among the identified hits was Paroxetine (Paxil), a type of selective serotonin reuptake inhibitor (SSRI) that blocks serotonin transporters on the presynaptic membrane, thereby increasing the concentration of this neurotransmitter at the synapse. Serotonin stabilizes our mood and feelings of well-being. Thus, SSRIs are used in the treatment of depression and other mental disorders. The connection between the SSRI Paroxetine and the activity of the UPR^{mt}, however, has not been previously reported. Consistent with suppression of the UPR^{mt}, Paroxetine further impaired mitochondrial function during stress but had negligible effects under standard conditions. Importantly, Paroxetine did not suppress other cellular stress response pathways including the endoplasmic reticulum UPR (UPR^{ER}). In addition, Paroxetine suppressed UPR^{mt}-associated phenotypes including extensions in lifespan and increased host survival during infection. Unexpectedly, genetically disabling the clinical target of Paroxetine, the serotonin reuptake transporter *mod-5*, did not suppress UPR^{mt}. Therefore, Paroxetine likely disables UPR^{mt} activity through an unconventional drug target.

1060A Identification of common lifespan-modulating genes through genomic comparison of diverse long-lived genetic mutants

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As the age of the world population rises, there is a concordant rise in age-associated diseases such as cancer, cardiovascular disease, and neurodegeneration. Understanding the aging process at the molecular level may aid development of preventative treatments for these diseases, as targeting lifespan-modulating pathways can be protective in models of age-onset diseases. However, lifespan-modulating pathways have wide ranging cellular effects, beyond delaying aging. To advance our understanding of genetic pathways contributing to longevity, and identify novel genetic targets to test in models of neurodegenerative disease, we identified “common” genes similarly modulated in a variety of long-lived genetic mutants.

We performed RNA sequencing on nine long-lived genetic mutants representing six different lifespan-modulating pathways: insulin-IGF1 signaling (*daf-2)*, dietary restriction *(eat-2),* germline deficiency *(glp-1)*, reduced mechanosensation (*osm-5),* reduced translation (*ife-2)*, elevated mitochondrial reactive oxygen species (*sod-2),* and weak mitochondrial impairment *(nuo-6*, *isp-1*, *clk-1*). There is a significant overlap in differentially expressed genes between almost all pairs of long-lived mutants. Thus, diverse lifespan-modulating pathways converge onto overlapping genetic targets. The genes upregulated by the lifespan-modulating pathways fall primarily into two disparate groups: genes upregulated in *eat-2, osm-5,* and *ife-2* worms, and genes upregulated in *daf-2*, *sod-2*, *isp-1,* and *nuo-6* worms. The two gene groups are enriched for different tissue expression

and different molecular pathways, further supporting that these genes extend lifespan through distinct means. To identify "common" genes potentially shared across these groups, we selected for genes similarly modulated in at least six of the nine mutants. We identified 196 "common" upregulated genes enriched for genes involved in immunity and metabolism, and 62 "common" downregulated genes enriched for genes associated with translation initiation. We will determine if the overarching common genes are required for enhanced longevity and then examine if the group-specific genes are required for longevity in their respective mutants. Developing a clearer understanding of the specific genes and pathways which directly modulate lifespan will advance our understanding of the aging process, and help to develop preventative treatments for our aging population.

1061B Investigation of the transcriptional response to starvation at the tissue level

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Competition for nutrients is a driving force in evolution. As such, organisms have evolved robust adaptive responses to withstand periods of starvation. In *C. elegans*, efforts have been made to map the acute starvation response at the transcriptome level using microarray and RNA-seq techniques. However, past studies used whole worms and thus represent an averaged response profile of all of the worms' tissues. Recently, Kaletsky and colleagues developed methods to isolate and study purified *C. elegans* cell populations from larvae, which allows studying tissue-specific transcriptomes. To gain a better understanding of the response to starvation and potentially reveal unique tissue-specific responses, we are adapting this technique to study how neurons, hypodermis, intestine, and body wall muscles respond to starvation. Specifically, we have used four strains that express GFP in each of these tissues of interest. We then dissociate worms into single cells and FACS-sort GFP-labeled tissues both fed and starved animals, followed by RNA isolation and RNA-sequencing. To date, we have sequenced mRNA from neuronal and body wall muscle tissues from fed animals and animals that were starved for six hours. Preliminary analysis indicates that starvation induces broader changes in neurons compared to body wall muscle. Interestingly, using a liberal significance threshold of P <.05 and FDR < 1, only 11% of genes are regulated by starvation in both tissues, suggesting that tissues respond in a highly specific manner to starvation. In neurons, we observed a positive enrichment for biological processes involved in guanylyl cyclase signaling and cell projection remodeling, whereas negatively enriched processes included protein translation and ribosome assembly. In muscles, we observed positive enrichment of the ER unfolded protein response and cuticle remodeling, and negative enrichment of protein synthesis. Our work demonstrates variation in responses to starvation between tissues. The methods described will allow us to assemble a profile for each tissue, which can be used to develop functional experiments to unravel the biology of the starvation response.

1062C Soluble Epoxide Hydrolase Inhibitor, AUDA, Recuses Neurodegeneration Induced by Amyloid β and Tau

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According to a United Nations report, the percentage of the population over the age of 65 is expected to increase from approximately 9% (2019), to roughly 20% by 2050. With this demographic change, we can expect a coinciding increased incidence of age-related neurodegenerative diseases (ND), including Alzheimer's Disease (AD), for which there is no cure. The two hallmark pathologies of AD are the deposition of the amyloid β (Aβ) and the neurofibrillary tangles of the microtubule-binding protein tau. Recent evidence suggests that increasing the epoxy-metabolites of polyunsaturated fatty acids (PUFAs) through pharmacological inhibition or genetic knock-out of soluble epoxide hydrolase (sEH), which metabolizes epoxy-PUFAs, could be an effective strategy in limiting AD neurodegeneration. Considering this, we seek to investigate the effect(s) of epoxy-PUFAs on tau- and Aβ-induced neurodegeneration using *Caenorhabditis elegans* (*C. elegans)*. The transgenic Tau (CK1441) and Aβ (CL2355) mutants show neurodegenerative behavior such as slow thrashing and decreased locomotion speed, respectively, and both strains show hypersensitivity to serotonin. Interestingly, supplementation of HE inhibitor, 12-[[(tricyclo[3.3.1.13.7] dec-1-ylamino)carbonyl]amino]-dodecanoic acid (AUDA), rescues neurodegeneration in both transgenic strains. Our results suggested that specific epoxy-PUFAs significantly affect neurodegeneration, in particular those mediated by deposition of Aβ and/or tau, and the mechanistic studies on how EH inhibition alleviates neurodegeneration induced by Aβ and/ or tau are underway.

1063A The PCP molecule Flamingo regulates body size and lifespan by controlling collagen content in *C. elegans*

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The CELSR/Flamingo homolog FMI-1 is well known for its key role in planar cell polarity (PCP) and axon guidance. The Adhesion-G protein-coupled receptor (aGPCR) acts as one of the PCP core proteins besides Frizzled, Dishevelled, Diego, Van Gogh or Prickle to realize the polarization of tissues such as epithelia. Interestingly, few studies report that aGPCRs as well as parts of the PCP core pathway components have also implications in other processes such as the regulation of metabolic processes.

Here, we show that FMI-1 is involved in body size regulation. Characterizing two different *fmi-1* loss-of-function mutants we observed a significantly smaller body size, a prolonged lifespan, higher fat levels, and abnormal movement in the mutants compared to wild-type animals. None of these phenotypes was caused by caloric restriction or defects in food intake. Transcriptome analyses revealed that a large number of collagen gens are significantly upregulated in the absence of FMI-1. This was verified by directly assessing collagen in the worms. We found that body size is regulated by FMI-1 exerting a signal from sensory neurons, thereby affecting hypodermal collagen synthesis. However, the respective phenotypes of *fmi-1* mutants are not a consequence of the previously described neuronal mismigration. Rescue experiments revealed that this function of FMI-1 is dependent solely on the N terminus of the GPCR, and thus, not on classical intracellular signaling into the same cell. Functional and expression analyses showed that FMI-1 signals are dependent on components of the insulin/IGF-1-like signaling pathway such as the DAF-16/FoxO transcription factor. Further, we tested a relation to the BMP signalling pathway. Interestingly, the role of FMI-1 in body size regulation seems to be caused by a different mechanism than its function in fat storage.

We hypothesize that the lack of the FMI-1 leads to higher levels of collagen in the worms' cuticle, restricting its growth and movement and making it more resistant towards external stress. Taking together, our data highly suggest the PCP molecule Flamingo to be an interesting player in collagen homeostasis and body size regulation.

1064B Investigating the role of *miro-1* in neurodegeneration using a *C. elegans* Alzheimer's disease model

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Alzheimer's Disease (AD) is a debilitating neurodegenerative condition affecting many elderly individuals. Although AD is generally considered a progressive, age-related condition, a subset of the population is genetically predisposed to developing AD early in life, a condition known as familial Alzheimer's Disease (FAD). FAD is primarily caused by mutations in one of three genes, Amyloid Precursor Protein (APP), Presenilin 1 (PSEN1), and Presenilin 2 (PSEN2), with most mutations occurring in PSEN1. PSEN1 functions the catalytic core of γ-secretase and is involved in the processing of APP to amyloid beta (Aβ) peptides. and the regulation intracellular Ca^{2+} signaling. Aβ plaque accumulation has long been regarded as the disease promoting agent controlling AD like neurodegeneration. However, recent studies are showing a lack of correlation between Aβ plaques and AD symptom severity, pointing to the possibility of other mechanisms controlling neurodegeneration. PSEN1 is also known to regulate intracellular Ca^{2+} signaling, and mutations in PSEN1 have been shown to result in increased reactive oxygen species (ROS) production, oxidative stress, and neurodegeneration as a product of intracellular Ca^{2+} dysregulation. The mechanisms underlying these intracellular changes however remain unclear. We are looking to investigate the relationship between endoplasmic reticulum (ER)-mitochondrial Ca^{2+} signaling, mitochondrial ROS production, and neurodegeneration using *C. elegans* strains harboring mutations in the gene encoding the *C. elegans* PSEN1 homologue, *sel-12*. *sel-12* mutants have been shown to display elevated intracellular Ca^{2+} levels and increased ER-mitochondrial contacts which are associated with increased mitochondrial Ca^{2+} uptake, mitochondrial ROS production, and neuronal dysfunction. To understand the mechanism underlying the *sel-12* mitochondrial phenotype, we are investigating the role a specific mitochondrial outer membrane protein, MIRO-1, which has been shown to have roles in mitochondrial trafficking, mitochondrial Ca^{2+} regulation, and mitochondrial metabolism. By introducing a *miro-1* null mutation into the *sel-12* mutant background, we are investigating whether *miro-1* has a role in the ER- mitochondrial Ca^{2+} homeostasis and if loss of *miro-1* improves *sel-12* mutant fitness. Thus far, our results indicate that disrupting *miro-1* in the *sel-12* mutant background restores the neurodegenerative phenotype observed in the FAD mutant model system.

1065C The AFD temperature sensing neurons adjust *C. elegans* defenses to match the temperature-dependent threat of hydrogen peroxide produced by bacterial pathogens

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Activation of cellular defenses can protect hosts from the macromolecular damage induced by hydrogen peroxide—the weapon of choice for many bacterial pathogens. Sensory systems can coordinate the induction of these protective host defenses, yet direct links between individual sensory mechanisms and these innate immune responses are only beginning to be understood. Understanding how sensory mechanisms regulate *C. elegans* defenses to bacterially produced hydrogen peroxide may reveal novel insights into how multicellular organisms coordinate their innate immune response to changing environments.

We investigated how the two AFD temperature sensing neurons in *Caenorhabditis elegans* use temperature information to coordinate resistance to hydrogen peroxide produced by the pathogen *Enterococcus faecium*. We found that hydrogen peroxide was more lethal to nematodes at higher temperatures, and that at those higher temperatures the action of the AFD neurons enabled the nematodes to deal with the increased lethality of peroxides. In addition, nematodes that developed at higher temperatures induced AFD-dependent hydrogen peroxide defenses that were lasting, nearly doubling the animals' survival to subsequent peroxide exposure at lower temperature, compared to animals grown continuously at lower temperatures. Remarkably, genetic ablation of the two AFD neurons pre-induced these defenses, leading to a three-fold increase in hydrogen peroxide resistance. Through mRNA-sequencing, we found that the AFD neurons regulated the activation of a broad set of DAF-16/FOXO and SKN-1/Nrf transcriptional targets. Genetic experiments demonstrated that in response to AFD ablation, and at higher temperatures, the partially redundant action of these two transcription factors in the intestine increased the nematode's hydrogen peroxide survival. Finally, we found that these AFD-dependent mechanisms conferred resistance to hydrogen peroxide produced by the pathogen *Enterococcus faecium*. As *E. faecium* grows in a temperature-dependent manner and produces hydrogen peroxide in a growth-dependent manner, we propose that this sensory system enables the nematodes to use temperature information to predict the likelihood of encountering bacterially produced hydrogen peroxide and adjust their innate immune defenses to match that threat.

1066A The heat shock transcription factor HSF-1 protects *Caenorhabditis elegans* from peroxide stress

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Cells induce conserved defense mechanisms that protect them from oxidative stress. How these defenses are regulated in multicellular organisms is incompletely understood. Here, we show that the heat shock transcription factor HSF-1 protects the nematode *Caenorhabditis elegans* from the oxidative stress caused by environmental peroxide. In response to a heat shock or a mild temperature increase, HSF-1 protects the nematodes from subsequent peroxide stress in a manner that depends on HSF-1's transactivation domain. At constant temperature, HSF-1 protects the nematodes from peroxide stress independently of its transactivation domain, likely by inducing the expression of *asp-4/cathepsin* and *dapk-1/dapk*. Thus, two distinct HSF-1-dependent processes protect *C. elegans* from peroxide stress.

1067B Collagen gene variants, endoplasmic reticulum homeostasis, and aging

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One third of all proteins are cotranslationally translocated into the endoplasmic reticulum (ER) during their biogenesis. A conserved homeostatic mechanism known as the ER unfolded protein response (UPR) has evolved to ensure protein folding homeostasis (proteostasis) in the ER lumen by tuning ER chaperone and protein degradative capacity to accommodate changes in unfolded protein load. Failure of the ER UPR can lead to protein misfolding and aggregation, a condition known as "ER stress" which is associated with common human diseases associated with aging such as Alzheimer's disease, Parkinson's disease, and diabetes. Therefore, insights into the molecular underpinnings of the ER UPR will likely lead to improved strategies to promote healthy aging through the maintenance of ER homeostasis.

In previous studies we discovered that *trap-1* null mutants exhibit constitutive expression of the *hsp-4::GFP* ER UPR reporter. To gain insight into how TRAP-1 contributes to ER homeostasis, we performed a genetic screen for modifiers of the *trap-1* mutant phenotype. We mutagenized *trap-1;hsp-4::GFP* worms and screened for F2 progeny with either increased or decreased GFP expression. One strain with increased GFP expression harbored a causal missense mutation in the *col-75* collagen gene *(dp691)*. 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. Surprisingly, while *col-75* is expressed primarily in the excretory cell, socket glia, and pharyngeal neurons, it induces *hsp-4::GFP* expression non-autonomously and specifically in intestine and spermatheca. Intriguingly, although germline missense mutations in human collagen genes that induce chronic ER stress typically cause disease, *col-75(dp691)* animals are healthy and retain wild-type levels of resistance to the ER stress inducer tunicamycin. A phenome-wide association study (PheWAS) of all common human COL missense alleles in the BioVU de-identified electronic health record-linked DNA biobank captured on the Illumina Exome BeadChip array revealed the association of several COL gene variants with protection from diagnoses of aging-related diseases such as heart failure, cancer, kidney disease, spinal stenosis, and inflammatory bowel disease. We hypothesize that collagen gene missense mutations that cause constitutive ER UPR induction have context-dependent effects on cellular and organismal fitness and may contribute to human health through constitutive ER UPR induction.

1068C Protective effects of caffeine intake on intestinal aging by regulating yolk protein production and autophagy-dependent intestinal atrophy in aged *C. elegans*

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Caffeine, a methylxanthine derived from plants is the most widely consumed ingredient in our daily life. Previous studies have shown that constant intake of a low dose of caffeine (<10 mM) has beneficial effects on aging-related disorders in *C. elegans*. In this study, we attempted to determine the possible anti-aging effects of caffeine on intestine using a *Caenorhabditis elegans* model. It was previously reported that intestinal atrophy was observed in aged worms due to the accumulation of vitellogenin. We also previously showed that caffeine intake reduces vitellogenin production. Vitellogenin production in *C. elegans* is highly temporal-, spatial- and sex-specific. It is produced only at the adult stage in the intestine of hermaphrodites. Therefore, we examined changes in the intestinal morphology and yolk protein production after caffeine intake in aged worms to see if caffeine can control the intestinal aging. Intestinal integrity including ACT-5 mislocalization and lumenal expansion were examined, and vitellogenin (VIT) production and pseudocoelomic lipoprotein pool (PLP) accumulation were measured after caffeine intake in aged worms. ACT-5 mislocalization and lumenal expansion were suppressed, and VIT production and PLP accumulation were significantly decreased by caffeine intake in aged worms. Furthermore, increased level of autophagy activity that is normally observed in aged worms for conversion of intestine to yolk protein was significantly reduced by caffeine intake. These findings suggest that caffeine intake suppresses a yolk-mediated intestinal atrophy in aged worms by reduction in yolk protein production and in autophagy activity. Taken together, caffeine appears to be a potential anti-aging agent that protects intestine from aging. This study was supported by NRF2018R1A2B6007915 and NRF2021R1A2C1011658.

Keywords: Caffeine; yolk protein; vitellogenin; pseudocoelomic lipoprotein pool; intestinal aging; autophagy

1069A Neuropeptide modulation of insulin signaling in bacteria-dependent survival

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Bacterial food sources will differentially affect *C. elegans* physiology and survival. For example, *C. elegans* fed two *E. coli* strains—the B type OP50 versus the K12 type CS180—exhibit different survival phenotypes. Wild-type *C. elegans* fed OP50 have a higher rate of early deaths compared to *C. elegans* fed CS180. The early deaths on OP50 are characterized by swollen pharynges (P-deaths) that resulted from bacterial accumulation within the tissue. In contrast, worms fed CS180 are more resistant to P-deaths. We find that the neuropeptide neuromedin U receptor *nmur-1* inhibits P-deaths on OP50, but not on CS180. Interestingly, however, *nmur-1* promotes the opposite response when the insulin receptor *daf-2* has reduced activity—where *nmur-1* now promotes P-deaths on OP50. Since both effects of *nmur-1* appear dependent on the FOXO *daf-16* transcription factor, we propose that *nmur-1* acts as a modulator of insulin signaling. Thus, NMUR-1 ensures that the insulin receptor DAF-2 signals at the appropriate level to promote pharyngeal health and optimal survival in response to specific bacteria.

1070B toluene-induced bioenergetics changes generate early aging and decreased healthspan in *caenorhabditis elegans*

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aging is a normal and inevitable cellular physiological process, however, in current days, individual or environmental factors are contributors to early aging, thus generating an impaired healthspan. barker, in an ecologic psychology context mentioned that “the environment always exerts influences in the individual”. if we adapt this to the toxicological sphere, daily we are exposed to many agents, whether intentional (drug’s abuse) or in a non-intentional manner (environmental or occupational), that induce countless toxic effects to the organism (influence). toluene is a major solvent used in industry of paints, gasoline and adhesives, therefore, workers are the non-intentional target public. however, toluene is also a psychoactive substance and epidemiologic data have shown its abuse as the third mostly consumed in comparison to others drugs there are many studies reporting the short-term effects of toluene, nevertheless, long-term effects after end of exposure are scarce. based on that, the aim of our work was to investigate the long-term impact of airborne exposure to toluene using c*aenorhabditis elegans*. we used two strains: n2 wild type and mutant pe255 feIs5 [*sur-5*p::luciferase::GFP + *rol-6*(*su1006*)] to measure atp levels. approximately 100 nematodes at l4 stage were exposed to toluene in a vapor chamber for 24h, mimetizing two scenarios of exposure: scenario 1 (mean conc. 792 ppm) and scenario 2 (mean conc. 1,094 ppm). the experiments were conducted 1, 48 and 96 hours after exposure. lipofuscin autofluorescence was measured as an aging bioindicator, neurobehaviors (head trashes, velocity and path length) to assess healthspan, and atp levels as mitochondrial function endpoint. we observed that worms exposed to both scenarios of exposure have shown increase of cellular lipofuscin accumulation, and that exposed worms demonstrated a progressive reduction of mobility in a significant manner. these findings corroborated with a decrease of mitochondrial functionality, demonstrated by atp levels reduction. here we demonstrate that toluene-induced early cellular aging can occur associate to accentuated motor loss that could be explained by a mitochondrial dysfunction, thus impacting on healthspan reduction. the literature supports this discovery, once long-term exposures either intentionally or occupationally are harmful to humans, however, here we observed that even long periods after withdrawal the toluene effects remain.

1071C HSP90 and HSF-1 regulate lipolysis in *C. elegans*

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Obesity is a risk factor for the 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. HSP90, an abundant heat shock protein plays an essential role in the maintenance of proteostasis through stabilizing the conformation of various proteins, thus affecting many different intracellular pathways. In our previous works we demonstrated that HSP90 is required for longevity in *C. elegans* through regulating the activity of the transcription factor DAF-16 isoform A, and in fat storage regulation in mammalian cells through its client PPARγ. We have also found a conserved chaperone-client interaction between HSP90 and SIR2 in mammalian cells and worms. Here we confirm that SIR-2.1 and further show that HSP90 are required for lipid mobilization in *C. elegans*. We found that loss and gene silencing of SIR-2.1, as well as gene silencing and pharmacological inhibition of HSP90 both hinder starvation-induced lipolysis and the subsequent increase in the free fatty acid concentration. Besides this, we identified the heat shock transcription factor HSF-1 as the mediator of the observed inhibitory effect by modulating the expression of lipases, such as ATGL-1. Studies aimed at elucidating the components at different levels of this regulatory pathway are under way. 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.

1072A Genetic basis of enhanced stress resistance in long-lived mutants

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In order to gain insight into the relationship between stress resistance and longevity, we directly compared resistance to multiple external stressors (heat, oxidative stress, bacterial pathogens, osmotic stress, and anoxia) with the magnitude of lifespan extension in nine long-lived *C. elegans* mutants representative of different pathways of lifespan extension. Furthermore, we have used RNA sequencing to analyse gene expression in each of these mutants to identify genes and pathways responsible for the enhanced resistance to stress. We find that all of the long-lived mutants examined have increased resistance to one or more type of stress. Resistance to each of the types of stress resistance examined exhibited a positive, significant correlation with lifespan, with bacterial pathogen resistance showing the strongest relationship. All of the long-lived mutants examined show upregulation of at least on stress response pathway, but differ in which pathway

shows the greatest enrichment. We used RNA sequencing data to identify which genes are most highly correlated with each type of stress resistance. In comparing these results to genes that are most highly correlated with longevity, we observed a highly significant overlap suggesting that the same genetic pathways are involved in both. This was especially true for genes correlated with bacterial pathogen resistance, which showed an 84% overlap with genes correlated with lifespan. Overall, our results demonstrate a strong correlation between stress resistance and longevity, and indicate that the same genes contribute to both phenotypes.

1073B Relating Behavioral Ageing and Lifespan with the Lifespan Machine v2

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Aging involves a gradual transition from youthful vigor to geriatric frailty and death. An ideal intervention in aging would increase the period of youthful vigor in absolute terms without extending the period of geriatric frailty. However, few lifespan-extending interventions appear to produce this effect. To better understand the interdependence between youthful vigor and lifespan, we developed a new version of "The Lifespan Machine" that can simultaneously measure outcomes of behavioral aging and lifespan at high precision in populations of thousands of *C. elegans*.

Across diverse interventions, we find that behavioral ageing and lifespan are not parsimoniously explained as manifestations of a single underlying aging process. Instead, we observe an inverse covariation between the duration of youthful vigor and remaining lifespan that more likely reflects a hierarchical structure in which partially independent aging processes progress during adulthood to separately drive behavioral aging and determine lifespan. Our results suggest there is substantial complexity in the pleiotropic, time-dependent action of lifespan-extending interventions, and suggest that a partitioning of lifespan into "healthy" an "unhealthy" periods may miss important distinctions present in the underlying physiology.

1074C Protective and reparative effect of dragon fruit upon central nervous system toxicity induced by copper

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Copper is an essential metal and is important in general metabolism. However, in high concentrations, it becomes toxic to the organism. The metal-induced toxicity is linked to many neurodegenerative diseases like Parkinson's, Alzheimer's, multiple sclerosis, occurring in senile aging. To prevent neurodegenerative diseases, some studies are being developed to find ways of healthy aging. Natural compounds and diets based on fruits are increasing. Dragon fruit (*Hylocereus undatus*) is a tropical and Latin American fruit that is gaining more popularity due to its antioxidant properties. This fruit still has low popularity, since its planting and large-scale commercialization are recent. Here we evaluate the protective and reparative effect of different doses of dragon fruit's microencapsulated pulp extract on copper-induced toxicity. The nematode *Caenorhabditis elegans* was used as a model, to research the effects of pitaya extract on the Cholinergic nervous system, behavior, lipidic peroxidation, and chaperon system. We observed that Cu severally increased directly the cholinesterase, chaperone, and peroxidation rates. When pitaya is applied, even to prevent or remediate, all those enzymes' rates are normalized again. Overall, the results have shown that the pulp fruit extract can be used on dietary supplementation to prevent and repair neural damage. The extract was able to regulates the chaperone system to reduce expression of heat shock protein (16.2) and reestablished the AChE levels avoiding lipidic peroxidation. Changes in the behavior, decreasing cell death biomarkers, and lipidic peroxidation caused by copper toxicity are showing, and, based on these results, we concluded that the pitaya fruit has an important role in gerontological issues.

1075A An autophagy activator extends healthspan and lifespan in *C. elegans*

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Autophagy is an evolutionarily conserved cellular recycling process with tight links to longevity and healthspan. In particular, autophagy function declines during aging, and is dysregulated in many age-related disorders such as in neurodegenerative diseases. Therefore, identifying interventions that can boost autophagy to prevent such chronic illnesses progression is crucial to improving organismal health.

Of note, autophagy is increasingly appreciated as a selective process by which specific types of cytosolic cargo, such as organelles, lipids and protein aggregates, are sequestered into double-membrane structures called autophagosomes that subsequently fuse with hydrolase-containing lysosomes to enable cargo degradation. Interestingly, accumulating evidence suggests that disruptions in selective autophagy can contribute to the development of age-related diseases. For example, chronic inhibition of lipophagy (selective lipid turnover) leads to increased accumulation of lipids, leading to obesity and diabetes. However, treatments that may target and improve selective autophagy to help relieve such illnesses remain underdeveloped.

To identify novel chemical compounds that may act as selective autophagy activators, we performed a high-throughput imaging screen in human adenocarcinoma cells to uncover small molecules that activate autophagy and increase lipid clearance. Given the previous links between autophagy and aging, we tested several autophagy activator hit compounds from the screen for autophagy- and lifespan assays in *C. elegans*. While we found that these compounds all increased autophagosome numbers, only animals fed with small molecule **A20**

exhibited life- and healthspan extension, along with reduced lipid levels, as observed in human cells. Importantly, this **A20**

-mediated lipid reduction and health benefits were not observed in autophagy mutants. Furthermore, we found that **A20**

could reduce PolyQ aggregate cargo load in multiple tissues, and we are currently investigating if **A20**

is affecting additional cytosolic cargos. Notably, inhibition of the nutrient sensor mTORC1 activates autophagy. However, **A20**

seemed to function independently of mTORC1, and we are currently performing studies to determine how A20 could mediate autophagy.

In conclusion, we have identified a new compound **A20**

, which may potentially be applied in future strategies to improve organismal health and alleviate age-related diseases by boosting autophagy.

1076B Investigating the effect of stress response pathways on *C. elegans* electrotaxis behaviour

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C. elegans is a leading animal model for understanding the mechanisms of stress-induced behavioural changes. Stress-inducing conditions activate the unfolded protein response (UPR) in different cellular compartments that include the cytosolic heat shock response (HSR) and the mitochondrial and endoplasmic reticulum UPR (MT-UPR and ER-UPR, respectively). Our group is using a microfluidic-based electrotaxis device to examine the roles of genes and pathways involved in maintaining the stress response in worms. Previous work from our lab showed that *C. elegans* exhibit directional locomotion in a microfluidic channel in the presence of a mild electric field. It was also found that both neurons, specifically dopaminergic neurons, and muscles are involved in mediating this behaviour. Using our electrotaxis device, we have investigated how various stressors affect the behavioral response of animals. The treatments included a range of chemical, genetic, and environmental stress-inducing conditions. Chronic exposure of paraquat or tunicamycin (activators of the UPR) affected the movement of animals, resulting in a lower electrotaxis speed. We then investigated mutations of the HSR, MT-UPR and ER-UPR pathways and saw a similarly abnormal response. Environmental stressors such as chronic heat exposure and dietary restriction, which can impact the UPR, were found to reduce the speed of animals significantly. We also investigated whether a daily exercise treatment could have a beneficial effect on worms and found that not only did it increase the electrotaxis speed but also led to improved muscle health. Together, these findings reveal the essential role of the stress response pathways in modulating the electrotaxis behaviour of animals. Additionally, our data show that chronic stress is detrimental, whereas transient stress can be beneficial to health.

1077C *C. elegans* TFIIH subunit GTF-2H5/TTDA is a non-essential transcription factor indispensable for DNA repair

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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.

1078A Perturbation of endosomal trafficking by *tbc-2* mutation decreases stress resistance and lifespan by altering nuclear localization of DAF-16

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The *C. elegans* homolog of the FOXO transcription factor, DAF-16, plays an integral role in insulin-IGF1 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 including antioxidants, chaperones and other stress response genes. To gain insight into the role of endosomal trafficking in the nuclear localization of DAF-16 in response to stress, we disrupted *tbc-2*, which encodes a GTPase activating protein involved in endosomal transport. We found that *tbc-2* mutants showed slower nuclear localization of DAF-16 in response to heat stress and anoxia, but faster nuclear localization in response to oxidative stress. To determine if the rate of nuclear localization of DAF-16, affected stress resistance in these animals, we examined survival after exposure to different exogenous stressors. Consistent with the pattern of nuclear localization of DAF-16, we found that *tbc-2* mutants have decreased resistance to heat stress and anoxia, but increased resistance to chronic oxidative stress. Finally, we examined the effect of *tbc-2* mutation on lifespan. As we have previously shown that DAF-16 has increased nuclear localization in long-lived mitochondrial mutants, and is required for their longevity, we examined the effect of disrupting *tbc-2* on their lifespan. We found that deletion of *tbc-2* markedly decreased the lifespan of three long-lived mitochondrial mutants (*clk-1*, *isp-1*, and *nuo-6*) whose longevity is dependent on DAF-16. 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.

1079B SKN-1 activity in ASI orchestrates cell non-autonomous stress resistance in peripheral tissues

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SKN-1 is an essential transcription factor in *C.elegans*. Originally identified as a transcription factor essential for the specification of the intestine during development, SKN-1 has been characterized as the *C.elegans* orthologue of NRF2 in mammals. One primary function of SKN-1 includes the transcriptional activation of phase two detoxification genes during

oxidative and xenobiotic stress, however it has also been implicated in lipid metabolism. All of these essential cellular processes are needed for the maintenance of normal lifespan and as such, SKN-1 is a master cytoprotective transcription factor. Recently, while screening for novel regulators of SKN-1, several gain-of-function (gf) alleles of *skn-1* were identified. While these mutants have increased stress resistance early in life, they also display shorter lifespans than wildtype animals. These early findings suggest a link between uncontrolled regulation of cytoprotective transcription factors and disease pathology. We seek to define the molecular and cellular mechanisms that govern the regulation of SKN-1 by exploiting a constitutively active mutant. We show via fluorescent microscopy that phenotypes caused by SKN-1gf are not the result of an intense soma-wide accumulation of SKN-1gf. Additionally we performed ChIP-seq to determine the difference in genome occupancy between wildtype SKN-1 and SKN-1gf. Lastly we show that target gene expression of SKN-1gf is diminished in mutants defective for neurotransmitter release, suggesting that phenotypes associated with SKN-1gf are controlled by signaling between two head sensory neurons and other somatic tissues where SKN-1gf is expressed

1080C Somatic Regulators of the Non-Cell-Autonomous CEP-1/p53-Mediated DNA Damage Response in Primordial Germ Cells

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The integrity of heritable genomes is a prerequisite for species maintenance. For more than a century, it was thought that the genomes in the germline of an organism are isolated from somatic influences by the so-called Weismann barrier. In *C. elegans*, the primordial germline consists of two somatic gonad precursors (SGPs) and two primordial germ cells (PGCs). We previously established that PGCs are relying on global genome nucleotide excision repair (GG-NER) to repair UV-induced DNA lesions. When GG-NER is compromised and the DNA damage persists the *C. elegans* p53-like, CEP-1, protein is induced in PGCs and keeps them arrested. Using a forward genetic approach, we established an unexpected non-cell-autonomous regulation of the CEP-1-mediated DNA damage response (DDR) in PGCs via the somatic niche. The niche control of the DDR in PGCs is mediated by the translation initiation factor IFE-4 operating in the SGPs and communicated via FGF-like signalling. Moreover, we determined that the niche control mechanisms of the p53 response is highly conserved from worms to mammals. In mammals, the IFE-4 orthologue eIF4E2 regulates the p53 induction in hair follicle stem cells upon UV-induced DNA damage. Therefore, we propose that a better understanding of the non-cell-autonomous control of the p53-mediated DDR in *C. elegans* will be highly relevant to better understand this central tumour suppressor mechanism in humans.

To elucidate the somatic regulators of the DDR in PGCs, we used a targeted genetic approach and interrogated pathways that we hypothesised to regulate the response to genotoxic stress. We determined an important regulatory role for nutritional interpretation and neuronal signalling. Impairment in these signalling processes suppressed the PGC cell cycle arrest and germline development upon persistent DNA damage. To dissect the mechanisms of this somatic influence in more detail, current emphasis is put on tissue-specific transcriptomics on fluorescence activated cell sorting (FACS)-based PGC/SGP isolations. We show for the first time a neuronal influence on the non-cell-autonomous DDR in PGCs and broaden the understanding of somatic regulations on the germline of *C. elegans* thus challenging the Weismann barrier.

1081A Identification and characterization of DNA repair complexes by proteomics in *C. elegans*

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The integrity of DNA is constantly threatened by DNA damage induced by endogenous metabolic processes, environmental factors like chemicals and irradiation, or spontaneously via deamination and depurination. In order to remove those DNA lesions fast and efficiently, multiple DNA repair pathways can be activated. Nucleotide excision repair (NER) is a major DNA repair pathway which repairs a wide variety of helix-distorting lesions, especially those induced by UV irradiation. Two subpathways in NER can be discerned: global genome NER (GG-NER), which detects DNA damage anywhere in the genome, and transcription-coupled NER (TC-NER) that recognizes DNA damage that stalls RNA polymerase II in transcribed strands. Following detection, both subpathways converge onto the common core NER verification step, followed by excision of the damaged DNA strand and gap filling by novel DNA synthesis. Hereditary mutations in NER genes cause multiple different diseases, including the progeroid Cockayne syndrome and the cancer prone xeroderma pigmentosum. However, their exact pathogenesis is not fully understood.

We use *C. elegans* to understand the *in vivo* impact of DNA damage and the biological relevance of NER in a multicellular, developing organism. Previously, we showed that GG-NER primarily acts in proliferative germ cells and embryos, while TC-NER acts in post-mitotic somatic cells to maintain transcription. To better understand how DNA repair is organized in this tissue-specific manner, we developed a label free quantification proteomics pipeline with which we are able to isolate and characterize endogenous DNA repair complexes, both in unchallenged conditions and after UV irradiation-induced DNA damage. Using this method, we identified several previously unknown proteins that might regulate both NER initiation as well as the verification step. Currently, we investigate whether these proteins function in GG-NER and/or TC-NER, at which NER step they might function and whether their function is evolutionary conserved.

1082B Mild impairment of mitochondrial function increases longevity and pathogen resistance through ATFS-1-driven activation of p38-regulated innate immunity

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While mitochondrial function is essential for life in all multicellular organisms, a mild impairment of mitochondrial function can extend longevity. By understanding the molecular mechanisms involved, these pathways might be targeted to promote healthy aging. In studying two long-lived mitochondrial mutants in *C. elegans,* we found that disrupting subunits of the mitochondrial electron transport chain resulted in upregulation of genes involved in innate immunity, which we found to be dependent on not only the canonical p38-mediated innate immune signaling pathway but also on the mitochondrial unfolded protein response. Both of these pathways are absolutely required for the increased resistance to bacterial pathogens and extended longevity of the long-lived mitochondrial mutants, as is the FOXO transcription factor DAF-16. This work demonstrates that both the p38-mediated innate immune signaling pathway and the mitochondrial unfolded protein response can act on the same innate immunity genes to promote resistance to bacterial pathogens, and that input from the mitochondria can extend longevity by signaling through these two pathways. Combined, this indicates that multiple evolutionarily conserved genetic pathways controlling innate immunity also function to modulate lifespan.

1083C Mitochondrial unfolded protein response transcription factor ATFS-1 increases resistance to exogenous stressors through upregulation of multiple stress response pathways

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The mitochondrial unfolded protein response (mitoUPR) is an evolutionarily conserved pathway that responds to various insults to the mitochondria through transcriptional changes that restore mitochondrial homeostasis in order to facilitate cell survival. Gene expression changes resulting from the activation of the mitoUPR are mediated by the transcription factor ATFS-1/ATF-5. To further define the mechanisms through which the mitoUPR protects the cell during mitochondrial dysfunction, we characterized the role of ATFS-1 in responding to organismal stress. We found that activation of ATFS-1 is sufficient to cause upregulation of genes involved in multiple stress response pathways, including the DAF-16-mediated stress response pathway, the SKN-1-mediated oxidative stress response pathway, the HIF-mediated hypoxia response pathway, the p38-mediated innate immune response pathway, and antioxidant genes. Moreover, ATFS-1 is required for the upregulation of stress response genes after exposure to exogenous stressors, especially oxidative stress and bacterial pathogens. Constitutive activation of ATFS-1 increases resistance to multiple acute exogenous stressors, while disruption of *atfs-1* decreases stress resistance. Although ATFS-1-dependent genes are upregulated in multiple long-lived mutants, constitutive activation of ATFS-1 in wild-type animals results in decreased lifespan. Overall, our work demonstrates that ATFS-1 serves a vital role in organismal survival of acute

stresses through its ability to activate multiple stress response pathways, but that chronic ATFS-1 activation is detrimental for longevity.

1084A The microbiome-muscle connection: Native microbiota affect muscle ageing and motility

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Large-scale human metagenomic sequencing has identified associations between gut microbiome composition and host physiology, including immunity, nervous system function, and ageing. New findings in humans and model organisms suggest that the gut microbiome affects healthy ageing, and that the microbiome could be used to develop interventions to improve the way we age, but underlying mechanisms are not understood.

To define host-microbiome interactions affecting ageing we have established a new model system consisting of the nematode *C. elegans* combined with an experimental microbiome of 11 bacterial isolates representing the most abundant genera of *C. elegans* in the wild. Cultivation with the experimental microbiome preserves age-related motility, an effect that requires components of the p38 MAP kinase pathway, including *nsy-1* and *pmk-1.* The experimental microbiome also induces mitochondrial fragmentation in body-wall muscle in a non-*pmk-1* dependent manner, suggesting multiple routes of communication by which the experimental microbiome may modulate age-related motility.

In a transgenic proteotoxicity model expressing human Aß$_{42}$ in muscle, age-associated paralysis is suppressed by the experimental microbiome. Cell-free supernatant from the experimental microbiome suppresses paralysis and reduces Aß$_{42}$ aggregation *in vitro*, suggesting secretion of microbial bioactive compounds capable of abrogating Aß$_{42}$-associated toxicity. Together these findings show that molecular host-microbiome interactions modulate muscle function, mitochondrial dynamics and proteostasis during ageing to delay age-related decline in motility.

1085B Tyramine modulates the systemic stress response by stimulating the release of intestinal insulin like-peptides (ILPs)

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Multicellular organisms trigger a complex and coordinated response against systemic stress. We have recently shown that in *C. elegans*, the neural stress-hormone tyramine supplies a state-dependent neural switch between acute flight- and long-term environmental-stress responses (De Rosa et al, 2019). Tyramine release during the flight response, stimulates the DAF-2/Insulin/IGF-1 signaling (IIS) pathway and precludes the nuclear translocation of the DAF-16/FOXO transcription factor through the activation of an adrenergic-like receptor TYRA-3 in the intestine. We hypothesize that tyramine stimulates the release of agonist ILPs from the intestine which acts as an autocrine and/or paracrine signal to systemically activate the DAF-2/IIS pathway. To test this hypothesis we are screening ILPs mutants for their resistance to environmental stressors (oxidative and thermal stress). The *C. elegans* genome encodes 40 ILPs, 28 of which expressed in the intestine. We performed a screening of intestinal peptides described as strong DAF-2 agonist, by silencing individual intestinal ILPs and testing worm resistance to environmental stressors (oxidative and thermal stress). Thus, so far we found that *ins-3* and *ins-7* mutants are resistant to environmental stress, like tyramine-deficient and *tyra-3* mutants. We are further testing whether tyramine directly stimulates the release of these ILPs from the intestine through a Gq-protein mediated signaling pathway. These studies will provide insight into how a neurohormone coordinates systemic cellular stress responses.

1086C Host-microbiome interactions with age on *Caenorhabditis elegans* reproduction

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Interactions with and alterations of the gut microbiome with age can have a dramatic impact on our physiology. Changes in the membership of the gut microbiome can dictate levels of immunity, stress resistance and vitality across systems. Linking changes in the microbiome to microbial factors that may influence them has been a challenge in many systems due to the complexity of the microbiomes and intractability of following these interactions over an individual's lifespan.

To address this gap, we leveraged recent advances in characterization of the natural microbiome of *C. elegans* to examine both the gut microbiome on the aging process in *C. elegans* and vice versa. First, we utilized a 64-member microbiome (BIGbiome) and asked whether microbiome membership changed as animals age. Compared to *E. coli* OP50 controls, the BIGbiome

community had several significant impacts on three genetically diverse strains (lab N2, plus wild JU1400 and ED3017), including extended lifespan (up to 10%), improved healthspans (e.g., 36% at d5) and greater reproductive output (23% higher at d1 adults). In addition, increases in the levels of *Ochrobactrum* BH3, a dominant colonizer of the *C. elegans* gut, from 0% to 25% in the BIGbiome community yielded a dose dependent promotion of reproductive rates. This indicates that specific members (such as *Ochrobactrum*) may promote reproductive rates and healthy aging in *C. elegans*.

Next, we determined how *C. elegans* age may influence microbiome membership. To do this, we monitored gut microbiomes of single animals for each of the *C. elegans* strains for the first 10 days of adulthood. Each of the host strains exhibited gut microbiomes distinct from the surrounding lawn in early adulthood, though they differed in (i) dominant microbes at that age, and (ii) the extent of the lifespan with enriched microbes. This suggests that the ability to maintain a specific microbiome community may be driven by changes in host responses in *C. elegans*.

Last, we sought to examine the genes that mediate *Ochrobactrum* impact on the aging process. We screened a ~1000 microbiome regulators by RNAi for defects in *Ochrobactrum* colonization with age, then looked for altered reproductive rates in candidates (32 clones). Hits from these studies suggest that *Ochrobactrum* may be promoting reproduction via germline interactions directly. Together, our results highlight use of *C. elegans* as a model system for investigating microbiome influence on host aging.

1087A Conserved roles for *alh-6/ALDH4A1* in muscle function over the lifespan

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Environmental stress can negatively impact the rate of aging across organisms, however the impact of endogenously derived reactive oxygen species (ROS) that can stem from normal cellular metabolism remains less clear. Previous work identified mutations in *alh-6*, a highly conserved enzyme in the mitochondrial proline

catabolism pathway, that drive oxidative stress and accelerated aging in *C. elegans*. Mutations in *alh-6* activate the cytoprotective transcription factor SKN-1 in muscle tissues. Here we demonstrate that of 95 mutations selected for activation of SKN-1 in the muscle, all harbor mutations in *alh-6*. These mutations cluster to specific regions in the ALH-6 polypeptide and impact muscle health across the lifespan. Lastly, we identify variants in ALDH4A1, the human ortholog of *alh-6*, that differentially impact large muscle group function in adult adults. Taken together, our work uncovers mitochondrial proline catabolism as a critical component of normal muscle aging across species.

1088B Isocitrate lyase protects *Caenorhabditis elegans* from mitochondrial superoxide stress through activation of the mitochondrial unfolded protein response

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Mitochondrial energy metabolism produces ATP to support cellular activities at the same time that superoxide, the byproduct of electron transport, can either trigger signaling responses or post threats to cellular functions. Conserved superoxide dismutase (SOD) functions in protection by scavenging superoxide and converting to H_2O_2.

Eliminating mitochondrial SODs causes neonatal/embryonic lethality in both mice and flies, but not in the nematode *C. elegans*. *C. elegans* have 2 mitochondrial *sod* genes (*sod-2* and *sod-3*) while mice and flies have one. Eliminating mitochondrial *sod-2* can actually increase *C. elegans* lifespan. The mechanism causing *C. elegans* to be resistant to mitochondrial superoxide stress is still unknown.

A metabolic pathway, named glyoxylate shunt, distinguishes *C. elegans* from mice and flies. As an alternative branch to the tricarboxylic acid (TCA) cycle, the glyoxylate shunt is missing in both mice and flies. The *icl-1* isocitrate lyase gene encodes the key enzymes of the glyoxylate shunt in *C. elegans*. We found that *icl-1* is critical for protection against embryonic lethality when mitochondrial SODs are absent.

The mitochondrial *sod-2;sod-3* double mutant exhibits embryonic lethality in the range of 5% to 9%, which is associated with elevated *icl-1* gene expression. We crossed the *sod-2;sod-3* mutant to an *icl-1* null allele, *icl-1(ok531)*, and found that the embryonic lethality rose to ~ 60%. Thus, icl-1 protects against embryonic lethality in the absence of mitochondrial SOD.

To explore the mechanism by which *icl-1* protects against embryonic lethality, we examined its role in activating the mitochondrial unfolded protein response (UPR^{mt}). We found that *icl-1* is required for the efficient activation of the UPR^{mt} and the UPR^{mt} is required to suppress embryonic/neonatal lethality. We also found that the UPR^{mt} plays a significant role in protecting against mitochondrial superoxide stress, as the constitutive activation of UPR^{mt} via *atfs-1(gf)*can bypass the *icl-1* requirement.

Our study reveals a pathway that involves the *C. elegans* specific glyoxylate shunt and a much-conserved UPR^{mt} to counteract mitochondrial superoxide stress. This pathway might be manipulated in developing synthetic biological tools or innovative pharmacological methods to treat diseases that are related to mitochondrial superoxide stress.

1089C Increased susceptibility to proteostasis collapse in C. elegans following consumption of UV-irradiated bacteria

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The maintenance of protein homeostasis (proteostasis) involves the regulated synthesis, folding, trafficking and degradation of proteins and is crucial to proper cell function. A breakdown in the regulation of proteostasis can lead to the accumulation and aggregation of mis-folded proteins, thereby causing cell and tissue dysfunction. As cells age, the ability to maintain proteostasis declines, leading to the emergence of age-associated diseases such as Alzheimer's, Parkinson's, Huntington's and cardiovascular disease. Therefore, understanding the factors that contribute to proteostasis collapse is crucial for the development of strategies to promote healthy human ageing.

Recent work has demonstrated that the composition and activity of the gut microbiota is a crucial determinant of several aspects of long-term health in both humans and *Caenorhabditis elegans*. However, the effects of bacteria on host proteostasis are largely unexplored. Our research investigates the interplay between microbiota stress and host proteostasis, by observing the effects of UV irradiated bacteria on the ability of *C. elegans* to maintain proteostasis and suppress proteotoxicity throughout life.

Our initial findings suggest that consumption of irradiated bacteria by *C. elegans* during early adulthood increases the rate of proteostasis collapse. The rate of collapse is linked to the dose of irradiation that the bacteria is subjected to and the effects are observed in both wild type and mutant *C. elegans* fed on various strains of E. coli that are commonly used in *C. elegans* research.

We intend to investigate how these effects are mediated, using transcriptomics and genetic screening to identify the pathways within both bacteria and host cells that

influence age-related proteostasis collapse, thereby defining a new fundamental aspect of proteostasis regulation with implications for human health, common practices in *C. elegans* research and frequently used disinfection methods.

1090A Characterization of a membrane stress response to stabilize intracellular trafficking in *C. elegans*

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Membranes form the outer boundaries of cells and their organelles. Some organelles, such as the endoplasmic reticulum (ER), mitochondria and Golgi apparatus, are defined by the structure and composition of their membranes. Dysfunction in the structure or function of membrane-defined organelles results in activation of stress responses to stabilize and restore the organelles. Organelle stress responses can affect lifespan and the aging process and may be activated in other physiological conditions such as during lipid accumulation in metabolic disease. Membrane stress responses in the ER and mitochondria can induce pathologies due to metabolic dysfunction, and as such constitute a public health concern. However, the Golgi stress response, a nascent field of study, has not been as extensively investigated. Using *Caenorhabditis elegans* and human

cell lines, we have found that altering levels of the membrane phospholipid phosphatidylcholine (PC) changes Golgi function by inactivating a key intracellular trafficking enzyme, the small GTPase ARF-1/ARF1. Furthermore, work in our lab has shown that dysfunctional COPI retrograde as well as COPII anterograde trafficking induces upregulation of the lowly expressed *warf-1*/*arf-1.1* in *C. elegans*. Additionally, our data indicates that upregulation of *warf-1* is dependent on LET-607/CREB3. We hypothesize that changes in the lipid composition of the Golgi apparatus, such as reduced levels of PC, activates a dual stress response to restore lipid homeostasis by SBP-1/SREBP1 and stabilize Golgi trafficking by the compensatory WARF-1. *Warf-1* is unique to *C. elegans*, however, in many respects its regulation and function are reminiscent of human ARF4. Like ARF4, *warf-1* is upregulated in response to dysfunctional COPI retrograde trafficking by LET-607/CREB3. Furthermore, the N-terminal end of WARF-1 resembles that of ARF4 more closely than ARF1. It has been shown that this domain is required for class II ARFs to associate with ERGIC membranes during dysfunctional intracellular trafficking Consequently, investigating the *warf-1* membrane stress response will enhance our understanding of how human ARF4 functions during altered membrane lipid composition and stress to intracellular trafficking.

1091B Neural G protein-coupled receptor OCTR-1 regulates temperature effects on lifespan in *C. elegans*

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We have previously demonstrated that OCTR-1, an octopamine G protein-couple receptor, functions in the sensory neurons ASH to suppress the innate immune response in *Caenorhabditis elegans* by inhibiting the expression of immune genes (Sun *et al*. 2011 Science 332:729-732). Here we discover that OCTR-1 also regulates temperature effects on lifespan in *C. elegans*. At the normal growth temperature 20°C, *octr-1(ok371)* mutant animals have similar lifespan to wild-type animals. However, at higher temperature 25°C, *octr-1(ok371)* mutants live significant longer than wild-type animals. These results suggest that OCTR-1 may mediate temperature effects on lifespan. RNA-seq data analysis showed that 63 immune genes were significantly down-regulated in *octr-1(ok371)* mutants relative to wild-type animals at 25°C. We further demonstrated that inactivation of several most-downregulated genes by RNA interference in wild type animals significantly extended their lifespan, similar to the phenotype of *octr-1(ok371)* animals. These suggest a new molecular regulation mechanism that downregulation of immune genes extend the lifespan of *C. elegans*, which is opposite to the general belief that an increase in defense immunity extends lifespan. We are currently investigating the underlying molecular mechanism in detail and also examining if the thermosensory neurons AFD and the chemosensory neurons ASH are involved in this OCTR-1-mediated regulation on lifespan.

1092C Protein kinase DRL-1 is required for activation of stress responses in cuticle furrow mutants

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Extracellular matrices (ECMs) mechanically support tissues of multicellular organisms. Barrier ECMs at the surface of animals act as a first line defense to many environmental stressors. ECM regulation of stress responsive gene expression remains poorly understood in animals but has the potential to influence development, aging, and clinically relevant conditions such as tissue fibrosis and tumorigenesis. We and others identified annular furrows in *C. elegans*, repeating circumferential bands of collagen in the cuticle, as an ECM structure required for regulation of stress responses. Genetic disruption of furrow collagen genes (*dpy-2, 3, 7, 8, 9,* and *10)* induces organic osmolyte, detoxification, and antimicrobial genes in the absence of environmental stress. Mutation or loss of four non-collagen proteins secreted from epidermal cells (OSR-1, OSM-7, OSM-8, and OSM-11) activates the same stress responses without obvious changes to the cuticle indicating that other extracellular proteins are involved.

To identify downstream signaling mechanisms, we conducted an RNAi screen of protein kinases in *dpy-7* worms. Loss of *drl-1,* Dietary Restriction-Like kinase, suppresses stress response gene expression and acute osmotic stress resistance in *dpy-7* and *dpy-10* worms. Loss of *drl-1* also suppresses the same phenotypes in *osm-7* and *osm-8* worms. Alternatively, loss of *drl-1* does not reduce stress response gene expression under basal conditions or during oxidative stress consistent with specificity to extracellular signals. DRL-1 has a kinase domain homologous to mammalian MEKK-3, which has been implicated in regulation of cell osmotic responses *via* p38 MAPK. RNAi of *drl-1* was previously shown to increase fat metabolism and extend lifespan in *C. elegans* by a mechanism similar to dietary restriction. Genetic interaction analyses suggest that dietary restriction and p38 MAPK have no effect or function parallel to DRL-1, respectively. Therefore, DRL-1 likely regulates stress responses downstream from the extracellular signals *via* a distinct mechanism. This work was supported by National Science Foundation grant IOS-1452948.

1093A Investigating the dietary restriction phenotype caused by disrupted intestinal cell-to-cell communication

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Mutations in the intestinal gap junction subunit innexin-16 (*inx-16*) result in poor cell-to-cell communication in the intestine. This disrupts a calcium wave that flows about once every minute controlling the defecation motor program. An abnormal *inx-16* calcium wave leads to what appears to be a dietary restriction phenotype: extended lifespan and a smaller brood size laid over a longer period of time. We hypothesized that *inx-16* mutant worms exhibit this dietary restriction phenotype due to a lack of nutrient absorption. Di- and tripeptide uptake is accomplished by the transporter PEPT-1, which is dependent on the proton gradient across the apical intestinal membrane. The proton gradient itself is maintained by a sodium proton pump whose activity is regulated by calcium flux. The interrelated nature of calcium dynamics, the proton gradient, and di- and tripeptide uptake led us to predict that the *inx-16* mutation would suppress PEPT-1 mediated nutrient uptake.

To delineate the genetic and phenotypic relationship between *inx-16* and *pept-1* we have created an *inx-16; pept-1* double mutant and begun to compare the phenotypes of the single and double mutants. *pept-1* mutants' failure to absorb di- and tripeptides results in a number of salient phenotypes including a reduced brood size, a shorter body length, and increased thermotolerance that we have analyzed. Brood size comparisons reveal already more than 50% reduction in both *inx-16* and *pept-1* single mutants when compared to wild-type, whereas the *inx-16;pept-1* double mutant strain shows a more than 80% reduced fecundity. Analyzing the developmental profiles also suggests an additive effect in the double mutant. Other parameters are different between the two single mutants. Both single mutants display thermotolerance relative to wild-type, yet *pept-1'*s tolerance is more profound. This magnitude difference between single mutants is mirrored in a fluorescently labeled dipeptide uptake assay. *inx-16* mutants significantly differ from wild-type worms in dipeptide uptake. However, *inx-16* mutants still absorb dipeptide in selected intestinal regions, unlike *pept-1* mutants. To learn more about the mechanistic differences between the mutants, we are conducting RT-qPCR experiments to investigate what pathways may be disrupted in these strains. Taken together, our results suggest that the dietary restriction phenotypes in *inx-16* and *pept-1* mutants are at least partially due to disruptions in different pathways.

1094B Identification of *ccf-1* as a novel regulator of stress response and aging in *C. elegans*

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In a genome-wide RNAi screen to identify activators of *numr-1*, a cadmium responsive gene involved in RNA splicing regulation, we isolated *ccf-1* as a gene that is required for cadmium-induced *numr-1* activation. The *ccf-1* gene encodes a deadenylase within the CCR4-NOT complex that generally serves to suppress gene expression by initiating mRNA degradation. However, our RNAi screen suggests a novel role for *ccf-1* in positively regulating gene expression during stress. Silencing of *ccf-1* inhibits various classes of cadmium-inducible genes including several *glutathione-s-transferase* (*gst*) and *heat shock protein* genes. RNAi knockdown of *ccf-1* significantly reduces lifespan and decreases survival in cadmium, implicating a role for *ccf-1* in regulating aging and stress protection. The *ccf-1* gene is also required for resistance against acrylamide toxicity with RNAi depletion of *ccf-1* inhibiting acrylamide-induced *gst* induction, decreasing survival in acrylamide stress, and increasing *C. elegans* sensitivity to acrylamide-induced neurodegeneration. Using RNA-sequencing, we observed that *ccf-1* regulates ~28-35% of all genes induced by cadmium (500 out of 1802 DEG) or acrylamide (296 out of 851 DEG) by >2-fold. Clustering analysis of *ccf-1* dependent cadmium or acrylamide up-regulated genes indicate significant enrichment to glutathione and cytochrome P450 metabolism, suggesting a central role for *ccf-1* in regulating antioxidant defense in response to different stressors. Using a CCF-1::GFP translational reporter, we find that CCF-1 is broadly expressed in the intestine, muscle, and hypodemis. Interestingly, CCF-1::GFP strongly localizes to the intestinal nuclei, implicating a potential nuclear role for CCF-1 in transcriptional regulation that is distinct for its deadenylase function in the cytoplasm.

1095C Identifying Metabolic Alterations That Activate the UPR-ER in vivo

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Endoplasmic reticulum (ER) stress due to protein misfolding is observed in many diseases such as cancer, diabetes, and metabolic diseases. ER homeostasis can be restored by activation of the unfolded protein response (UPR-ER), which alleviates the molecular stress or induces cell death when damage is too severe. In higher eukaryotes, the UPR consists of three branches: The Inositol-Requiring-Enzyme 1 (IRE-1) branch, the protein kinase RNA-like ER kinase (PEK-1) branch, and the Activating Transcription Factor 6 (ATF-6) branch. When misfolded proteins accumulate in the ER lumen, these sensors activate downstream effectors, which together attenuate global translation and transcriptionally upregulate genes that restore homeostasis. Interestingly, abnormal membrane lipid composition also induces the UPR-ER, independent of protein misfolding. However, to date we lack a global view of genetic perturbations that activate the UPR-ER in metazoans. To identify proteotoxicity-independent metabolic pathways that affect ER homeostasis, I used RNA interference (RNAi) to inactivate 1247 metabolic genes in *Caenorhabditis elegans* with the IRE-1 branch specific transcriptional reporter, *hsp-4p::gfp*. After screening and rigorous validation, I obtained 34 high-confidence *xbp-1*-dependent hits that also activate the PEK-1 branch. Next, I used quantitative real-time PCR to show that 11 of 15 tested RNAi clones induce the endogenous UPR-ER in wild-type worms. Then I tested whether dietary choline supplementation, which suppresses UPR-ER in worms defective for phosphatidylcholine (PC) synthesis pathway, is sufficient to suppress UPR-ER activation in our hits. Of the 34 hits, 3 were partially rescued by dietary supplementation of choline along with the complete rescue of *sams-1* RNAi-treated animals, suggesting majority of the hits does not activate the UPR-ER via defective PC synthesis. Finally, I performed follow-up studies on one of the hit pathways on DNA replication. In early embryos, DNA replication stress induces UPR-ER activation, but not the mechanistically distinct cytosolic or mitochondrial UPRs, in a partially *ire-1*-, *xbp-1*-independent manner. This suggests that replication stress does not trigger global protein misfolding. Interestingly, genomic instability caused by loss of DNA repair pathways such as mismatch repair, nucleotide excision repair, and cohesin, which is essential for proper chromosome segregation, did not activate the UPR-ER, suggesting that molecular events specific to replication stress activate *hsp-4* and the UPR-ER in the embryo. In sum, by identifying new genes that affect UPR-ER homeostasis in *C. elegans*, my project provides new insights into UPR-ER regulation and may serve as a starting point for the discovery of drug targets for human diseases featuring UPR-ER dysfunction.

1096A Identifying Downstream Factors in *efk-1*/eEF2K-mediated Starvation Resistance in *C. elegans*

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During nutrient deprivation, cells protect themselves by shutting down energy-demanding processes such as protein synthesis. EFK-1/eEF2K (eukaryotic elongation factor 2 kinase) is a conserved kinase that responds to starvation by inactivating EEF-2/eEF2, the rate-limiting driver of translation elongation, thus blocking mRNA translation to conserve energy. In *C. elegans*, the eEF2K ortholog *efk-1* is transcriptionally induced in starvation and is essential for L1 starvation survival. However, little is known about the factors that promote starvation survival downstream of *efk-1*. Interestingly, *Pseudomonas aeruginosa* virulence factor ToxA also causes EEF-2 inhibition, which activates the transcription factors (TFs) ZIP-2/bZIP and CEBP-2/CEBPG downstream; additionally, eEF2K also regulates p53 in cancer cells. Thus, we asked if the TFs *zip-2*, *cebp-2*, and *cep-1* function in starvation survival downstream of *efk-1*. Using the population starvation survival assay, we found that these factors are indeed required for L1 starvation survival. Next, we asked whether these TFs function in the *efk-1* pathway. We found that *efk-1;zip-2* and *efk-1;cep-1* double mutants do not exhibit synthetic starvation survival defect compared to single mutants, indicating that these TFs act in the *efk-1* pathway. Next, to characterize the pathways regulated by EFK-1, ZIP-2, and CEP-1 in starvation, we performed gene expression profiling by RNA-seq on wildtype and *efk-1*, *zip-2*, and *cep-1* null mutants in fed and starved conditions. Consistent with functional data, the transcriptomic profiles of starved *zip-2*, *cep-1*, or *efk-1* mutants correlate well with each other. Additionally, we found that expression of DNA repair pathways, such as nucleotide excision repair (NER), were elevated in starved wildtype worms, but attenuated in *efk-1*, *zip-2*, and *cep-1* mutants, suggesting that EFK-1 activates DNA repair during starvation via ZIP-2 and CEP-1. As follow-up, we used the starvation survival assay to confirm that NER factors such as XPA-1 and others are also required for L1 starvation survival. Furthermore, by assaying starvation survival of the *efk-1;xpa-1* double mutant, we found that *efk-1* and *xpa-1* function in the same pathway. In the future, we plan to confirm the role of EFK-1 in starvation-induced DNA repair by quantifying DNA damage in fed and starved wildtype and *efk-1* mutant worms. Overall, our study has identified a new possible downstream mechanism by which *efk-1*/eEF2K promotes starvation stress resistance.

1097B Recovery of Muscle Function Dependent on the Impaired Cell Death in a *C. elegans* Premature Aging Model

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In mammals including humans, the age-related losses of muscle mass and function are known as sarcopenia. Although the mechanisms underlying the development of sarcopenia are multifactorial, apoptosis has been shown to play a role in muscle loss during aging [1]. Recently, age-related muscle loss has also been found in the nematode *C. elegans* during normal aging. As in humans, age-related muscle loss in *C. elegans* leads to decreased mobility and serves as a marker for increased mortality [2, 3]. Here we examined the difference in age-associated pharyngeal muscle function between the short-lived *mev-1(kn1)* and the phenotype of deficient cell death (*mev-1;ced-3* mutant), however, have shown no significant histopathological change in the loss of pharyngeal muscle mass. 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 shortenes the lifespan [4]. Moreover, we aim to detect age-related difference in gene expression concerned with the cell death signaling using both mutants.

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1098C Developing a System to Screen Microbiome-Targeted Neurodegeneration Therapeutics Using Automated Monitoring of *C. elegans*

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Neurodegenerative diseases predominantly affect older cohorts, but the first misfolding processes occur earlier in life, prior to diagnosis. Therefore, the ability to monitor health prior to the onset of severe symptoms will be informative in the search for preventative interventions and in understanding disease onset. There are several *C. elegans* models of neurodegeneration but manual assays are subjective, labour intensive and provide binary data at single time points per animal – namely time of paralysis. An automated imaging system to monitor worm movement can assist by simultaneously revealing in-depth movement data unavailable to manual methods and scaling to study hundreds of plates of animals more easily.

We have developed a technology that monitors plates of 6 cm petri dishes, with 30 worms on each. Using strains expressing polyglutamine tracts and amyloid-beta, we show that the presence of these transgenes is associated with early-life movement decline. Furthermore, there are differences in decline pattern dependent on whether the polyQ tract is expressed in body wall muscle or pan-neuronally, and on what treatments are used to prevent progeny such as FUdR or temperature sensitive sterile backgrounds. These findings allow the development of a robust system on which to screen mutants and pharmaceutical therapies. Our particular interest is drugs that influence *E. coli* as a model for microbiome interventions. We have found that inhibiting bacterial folate synthesis using sulfamethoxazole (SMX) extends *C. elegans* lifespan and healthspan, and here we also show the effect of SMX on various neurodegenerative models. This approach therefore becomes a model of gut microbiome-host interaction which can streamline the selection of therapies for further testing on mammal models in the drug discovery pipeline.

1099A Elongator complex modulates longevity by modifying tRNA nucleotide in C.elegans

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Animals adjust their protein synthesis according to nutrient availability. Protein translation is a key cellular process that couples the environment signals to the animal physiology. Here we show that Elongator complex (ELPC) is essential for the formation

of mcm side chain on the uridine at the wobble position of tRNA in *C. elegans*. Lack of uridine modifications in the Elongator mutants leads to reduced translation efficiency. The thiolation of wobble uridine is mediated by NCS-1. A dramatic reduction of protein synthesis is observed when both mcm side chain and thiolation are abolished at wobble uridine in *elpc; ncs-1* double mutants. Both *elpc* and *ncs-1* mutants have increased lifespan, which is further extended in the *elpc; ncs-1* double mutants. We did not observe an additive effect on longevity of tRNA modification mutants together with a reduced TOR signaling, both of which affect protein synthesis. Similar to the other mutants with reduced translation efficiency, *elpc* and *ncs-1* mutants also display increased tolerance to several stress conditions including starvation, heat and oxidative challenges. Wobble uridine modifications are used to decode AAA and CAA, and we confirmed that the translation of the genes enriched in AAA and CAA codons are severely impaired in *elpc*; *ncs-1* double mutants. Our observations demonstrate that Elongator complex modulates multiple cellular processes by regulating protein synthesis.

1100B Steroid Hormone Pathways Coordinate Developmental Diapause and Olfactory Remodeling

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Developmental and behavioral plasticity allow animals to prioritize alternative genetic programs during fluctuating environments. In nematodes that interact with host organisms, reproductive adults and the developmentally arrested, host-seeking larvae are likely to have different responses to odorants. To understand the genes that coordinate development and behavior, we used *Pristionchus pacificus* to characterize two dauer-constitutive mutants that form dauer larvae in the presence of food and at ambient temperatures (Daf-c). Both Daf-c alleles are recessive, temperature-sensitive, and incompletely penetrant, with a subset of the emerging DL exhibiting a transient, dauer-specific exsheathment defect. *csu60* mutant larvae committed to dauer entry spend a prolonged time as pre-dauer J2 larvae (J2d). Like dauers, the mutant J2d rarely ingest fluorescent latex beads, but they lack resistance to SDS. Both *P. pacificus* Daf-c alleles disrupt steroid synthesis required for proper regulation of the conserved canonical steroid hormone receptor DAF-12, whose dauer-constitutive and cuticle exsheathment phenotypes can be rescued by the feeding of Δ7-dafachronic acid (7DA), a ligand for DAF-12. The *csu60* allele has a deletion of the sole HydroxySteroid Dehydrogenase (HSD) in *P. pacificus*, and a transgenic *Ppa-hsd-2* reporter shows expression in CAN neurons, intestine, and excretory gland cells in all stages, and excretory canal expression in dauers only. *csu60* mutants exhibit ectopic expression of *Ppa-odr-3p::rfp* in an extra pair of amphid neurons. Unlike *C. elegans*, both the wild-type and mutant DL show repulsion to CO_2. Both *hsd-2*(*csu60*) adults and dauers show enhanced attraction to a beetle pheromone, possibly due to the heterochronic activation of dauer-specific neuronal development in the adults. While this enhanced odor attraction can be rectified by a *Ppa-hsd-2* rescue transgene, the enhancement acts independently of *Ppa-daf-12*, revealing unexpected decoupling of dauer formation and dauer-specific host-seeking behavior upstream of the conserved 7DA/DAF-12 module. Given that mutations in *Ppa-daf-22*, *Ppa-daf-19*, *Ppa-daf-21*, and several TGF-β homologs do not exhibit dauer formation phenotypes, we speculate that the genetic regulation for dauer entry has significantly diverged between *C. elegans* and *P. pacificus*.

1101C Peptidergic modulation of dispersal behavior in pathogenic and free-living nematodes

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Emerging evidence suggests that behavioral changes associated with host finding of pathogenic nematodes may be regulated by neuropeptides. Hence, we started from peptidomic discovery in such a species, *Steinernema carpocapsae*, to direct functional discovery. We observe numerous similarities between the well-studied *C. elegans* peptidome and the *S. carpocapsae* peptidome, which we further exploit to understand neuropeptidergic contributions to regulating nictation, an evolutionary conserved behavior for foraging in (these) nematodes.

An in-house method based on acidified methanol was used to extract endogenous neuropeptides of *Steinernema carpocapsae* infective juveniles, a life stage similar to the *C. elegans* dauer stage. Neuropeptide identification was done by state-of-the-art UHPLC-MS/MS. We detected 30% (139) of the predicted peptidome in these infective juveniles, which provides a resource for comparison with the *C. elegans* dauer peptidome (in house). We hypothesized that nictation-relevant peptides will be abundant in infective juveniles and/or dauers, and prioritized these for functional assays.

Out of several tested target genes, we found at least one neuropeptidergic signaling system that is involved in modulation of nictation behavior, which we assayed using microdirt arenas. In addition, using phylogenetic analyses, we aim to get a better global understanding of conserved peptidergic signaling systems in parasitic and free living nematodes.

Steinernema spp. are used as eco-friendly alternative for chemicals to combat pest insects. Knowledge on neuropeptidergic regulation of host-finding strategies will help understand how entomopathogenic nematodes regulate their behavior. This should contribute to improving their applicability and host specificity in the field.

1102A Molecular and neuronal mechanisms underlying early experience-dependent chemosensory plasticity in *C. elegans*

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Responses of animals to food or chemical stimuli can vary based on their internal state and experience. Work from our lab and others previously showed that adult animals which transiently experienced stress-induced dauer arrest during development (post-dauer) exhibit distinct gene expression profiles and behavioral responses compared to control adults which bypassed the dauer stage. We show that post-dauer adults exhibit significantly enhanced responses to volatile attractive odors, including odors sensed by the AWA and AWC olfactory neurons, as compared to control adults that bypassed the dauer stage. In contrast, responses of post-dauer adults to repellents is unaffected. We find that dauer larvae and post-dauer adults upregulate the diacetyl olfactory receptor ODR-10 in the AWA chemosensory neuron pair. Expression of this receptor in AWA neurons is known to drive food searching behavior in adult worms and is regulated in a sexual dimorphic manner. The dauer-induced upregulation of this receptor in AWA neurons requires the stress response transcription factor DAF-16/FOXO. Via spatiotemporally regulated depletion of DAF-16 in AWA, we have established that DAF-16 acts early in development prior to dauer entry to transcriptionally upregulate *odr-10* expression, and that it is dispensable for the maintenance of higher expression levels in post-dauer adults. Our data suggest a model in which early developmental stress is encoded at the level of regulation of olfactory receptor gene expression, and may modulate behavioral differences between adult animals based on their respective developmental experiences. To determine whether additional sensory genes are also subject to similar experience-dependent regulation, we have isolated AWA neurons via FACS from control and post-dauer L4s and are performing differential expression analyses. Future goals will be to determine the contributions of these differentially expressed genes to sensory behavior, and to define the *cis*- and *trans*-regulatory mechanisms that underlie this plasticity in gene expression and behavior.

1103B *daf-42* is an Essential Gene for development into diapause stage in *Caenorhabditis elegans*

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Dauer is a crucial diapause stage in nematodes as it facilitates phoresy from adverse to suitable and reproducible environment. In *C. elegans*, young larvae are able to integrate surrounding environmental conditions and develop into dauer stage instead of reproducing adult in adverse conditions. The worms are then arrested at dauer stage until they encounter favorable environment and resume development into adult stage. While studies on dauer formation *in C. elegans* have provided insights in the decision processes prior to dauer commitment, the downstream factors that directly participate in dauer development have remained elusive. This is in part due to difficulty in generating dauer stage-specific lethal mutants by random mutagenesis. We have discovered a novel mutant strain that exhibits developmental defect specifically during dauer development and identified its causative nonsense mutation in *daf-42* gene. *daf-42* mutants develop normally into reproducing adult animals under standard laboratory conditions, but they become trapped in their own cuticle and fail to molt into dauer larvae in dauer-inducing conditions. DAF-42 is expressed during dauer entry in hypodermis, according to RNA-seq and transcriptional reporter analysis. As *daf-42* is known to have homologs in other *Caenorhabditis* species, we seek to elucidate both molecular mechanism of *daf-42* in dauer development and its evolutionary conservation among nematode species. [This work is supported by research grants through Samsung Science and Technology Foundation under Project number SSTF-BA-1501-14 and the National Research Foundation of Korea (NRF-2014-Global Ph.D. Fellowship Program)]

1104C An excitatory GABA receptor, EXP-1 switches odor preference and regulates metabolic plasticity in *C. elegans*

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C. elegans like many other organisms have the remarkable ability of sensing changes in the external environment and accordingly shifting their metabolism from the reproductive state to the dauer diapause state that occurs during adverse

conditions. This regulatory plasticity is largely dependent upon olfaction since it provides essential dietary cues to choose between reproduction or long lasting survival strategy by shifting to developmental arrest. Innate sensory preferences towards attractants and repellents are defined by specific sensory neurons. The AWC and AWA sensory neurons detect volatile attractants, whereas AWB, ASH and ADL sense repellents. I will present data showing that EXP-1, an excitatory GABA receptor functions in a non-cell autonomous manner to regulate AWC dependent chemotaxis. We found that in the absence of *exp-1* from the ASI neuron, there is an attractant to repellant switch towards AWC sensed odorants. This extrinsic defect leads to changes in the intrinsic metabolism that results in a shift towards stress resistant dauer stage in a DAF-7 (TGF-ß) dependent manner. Our results also reveal that in the absence of *exp-1*, AMPA-type glutamate receptor levels are altered in amphid neurons and we are investigating how this process allows for a switch in the AWC dependent response to odorants. Together our data indicates that EXP-1 regulates a complex signaling network by integrating signals from multiple cells to perceive the environment and adapt to it by choosing between growth or dauer formation.

1105A Disruption of mitochondrial factor SDHA-2 affects sperm motility and male fertility

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Sperm quality is essential to male fertility. Mitochondrial health contributes to sperm quality, but the precise role of mitochondria in sperm function is not fully understood. SDHA is a component of the succinate dehydrogenase (SDH) complex and plays a critical role in mitochondria in both the citric acid cycle and mitochondrial respiration. In the citric acid cycle SDHA converts succinate to fumarate. Additionally, this reaction contributes electrons to the electron transport chain, responsible for driving ATP synthesis. *C. elegans* have two orthologues of SDHA, SDHA-1 and SDHA-2.

Here, we show that mutation in *sdha-2* results in dramatically reduced male fertility due to defective sperm activation. We found that *C. elegans* harbouring a *sdha-2* SNP produce a significantly diminished brood size, one fifth of that of wild-type animals. In vitro sperm activation assays reveal that most mutant sperm do not activate from spermatids to spermatozoa, failing to grow the pseudopod required for motility. As a result, mutant sperm fail to localise to the spermatheca in hermaphrodites, the site of oocyte fertilisation. Instead, sperm display aberrant localisation throughout the uterus. We repaired the *sdha-2* SNP in the endogenous locus to wild-type sequence using CRISPR-Cas9 and demonstrated complete rescue of brood size and sperm activation.

We identified similar sperm motility defects in *sdha-1* mutant animals, further implicating succinate dehydrogenase function, and in *icl-1* mutant animals. ICL-1 catalyzes the cleavage of isocitrate to succinate and glyoxylate in the glyoxylate cycle. This suggests that an imbalance in succinate may underlie the dramatic brood size defect, a hypothesis that we have tested by performing metabolomics on these strains.

Our results demonstrate a role for SDHA-2 in sperm motility and male reproductive health. In humans, SDH activity is positively correlated with sperm quality, and mutations in SDHA are associated with Leigh Syndrome. This strain may provide a new animal model of human SDH deficiency-associated infertility and other pathologies.

1106B Determining the Effects of 1-Hydroxyphenazine Exposure on UGT Mutants in *Caenorhabditis elegans*

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Caenorhabditis elegans is an ideal model organism for studying the xenobiotic detoxification pathways of various natural and synthetic toxins. One such toxin that has been shown to cause death in *C. elegans* is 1-hydroxyphenazine (1-HP), a molecule produced by the bacterium *Pseudomonas aeruginosa.* Prior research in our lab has shown the median lethal dose (LD50) for 1-HP in *C elegans* is 179 µM in PD1074 and between 150-200µM in N2.(Asif et al., 2021; Stupp et al., 2013). Prior research has also shown that *C. elegans* detoxifies 1-HP by glycosylating it with one, two, or three glucose molecules in N2 worms.(Stupp et al., 2013) We hypothesize that UDP-Glucuronosyltransferase (UGT) enzymes are responsible for glycosylating 1-HP in *C. elegans*. To identify UGT enzymes implicated in the glycosylation of 1-HP in PD1074, we have implemented our plate-based toxicity assay developed in our prior work on available UGT strains. We began by testing eight UGT mutants, UGT-1, UGT-6, UGT-9, UGT-23, UGT-49, UGT-60, UGT-62, and UGT-66, at the LD50 concentration of 1-HP in PD1074. We screened for mutants with a different mortality rate to N2 and PD1074 worms. Additionally, we will perform HPLC/UV analysis and NMR analysis in order to describe the differences in glycosylation patterns and the ratios of glycosylated and unglycosylated products in mutant strains with differential susceptibility to 1-HP than N2 and PD1074 worms. This could help explain the variation in mortality rates between the different strains and help us understand the complexity of UGTs in *C. elegans.*

1107C Lipid-Metabolic Genes that Coordinate Innate Immunity and Fertility

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Fertility and immunity are both energy intensive metabolic programs. Energy allocation by an organism to one process is a trade-off at the cost of the other. Lipids are an energy-rich resource whose mobilization is integral to both reproductive success and pathogen defense. We are investigating the role of lipid-metabolic pathways in the immunity-fertility dialogue through infection of the nematode *Caenorhabditis elegans*, a well-established molecular-genetic model organism, by the human opportunistic pathogen *Pseudomonas aeruginosa* (PA14).

Previously, we demonstrated that PA14 infection dramatically reduces fertility and that TCER-1, worm homolog of a human transcription elongation and splicing factor TCERG1, protects host reproductive fitness in the presence of pathogen. Our recent data suggest that PA14 infection causes rapid depletion of stored lipids in somatic tissues, but that lipid stores in developing oocytes are protected from depletion by TCER-1. Thus, TCER-1 diverts energetic resources toward reproduction. In a preliminary RNA-seq study, we identified several lipid-metabolic genes antagonistically regulated by PA14 infection and TCER-1 activity. We continue to interrogate the role of TCER-1 and its targets in balancing resource allocation between the competing demands of immunity and reproduction. These results will be discussed.

1108A Unravelling the identity of phosphorylcholine-transferring enzymes in *C. elegans*

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The attachment of phosphorylcholine on carbohydrates (PC-glycans) is a common modification for nematodes including *C. elegans*. The presence of PC appears to be important for the development of nematodes and for immunomodulation by parasitic nematodes. PC-glycoprotein ES-62 of *Acanthoceilonema vitae* directs immune cells towards an anti-inflammatory phenotype and this immunomodulatory property appears to be dependent on the presence of PC-glycans. This makes glycoproteins with PC-glycans interesting therapeutic agents to combat immune disorders such as rheumatoid arthritis, systemic lupus erythematosus and asthma. However, the biosynthetic pathway of PC-glycans is not completely understood, where especially the identity of the PC-transferring enzyme, or PC-transferase, is not fully elucidated. There are strong indications suggesting that fukutin-related genes potentially encode for this PC-transferase. In this study, we examined whether four selected fukutin-related genes of *C. elegans* (W02B3.4, T07A5.1, T07D3.4 and Y22D7AL.11) are involved in the biosynthetic pathway of PC glycans. With CRISPR/Cas9 technology we created *C. elegans* knock-out lines for each fukutin-related gene, but no significant reduction of PC was observed. Interestingly, one mutant line of W02B3.4 showed an increase of PC due to a potentially introduced signal peptide, indicating that the W02B3.4 gene could encode for a PC-transferase. Currently, we are combining mutant lines into double/triple/quadruple knock-out lines, which may provide more insight whether these fukutin-related genes encode for PC-transferases. These findings will contribute to our understanding of the pathway for PC-glycan biosynthesis, offering potential opportunities for design and synthesis of PC-glycan therapeutics.

1109B Selective Control of Parasitic Nematodes with Bioactivated Nematicides

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Global food security is threatened as the world amasses 10 billion people amid limited arable land. While nematode pests are a major barrier to agricultural intensification, most traditional nematicides are now banned because of poor nematode-selectivity, leaving farmers with inadequate controls. Here, we describe a screen carried out in the model nematode *Caenorhabditis elegans* that enriches for selective nematicides by identifying molecules that are bioactivated by cytochrome

P450s, which are phylogenetically diverse. We identify a family of structures, called nemactivins, that are robustly bioactivated to a toxic metabolite selectively in nematodes. At low parts-per-million concentrations, nemactivins perform comparably well with commercial nematicides at controlling infection by the world's most destructive plant-parasitic nematode *Meloidogyne incognita*. Hence, nemactivins are first-in-class bioactivated nematicides that provide much needed nematode-selectivity.

1110C Developing *C. elegans* models for SRD5A3-CDG and Cori rare congenital diseases

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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 defined genome and because many genes are highly conserved between *C. elegans* and humans, it is possible to investigate the underlying pathways of these diseases. In this study, we characterize two rare congenital diseases: SRD5A3-CDG, a congenital disorder of glycosylation type 1q and

Cori disease, a glycogen storage disease type III. Current literature mainly shows clinical data concerning these diseases.

SRD5A3-CDG disease is caused by a mutation in steroid 5 alpha-reductase 3 gene (*SRD5A3*), leading to significant psychomotor, cognitive and visual impairments. *C. elegans* has an orthologue of *SRD5A3* called *B0024.13* along with deletion (*B0024.13(deletion))* and point mutation (*B0024.13(W6X)*) resulting in viable animals suitable for experimentation. *B0024.13 C. elegans* strains show significant motor impairments, neurodegeneration as well as neurodevelopmental delay.

Cori disease is caused by a mutation in *AGL* gene coding for glycogen debranching enzyme which is involved in the glycogenolysis pathway. Clinically, Cori patients have significant hyperplasia of the liver, hypoglycemia, mental retardation and myopathy. The *C. elegans* orthologue of the Human *AGL* gene is *agl-1*. Our work with whole gene deletion (*agl-1 (deletion))* and point mutation strains (*agl-1 (W1044X), agl-1(S1444R*)) showed significant movement impairments, neurodegeneration, and interestingly, a glycogen accumulation phenotype which make them suitable for drug screening experiments. An update of our work will be presented.

1111A UFD-2 modulates the E4 activity of the chaperone-assisted E3 ligase CHN-1/CHIP to regulate organismal proteostasis and lipid metabolism.

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E3 ubiquitin ligases mediate the transfer of ubiquitin to a target protein (ubiquitylation). This process can be assisted by the ubiquitin chain elongation factor (E4) that enhances the polyubiquitylation of substrates. Chaperone-associated U-box protein CHIP can be involved in E4-like function when interacting with other ubiquitin ligases. However, little is known about CHIP direct interplay with E3s and the occurrence and regulation of its E4 activity. Here, we apply an integrative in vitro and in vivo approach to show that UFD-2, a U-box E3 enzyme, triggers the E4-like action of CHIP. Our data indicate that UFD-2 uses short, acidic peptide sequences to interact with the TPR domain of CHIP. This changes the flexibility of the U-box domain, allowing CHIP to work more efficiently with E2 conjugating enzymes boosting the synthesis of polyubiquitin chains. Hsp70 chaperone, a partner protein of CHIP, can negatively regulate the E3/E4 activity of CHIP and its interaction with UFD-2. By employing *Caenorhabditis elegans* as a model system, we show that the cooperation of CHIP and UFD-2 affects global proteostasis. Moreover, we demonstrate that the CHIP/UFD-2 pair influences lipids metabolism by directly regulating S-Adenosylhomocysteinase.

1112B A Genetic Titration of Membrane Composition Reveals its Importance for Multiple Cellular and Physiological Traits

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The composition and biophysical properties of cellular membranes must be tightly regulated to maintain the proper functions of myriad processes within cells. To better understand the importance of membrane homeostasis, we assembled a panel of five *C. elegans* strains that show a wide span of membrane composition and properties, ranging from excessively rich in saturated fatty acids (SFAs) and rigid to excessively rich in polyunsaturated fatty acids (PUFAs) and fluid. The genotypes of the five strain are, from most rigid to most fluid: *paqr-1(tm3262) paqr-2(tm3410), paqr-2,* N2 (wild-type), *mdt-15(et14) nhr-49(et8),* and *mdt-15(et14) nhr-49(et8) acs-13(et54)*. We confirmed the excess SFA/rigidity-to-excess PUFA/fluid gradient using the methods of fluorescence recovery after photobleaching (FRAP) and lipidomics analysis. The five strains were then studied for a variety of cellular and physiological traits and found to exhibit defects in: permeability, lipid peroxidation, growth at different temperatures, tolerance to SFA-rich diets, lifespan, brood size, vitellogenin trafficking, oogenesis and autophagy during starvation. The excessively rigid strains often exhibited defects in opposite directions compared to the excessively fluid strains. We conclude that deviation from wild-type membrane homeostasis is pleiotropically deleterious for numerous cellular/physiological traits. The strains introduced here should prove useful to further study the cellular and physiological consequences of impaired membrane homeostasis.

1114A Loss of adaptor protein complexes bypasses *mrp-5* deficiency and restores heme deficit in *Caenorhabditis elegans*

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Metazoans require heme as an essential cofactor and synthesize it in the mitochondria. *Caenorhabditis elegans* and related helminths are an exception as they are unable to synthesize heme *de novo* but instead acquire heme from dietary sources for their growth and development. Heme is imported from the apical surface of the intestine by HRG-1-related importers and exported by MRP-5/ABCC5 from the basolateral surface. Loss of *mrp-5* causes heme deficiency in extra-intestinal tissues and embryonic lethality, which can be suppressed by dietary heme supplementation raising the possibility that heme export in the absence of *mrp-5* could be mediated by alternate pathways. Here we show, by performing a forward genetic screen in an *mrp-5* null mutant, that loss-of-function in vesicular transport adaptor protein complexes fully restores heme homeostasis and normal growth and development in *mrp-5* mutants. We screened over 160,000 haploid genomes and identified thirty-two bypass suppressors of *mrp-5(ok2067)*. Deep-sequencing and variant analyses revealed three suppressors, each containing mutations in different adaptor protein 3 (AP3) complex subunits. RNAi depletion of individual subunits for either AP3 or AP2 phenocopies the *mrp-5* bypass suppressors. Remarkably, loss of AP3 subunits in the *mrp*-5 mutants reversed intestinal accumulation of fluorescent heme analog; resistance to gallium protoporphyrin IX toxicity; extra-intestinal heme deficiencies; and neuronal abnormalities including glial migration and synaptogenesis. Taken together, our results show that cargo transport by vesicular adaptor proteins play a previously unanticipated role in systemic heme homeostasis and that multiple routes govern intra- and intercellular heme trafficking in metazoans.

1115B Characterizing the roles of ETS-4 transcription factor in fat metabolism

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Obesity is a global health problem associated with many comorbidities, sparking interest in molecular mechanisms controlling body fat. Previously, we found that fat accumulation in *C. elegans* requires the endoribonuclease REGE-1/Regnase-1, which inhibits the expression of transcription factor ETS-4. Analyzing gene expression dependent on ETS-4, we noticed increased expression of a peptide transporter, PEPT-1, previously implicated in fat regulation. Currently, we investigate the potential link between ETS-4 and PEPT-1-mediated fat loss, and will present our results so far.

1116C A fat-promoting botanical extract from *Artemisia scoparia* acts as longevity modifier in *C. elegans*

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Like other biological processes, aging is not random, but subject to molecular control. Natural products that act on conserved metabolic pathways may provide entry points to extend animal lifespan and promote healthy aging. Here, we show that a botanical extract from *Artemisia scoparia* (SCOPA), which has previously been reported to promote fat storage and metabolic resiliency in mice, exerts pro-longevity effects on the nematode *Caenorhabditis elegans*. We find that wild-type *C. elegans* treated with SCOPA show significantly higher fat levels than controls but live nearly 40% longer. The SCOPA-mediated changes

to fat accumulation and lifespan require the transcription factor DAF-16/FOXO, which we find shuttles into the nucleus upon SCOPA treatment. Notably, the expression of DAF-16-targeted delta-9 desaturases dramatically increases in SCOPA-treated animals; these desaturases govern SCOPA's effects on fat content and are partly required for the SCOPA-dependent lifespan extension. We also find that SCOPA can enhance lifespan even when its administration is initiated at mid-adulthood, supporting its potential application as an intervention. In addition to their extended lifespan, SCOPA-treated animals show some signs of improved health during aging, including superior stress resistance. Thus, our data suggest that SCOPA-treated worms are fatty but metabolically healthy, and that they live long in part due to altered fat regulation. These findings add to emerging evidence indicating that elevated fat can be pro-health, and even pro-longevity, in some contexts.

1117A Synthesis and trafficking of mitochondrial phospholipids determines survival under hypoxia

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Oxygen deprivation, referred to as hypoxia, entails extensive reprogramming of intracellular energy metabolism. For example, reduction of mitochondrial respiration and enhancement of glycolytic metabolism are commonly observed in hypoxic cells. These adaptations are mainly orchestrated by the hypoxia-inducible factor 1 (HIF-1). Interestingly, recent studies have shown that mitochondrial respiration is necessary for tumor growth under hypoxic conditions. However, the molecular mechanisms that adjust mitochondrial function upon oxygen deprivation remain elusive. We find that, while impairment of mitochondrial oxidative phosphorylation (OXPHOS) triggers HIF-1 activation, this is not sufficient for survival under oxygen limitation. By contrast, both mitochondrial OXPHOS and glycolysis are required for hypoxia resistance. To gain further insight into hypoxia resistance mechanisms, we conducted a genetic screen for mitochondrial genes involved in HIF-1-independent survival under hypoxia. We found that the mitochondrial phospholipid trafficking mediator MDMH-35 and its interacting partners B0334.4 (PRELID-1) and F15D3.6 (PRELID-3), contribute to preserve mitochondrial function and confer resistance to hypoxic stress. In addition, we show that PRELID-1 and PRELID-3 antagonistically regulate mitochondrial phosphatidylserine (PS) trafficking and phosphatidylethanolamine (PE) synthesis. Together these findings indicate that balancing mitochondrial phospholipid content is necessary for survival under hypoxia, independently of HIF-1.

1118B The role of O-GlcNAc in fertility of *C. elegans* males

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Mutations in the gene encoding O-GlcNAc transferase lead to embryonic lethality or a severe form of X-linked intellectual disability in humans. This disorder presents with developmental delay, low IQ, and several other developmental defects. To model this disorder, we turned to *C. elegans*, unique in model organisms in its fully mapped development and in that worms survive without O-GlcNAc. This post-translational protein modification is involved in diverse biological processes including nutrient signaling, signaling, and development. Deletion of the O-GlcNAc transferase gene (*ogt-1*) results in *C. elegans* males with reduced ability to successfully mate. This phenotype likely impacts signaling or development and may provide insights into which pathways are involved in this disorder.

Compared to wild-type male controls, *ogt-1* males have a four-fold reduction in mean offspring, with nearly two thirds failing to produce any offspring at all. Fluorescent sperm tracking assays revealed that *ogt-1* males transfer fewer sperm to their mates. We also determined that *ogt-1* males show an increased incidence of developmental defects in male tail structures important to mating. Together, these data suggest a defect in mating behavior is the most likely explanation for the phenotype. In support of this, *ogt-1* males are less likely than wild-type males to initiate mating when presented with mates. Compared with wild-type males, *ogt-1* males are less likely to actively search for mates when isolated from other worms. This indicates these males have an imbalance between mating drive and food-seeking behavior.

The genetic amenability of *C. elegans* allows incorporation of transgenes, which we have exploited to determine the tissue-specific effects of *ogt-1* on the mating phenotype. As a positive control, we injected a wild-type copy of *ogt-1* into deletion worms and demonstrated it was sufficient to rescue both the lost enzymatic activity and male infertility associated with *ogt-1* deletion. Fertility was also rescued by expressing *ogt-1* in a hypodermis-specific manner, which further implicates the development of the male copulatory organ, which is largely derived from the hypodermal lineage. Alternatively, the infertility may arise from disruption of signaling pathways within the hypodermis.

This study reveals a crucial role for O-GlcNAc in complex developmental and behavioral processes in the *C. elegans* male. Genetic and molecular studies will further define the developmental and neuronal mechanisms impacted by O-GlcNAc.

1119C Investigating the mechanism of the cell-nonautonomous roles of the nuclear hormone receptor NHR-49 in the nervous system of *Caenorhabditis elegans*

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The central nervous system plays a key role in regulating and coordinating whole-body metabolism. In *C. elegans*, the nuclear hormone receptor NHR-49 is ubiquitously expressed and serve as an important regulator of fat metabolism. In addition to altered lipid composition, *nhr-49* mutants display a pleiotropy of defects, including shorter lifespan, impaired starvation response, and increased susceptibility to oxidative stress and pathogenic bacteria. Interestingly, NHR-49 expression in the neuron alone is sufficient to nearly restore lifespan. In light of recent findings of the cell-nonautonomous effects of neurons in cell stress and energy homeostasis in *C. elegans*, we wondered whether NHR-49 function in the neuron alone could also reverse other known *nhr-49* mutant defects. We confirmed that expressing NHR-49 exclusively in the neurons restores lifespan to a near-wild type level and found that it was also sufficient to restore resistance to gut commensal bacteria. In contrast, neuronal NHR-49 did not restore resistance to oxidative stress. We are currently trying to identify the circuitry and signaling mechanism of this neuron-to-periphery communication.

1120A LPIN-1/Lipin 1 moderates the lifespan-shortening effects of dietary glucose by maintaining ω-6 polyunsaturated fatty acids

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Glucose is an essential energy source but excessive glucose causes diseases and accelerates aging in many species. We previously reported that a glucose-rich diet shortens the lifespan of *C. elegans* via accumulating saturated fatty acids (SFAs) and toxic metabolites (Lee et al., 2015, *Genes Dev.*). However, underlying mechanisms by which high-glucose diets shorten lifespan by affecting lipid metabolism remain incompletely understood. Here, we find that LPIN-1/Lipin 1, a phosphatidic acid phosphatase and a potential transcriptional coregulator, prevents worms from the lifespan-shortening effects of high-glucose diets. Through performing RNA-sequencing analysis, we showed that the transcriptomic changes caused by *lpin-1* RNAi were partially reversed by glucose-rich diet feeding. Consistent with a previous report showing that LPIN-1 is a negative regulator of SBP-1/sterol regulatory element-binding protein (SREBP), a key transcription factor for fat synthesis (Smulan et al., 2016, *Cell Rep.*), we found that *lpin-1* RNAi upregulated fat-metabolic SBP-1 target genes. In contrast, we showed that overall transcriptomic changes caused by *lpin-1* RNAi positively correlated with those by RNAi targeting *sbp-1* or *mdt-15*/mediator 15, a transcriptional coregulator of SBP-1. We also found that *lpin-1* RNAi reduced lipid levels in glucose-rich diets, leading to compensatory activation of SBP-1. Specifically, we showed that *lpin-1* RNAi upregulated lipid synthesis/desaturation genes and downregulated lipolysis genes in glucose and lipid metabolic pathways. Overall, these data suggest that LPIN-1 maintains lipid homeostasis under glucose-rich diets by altering lipid levels and composition in worms. Consistently, we found that *lpin-1* RNAi reversed the composition of fatty acids altered by glucose-rich diet feeding; the levels of SFAs and monounsaturated fatty acids (MUFAs) were decreased by *lpin-1* RNAi but were increased by glucose-rich diets. In contrast, the levels of two ω-6 polyunsaturated fatty acids (PUFAs), linoleic acid (18:2n-6) and arachidonic acid (20:4n-6), were increased by *lpin-1* RNAi but decreased by glucose-rich diet feeding. Importantly, supplementation with linoleic acid or arachidonic acid suppressed the shortened lifespan of *lpin-1(RNAi)* worms under glucose-rich conditions. Thus, proper metabolic flow from glucose to ω-6 PUFAs via LPIN-1 is crucial for maintaining health and normal lifespan against dietary glucose toxicity in animals.

1121B The Search for Novel Anthelmintic Targets: Characterizing Alternative Metabolic Pathways in Caenorhabditis elegans

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Around a quarter of the human population is infected by parasitic helminths and this places a large economic burden on the agricultural industry. Unfortunately anthelmintic resistance is a growing problem. Helminths survive long periods of hypoxia

in their hosts, but their anaerobic metabolism has yet to be fully characterized. These anaerobic metabolic pathways are a promising, selective target for new drugs.

Fumarate reduction, a rewiring of the TCA cycle, has long been identified as a key pathway for parasitic worms. We have previously identified the metabolic pathway for the synthesis of the essential small molecule involved in fumarate respiration, rhodoquinone (RQ) which combines two important pathways, the kynurenine metabolism pathway and the ubiquinone synthesis pathway and found that COQ-2 is the key connection between both. We have previously found that a mutually exclusive exon in coq-2 which is exclusive to RQ-species is what determines whether RQ or the closely related ubiquinone (UQ) is synthesized. coq-2 has two key residues near the active site which are conserved in RQ-species but not in other invertebrates or in hosts. Furthermore, mutating these two residues is sufficient to change the UQ-exon so that it can recover in a RQ assay developed by our lab.

This finding combined with previous research which suggested that parasitic worms rely on extensive rewiring of existing pathways led to a focus on known metabolites and known pathways which often have existing drugs and crystal structures. By building from previous work, drug targets can be more easily found than if a novel pathway had to be characterized. The most effective drug targets will be those genes which are funadamental to parasitic metabolism and which have had divergence between hosts and parasites so that they can be targeted selectively. More specifically, four pathways which have been previously linked with anaerobic metabolism were tested for importance in RQ-dependent metabolism to narrow down to specific targets. For each of these targets, protein sequences were compared between hosts, parasites and RQ-containing mollusks and annelids to find conserved divergent residues in proximity to druggable sites. In total four known enzymes, including COQ2, have been found which are required for RQ-dependent metabolism and which have parasite specific residues near active sites which may act as selective drug targets. Humanized mutants have been generated for those residues. Moving forwards, in silico drug design will be carried out to find drugs predicted to be specific to the helminths which can be then be assayed against wild-type as well as the humanized mutants.

1122C Characterization of a third SHC adaptor protein in *Caenorhabditis elegans*

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SHC proteins are a family of adaptor proteins that play an important role in signal transduction, they are characterized by three crucial domains: the phosphotyrosine binding (PTB) domain, a Src2 homology (SH2) domain and a less conserved collagen homolog (CH1) domain. Two *Caenorhabditis elegans* SHC proteins have been described: SHC-1 and SHC-2. We have identified a third SHC protein, K11E4.2, that is intestinally expressed. Our analysis revealed that K11E4.2 null mutant animals suffer from a diet-dependent change in fat accumulation and increased sensitivity to starvation and oxidative stress. *C. elegans shc-1* plays a role in stress response and lifespan regulation through the insulin signaling pathway. Our data suggest that *shc-1* and K11E4.2 do not act redundantly to regulate stress or starvation response, but rather each plays a distinct role in these processes.

1123A The role of ceramide metabolism enzymes, *hyl-2*/ceramide synthase and *asm-3*/acid sphingomyelinase, on lipid metabolism

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The increase in our aging population presents a need for research on healthy aging. Here, we present work examining the role of ceramide and sphingomyelin metabolism on aging. Ceramide, which can be synthesized de novo or by recycling from sphingomyelin, is a sphingolipid that is important in responding to oxidative stress. Furthermore, it is known that ceramide levels increase with age, making ceramide an important lipid mediator of aging. We utilized *C. elegans*' mutants lacking acid sphingomyelinase (*asm-3*) and ceramide synthase (*hyl-2*), which are previously found to be long- and short-lived, respectively. We performed a lipidomic analysis to explore longitudinal changes in lipid concentrations in wild type (N2) worms at 1, 5, and 10 days of age, compared to long-lived worm models (*eat-2/mAChR* and *asm-3/acid sphingomyelinase)* as well as short-lived worm models (daf-16/FOXO and *hyl-2/ceramide synthase)*. Among our samples, we detected 700 different lipids, including fatty acids and sphingomyelins. Interestingly, 10-day old *hyl-2* mutants, which have a reduced life-span, showed an increased concentration of the putative omega-3 fatty acid, eicosapentaenoic acid (EPA), which has been shown to increase with longevity in worms. Conversely, *asm-3* mutants, which are long-lived animals, have reduced levels of EPA. To expand upon

our lipidomic data with enzymatic findings, we utilized RT-qPCR analysis to longitudinally analyze fatty acid desaturases (*fat-1* and *fat-4*) involved in EPA metabolism in worms. We found that *fat-4* expression is reduced in 1, 5, and 10 day *hyl-2* animals, suggesting that ceramide metabolism may impact fatty acid genes involved in aging. Further work will examine the role of fatty acid tail elongases (*elo-5* and *elo-6*) in *hyl-2* and *asm-3* mutant animals. This analysis will better define the intrinsic biochemical lipid processes associated with ceramide metabolism in aging animals.

1124B Rescue of Complex I mutants by hypoxia and intra-Complex I mutation

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Inherited disorders of mitochondrial oxidative phosphorylation can be caused by mutations in at least 290 genes and affect approximately 1 in 5,000 live births, with no proven therapies to date. In a mouse model of Leigh Syndrome harboring a deletion of the Complex I subunit Ndufs4 (LPD-5 in *C. elegans*), breathing hypoxia extends lifespan and prevents neurodegeneration, while exposure to modest hyperoxia exacerbates the disease and results in rapid death. The precise mechanism underlying the Complex I rescue by hypoxia and sensitivity to hyperoxia remains elusive. Here, we show that Complex I mutants in *C. elegans* including *lpd-5* and *nduf-7* are similarly rescued by hypoxia and sensitive to mild hyperoxia with respect to growth and development. Genetic activation of HIF-1 is not sufficient to rescue Complex I mutants, nor is HIF-1 necessary for the beneficial effect of hypoxia. To gain further insight into the interaction between Complex I dysfunction and oxygen we used forward genetics to isolate genetic suppressors of *nduf-7* and *gas-1* in hyperoxia. We identified an intra-complex missense mutation in a supernumerary subunit of Complex I, which confers dominant suppression of *nduf-7*, *gas-1*, and *lpd-5* in hyperoxia. Ongoing studies, including additional forward genetic screens, promise to shed light on the mechanisms underlying the interaction between Complex I and oxygen.

1125C Global profiling of distinct cysteine redox forms reveals wide-ranging redox regulation in *C. elegans*

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Post-translational changes in the redox state of cysteine residues can rapidly and reversibly alter protein functions, thereby modulating biological processes. The nematode *C. elegans* is an ideal model organism for studying cysteine-mediated redox signaling at a network level. Here we present a comprehensive, quantitative, and site-specific profile of the intrinsic reactivity of the cysteinome in wild-type *C. elegans*. We also describe a global characterization of the *C. elegans* redoxome in which we measured changes in three major cysteine redox forms after H_2O_2 treatment. Our data revealed redox-sensitive events in translation, growth signaling, and stress response pathways, and identified redox-regulated cysteines that are important for signaling through the p38 MAP kinase (MAPK) pathway. Our in-depth proteomic dataset provides a molecular basis for understanding redox signaling in vivo, and will serve as a valuable and rich resource for the field of redox biology.

1126A Oleic acid modulates reproductive plasticity via DAF-12 in postdauer adults

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Environmental stress experienced during critical periods of development has been shown to result in reproductive plasticity in both plants and animals, although the molecular mechanisms regulating these phenotypes are not well understood. In previous work, we showed that different environmental stresses experienced during early development in *Caenorhabditis elegans* resulted in distinct reproductive outcomes. *C. elegans* larva experiencing early-life stress (starvation or high pheromone) may enter the stress resistant, developmentally arrested dauer stage. When environmental conditions improve, larva will exit dauer and proceed with reproductive development (postdauers, PD). We have shown previously that hermaphrodites that experienced starvation-induced dauer (PD_{Stv}) exhibited a lower brood size compared to continuously fed animals (control, CON). The set of genes with the most significant differential expression between PD_{Stv} and CON adults have functions in fatty acid metabolism, and we found that double mutant combinations of the delta 9-desaturase genes, *fat-5*, *fat-6*, and *fat-7*, as well as mutations in their transcriptional regulators, *nhr-49*, *sbp-1*, and *mdt-15*, abrogated the decreased brood size phenotype. Using Oil Red O staining to examine levels of stored lipids, we found that PD_{Stv} adults displayed lower

lipid stores in their intestines, but showed increased stored lipids in their embryos, when compared to CON adults. These results suggested that PD_{Stv} adults prioritize lipid production for reproduction rather than somatic maintenance after dauer exit. To test this hypothesis, we examined the brood sizes of PD_{Stv} adults that were cultivated on OP50 supplemented with different fatty acid molecules. We observed that supplementation with oleic acid (OA) resulted in a significant increase in brood size compared to animals fed only OP50, while other fatty acids resulted in no change or a decrease in brood size. We found that the OA-dependent increase in brood size required FAT-7, as well as the dafachronic acid (DA)-dependent activity of the DAF-12 steroid signaling pathway. Furthermore, we demonstrated that OA is required after dauer exit for the increased brood size phenotype. Together, these results suggest that OA may be acting as a signaling molecule to modulate PD_{Stv} reproductive plasticity through regulation of the DA-dependent DAF-12 pathway rather than acting in a nutritional capacity for reproduction.

1127B Mitochondrial complex I redox signaling mediates hypoxic responses in *C. elegans*.

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Oxygen is essential for organismal function due to its role in cellular metabolism. Mitochondria are organelles that utilize substrates and oxygen to produce ATP. Dysregulation in oxygen supply caused by hypoxia, a condition of low oxygen tension, followed by the restoration of oxygen or reoxygenation, leads to mitochondrial dysfunction and reactive oxygen species (ROS) production. However, organisms have evolved various ways to sense and respond to changes in oxygen concentration. For example, *C. elegans* surveil their environment and respond to mitochondria distress and changes in oxygen concentration through behavioral avoidance responses but the mechanisms are unknown. Mitochondrial complex I of the electron transport chain is a major site of ROS production and is canonically associated with oxidative damage following hypoxic exposure. However, ROS also play important role in hypoxic signaling and we sought to untangle the dual role of complex I ROS in hypoxic signaling. We found that ROS is required for mediating behavioral responses to hypoxia and that ROS generated by the complex I inhibitor, rotenone, is sufficient. We then developed an optogenetic approach to spatiotemporally control complex I ROS production with light to demonstrate the role of site-specific ROS signaling. Light-induced complex I ROS rapidly and reversibly increased behavior. We showed that the bioenergetic effect of light induced complex I ROS was selective to complex I activity. Using genetic and pharmacologic approaches, we then characterized the ROS species and found that the matrix generation of H_2O_2 mediated the behavioral response. Surprisingly, this effect was mediated through a single thiol modification on complex I. We showed that mutants lacking the thiol residue were not responsive to acute hypoxic signaling. The hypoxic signaling mediated through the thiol extended to protection against prolong hypoxia-reoxygenation. Overall, we demonstrate that site-specific ROS can result in a pro-survival response to hypoxia through single thiol modification.

1128C Bacterial D-alanine metabolism affects *C. elegans* lifespan under high glucose conditions

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Microbiome-derived metabolites are able to impact the host's nervous system through the gut-brain axis. D-amino acids (D-AAs), the D-enantiomers of prevalent L-amino acids, have unique functions such as neuromodulators in animals including mollusks, rodents, and primates, while microbiota is a known contributor to the source of D-AAs in animals. Among all D-AAs, D-Ala is a potent agonist of the glycine binding site of N-methyl-D-aspartate (NMDA) glutamate receptors *in vitro.* Additionally, D-Ala immunoreactivity in pancreatic β-cells and adrenocorticotropic hormone (ACTH)-secreting cells suggests its involvement in glucose homeostasis. While several researches showed microbiota as a major source of D-Ala in animals, the effect of bacterial D-Ala synthesis and metabolism on host physiology has not been studied.

In this study, we fed wild type *Caenorhabditis elegans* N2 with *Escherichia coli* mutants with knockout of genes relevant to D-Ala biosynthesis (*dadX*, *alr*) and D-Ala metabolism (*ddlA*), and then examined changes in *C. elegans* phenotypes with or without the presence of high glucose. All three bacterial mutants displayed similar growth curves in nutrient-rich liquid media, but decreased D-Ala/total alanine ratio compared to the parental *E. coli* strain. To prevent the introduction of exogenous D-Ala, we cultured *C. elegans* on peptone-free NGM media. We found no statistically significant differences in life span when fed on the selected *E. coli* mutants. However, we observed a slight avoidance to all three bacterial mutants compared to the parental strain. When amended with 40 mM glucose, *ΔddlA* significantly decreased the life span of N2. No food preference was observed on glucose-amended plates. These results indicate that the combination of deficiency in bacterial DdlA activity and high glucose led to a decreased life span in *C. elegans*.

1129A Role of branched chain amino acid metabolism in ubiquitin-dependent proteolysis

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The role of ubiquitin in mitochondrial surveillance is increasingly gaining attention with numerous stress pathways being recently described. Our group observed that mitochondrial stress affects ubiquitin-dependent proteolysis both in *C. elegans* as well as in mammalian cells. We performed a candidate screen and identified that metabolic defects could result in reduced UPS functionality independently of the mitochondrial unfolded protein response (UPR^{mt}). Especially defects in the catabolism of branched chain amino acids (BCAAs) affects the ubiquitin-dependent turnover of a GFP-based model substrate in *C. elegans*. Surprisingly, a newly generated mutant of the enzyme responsible for the first step of the BCAA catabolism, *bcat-1 (hh58)*, rescues this defect. We have indications that this mutation impairs mitochondrial import of BCAT-1, resulting in increased levels of this enzyme in the cytosol, where, its mammalian homolog BCAT1 is reported to be localized, in contrast to the other homolog BCAT2, which localizes in mitochondria. Through multiple omics analysis, we explored the general transcriptional, translational and metabolic regulation deriving from BCAA defects in combination with mislocalization of BCAT-1. Considering that balanced regulation of proteolysis and metabolism is crucial for organismal health, understanding the mechanisms underlying *bcat-1 (hh58)* increased proteolytic capacity might represent a novel starting point for the development of new therapeutic strategies or nutritional guidelines to treat metabolic disorders as well as neurodegenerative diseases.

1130B *N. parisii* compensates for genomic loss of dihydroceramide desaturase through reliance on *C. elegans* sphingolipid biosynthesis

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Microsporidia are obligate intracellular parasites that have lost many metabolic enzymes, resulting in the smallest known eukaryotic genomes. Microsporidia are highly dependant upon host nutrients, but it is poorly understood the effect that these parasites have on host metabolism. Using *N. parisii*, a natural microsporidian parasite of *C. elegans*, we describe how infection alters host lipid metabolism. We show that the *C. elegans* lipase ATGL-1 is upregulated in response to *N. parisii* infection and that host fat stores are depleted. Metabolic profiling of infected animals reveals large changes in lipid composition including changes consistent with nutrient starvation. Additionally, we identify novel ceramides only generated in animals infected with *N. parisii*. Genomic analysis reveled that *N. parisii* has lost the enzymes necessary for the de novo synthesis of these sphingolipids. Mutations in *C. elegans* dihydroceramide desaturases *F33D4.4* and *ttm-5* or acid ceramidase *asah-2*, reduce *N. parisii* growth. Supplementation with sphingosine, a substrate for ceramide synthesis, enhances *N. parisii* proliferation. Together, our data demonstrate that *N. parisii* exploits sphingolipid biosynthesis in *C. elegans* and reveals an evolutionary strategy used by microsporidian parasites to cope with extreme genomic reduction.

1131C Downregulation of SEMO-1, a novel hydrogen sulfide-generating *C. elegans* enzyme, enhances lifespan: role of AAK-1/-2

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The *C. elegans* ortholog of human selenium-binding protein 1 (SELENBP1), Y37A1B.5, is a pro-aging factor that confers resistance to high doses of selenite (1). SELENBP1 was recently identified as a methanethiol oxidase (MTO), catalyzing the conversion of methanethiol to hydrogen sulfide (H_2S), hydrogen peroxide (H_2O_2) and formaldehyde. Here, we tested whether Y37A1B.5 has MTO activity and whether the AMPK orthologs AAK-1/-2 are involved in the effects of the protein on *C. elegans* lifespan.

We developed an MTO activity assay that is based on *in situ*-generation of methanethiol from methionine as catalyzed by a bacterial recombinant L-methionine gamma-lyase, followed by detection of two methanethiol oxidation products, H_2S and H_2O_2. Using this assay, we demonstrate MTO activity of isolated recombinant Y37A1B.5, similar to recombinant human SELENBP1. Moreover, MTO activity was detected in lysates from wild-type nematodes but not in lysates from a newly generated Y37A1B.5-deficient mutant strain, suggesting that the Y37A1B.5 protein is the major *C. elegans* MTO. Thus, Y37A1B.5 was named SEMO-1 (SELENBP1 ortholog with MTO activity). It is a novel methanethiol oxidase and therefore a novel potential source of H_2S and H_2O_2, two molecules known to affect lifespan in *C. elegans*.

A Y37A1B.5/SEMO-1-deficient mutant strain showed an extended lifespan similar to the previously reported worms exposed to Y37A1B.5-specific RNAi (1). SEMO-1, therefore, is a factor apparently shortening *C. elegans* lifespan. Interestingly, lifespan extension following SEMO-1 depletion was abrogated in an AAK-deficient strain (NB245; deficient in both isoforms of the catalytic AAK subunit), and *vice versa*, SEMO-1 depletion through RNAi appeared to enhance AAK phosphorylation in wild-type worms. As AAK activity is known to be related to *C. elegans* lifespan, we propose that the extended lifespan of SEMO-1-depleted worms is caused by AAK activation. The mode of AAK activation following SEMO-1 depletion remains to be identified.

(1) Köhnlein K, Urban N, Guerrero-Gómez D, Steinbrenner H, Urbánek P, Priebs J, Koch P, Kaether C, Miranda-Vizuete A, Klotz LO. Redox Biol. 28:101323 (2020).

1132A Deciphering endogenous formaldehyde-induced cytotoxicity mechanisms in *C. elegans*

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Formaldehyde (FA) is an endogenous and environmental metabolite genotoxin that causes DNA and DNA-protein crosslinks. Endogenous FA has been identified as a major genotoxic and carcinogenic agent involved in the onset of the cancer-prone genetic disease Fanconi Anemia and in the development of a type of inherited bone marrow failure syndrome (IBMFS). Here, we employ *C. elegans* and human carcinoma cells to reveal that FA causes cellular damage by triggering oxidative stress, which is prevented by the alcohol dehydrogenase enzyme ADH5/GSNOR. We identify the ADH5 orthologue in *C. elegans* (*adh-5*; H24K24.3) and show that *adh-5* mutants are hypersensitive to FA. Oxidative stress exacerbates FA sensitivity in ADH-5 deficient worms. Moreover, both worms and cells display ROS accumulation and the activation of oxidative stress responses, which is suppressed by supplementation with the GSH precursor N-acetylcysteine (NAC). Further, we demonstrate that endogenous GSH can protect cells lacking the Fanconi Anemia DNA repair pathway, or worms that are deficient in Nucleotide Excision Repair (NER), from endogenous or environmental FA. Hence, our findings establish a conserved mechanism that protects from FA cytotoxicity, which has broad implications for developing novel intervention strategies for Fanconi Anemia patients and against certain cancers.

1133B Interaction of BMP and Insulin Signaling in *C. elegans* Lipid Metabolism

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A small number of peptide growth factor ligands are used repeatedly in development and homeostasis to drive programs of cell differentiation and function. Cells and tissues must integrate inputs from these diverse signals correctly, while failure to do so leads to pathology, reduced fitness, or death. We have demonstrated that DBL-1, a bone morphogenetic protein (BMP) ortholog, is required for normal lipid accumulation, in part by downregulation of insulin/IGF-1-like signaling (IIS). We have investigated the molecular mechanisms for this interaction. DBL1/BMP signaling represses expression of insulin-like peptide (ILP) INS-4, increases expression of anabolic fatty acid desaturases, and reduces expression of catabolic β-oxidation enzymes, thereby promoting storage of neutral lipids. Both loss- and gain-of-function of the DBL-1/BMP pathway leads to decreased fat accumulation. Although the mechanism for low fat accumulation caused by excess DBL-1 signaling is unknown, it differs from that of reduced DBL-1 signaling at the level of lipid droplet size. DBL-1/BMP signaling alters the sensitivity of IIS transcription factors to DAF-2/Insulin Receptor activity in opposite directions, increasing the sensitivity of DAF-16/FoxO, while reducing the sensitivity of SKN1/Nrf. Interestingly, DBL-1/BMP low-fat phenotype can be partially suppressed by overexpression of DAF16/FoxO but not by SKN1/Nrf. Finally, we have shown that *ins-4* mutants have a high-fat phenotype that is also dependent on DAF-16/FoxO but not SKN-1/Nrf. The molecular interactions we identify provide new insight into mechanisms of signaling crosstalk and potential therapeutic targets for IIS-related pathologies such as diabetes and metabolic syndrome.

1134C *Caenorhabditis elegans* Fluorescent Mutants: Tryptophan Kynurenine Pathway

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Caenorhabditis elegans fluorescent mutants *(flu-1- flu-4*) show altered fluorescence of the lysosome like intestinal granules, when observed under UV light [Babu, 1974, Siddiqui, 1978; Siddiqui and von Ehrenstein, 1980; Siddiqui and Babu, 1980 a, b;

and Babu and Bhat, 1980]. Using biochemical techniques, it was shown that the *flu-1* mutant alleles characterized by a bluish purple gut fluorescence possess reduced kynurenine hydroxylase enzyme activity [Siddiqui and Babu, 1980], and the *flu-2* mutant alleles characterized by a pale dull green fluorescence had a block in kyunreniase enzyme activity [Babu and Bhat, 1980]. The *C. elegans* genomic sequence encodes these two enzymes in the proximity of *flu-1* and *flu-2* loci. R07B7.5 encodes kynurenine hydroxylase, and C15H9.7 encodes kynureninase, respectively [Altschul et al., 1990; Kanehisa, 2012].

Kynurenine pathway is the primary route for tryptophan catabolism in and the starting point for the synthesis of nicotinamide adenine dinucleotide (NAD) in mammals. Dysregulation or overactivation of this pathway can result in immune system activation and accumulation of potentially neurotoxic compounds, resulting in inflammation, depression, cancer, neurodegenerative and behavioral alterations, SARS-COV-2 resistance, and epigenetic modifications. Kynurenine pathway genes are also conserved in *C. elegans* [van der Goot and Nolan, 2013].

We are investigating genetic and epigenetic consequences of manipulating kyunrenine pathway in *C. elegans* to reveal key components of the catabolic pathway in *C. elegans,* to reveal molecules and mechanisms that are shared in human disease. For instance, we may use sequence optimized human ortholg cDNA in *a flu-1* deletion knockout mutant that shows altered bluish purple intestinal fluorescence and reduced kynurenine hydroxylase activity. Human gene variants cDNA can be used to rescue and be examined for the restoration of intestinal fluorescence and the kynurenine hydroxylase enzyme activity, if the cDNA carrying human variant can restore the normal function in the nematode. Such approach may allow examining human lethal and sublethal variants using the *C. elegans* model, and could elucidate critical molecular mechanisms underlying human disease.

1135A Neuronal control of lipid metabolism by STR-2 G protein-coupled receptor promotes longevity in *C. elegans*

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The G protein-coupled receptor (GPCR) encoding family of genes constitutes more than 6% of genes in *Caenorhabditis elegans* genome. GPCRs control behavior, innate immunity, chemotaxis, and food search behavior. We find that *C. elegans* longevity is regulated by a chemosensory GPCR STR-2, expressed in AWC and ASI amphid sensory neurons. STR-2 function is required at temperatures of 20°C and higher on standard *Escherichia coli* OP50 diet. Under these conditions, this neuronal receptor also controls health span parameters and lipid droplet (LD) homeostasis in the intestine. We show that STR-2 regulates expression of delta-9 desaturases, *fat-5*, *fat-6* and *fat-7*, and of diacylglycerol acyltransferase *dgat-2*. Rescue of the STR-2 function in either AWC and ASI, or ASI sensory neurons alone, restores expression of *fat-5*, *dgat-2* and restores LD stores and longevity. Rescue of stored fat levels of GPCR mutant animals to wild-type levels, with low concentration of glucose, rescues its lifespan phenotype. In all, we show that neuronal STR-2 GPCR facilitates control of neutral lipid levels and longevity in *C. elegans*.

1136B Identification of mitochondrial dysfunction as a key modifier in Myotonic Dystrophy Type 1

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Myotonic dystrophy type 1 (DM1) is a RNA repeat-based disorder caused by transcripts bearing expanded CUGs in the 3'UTR. Few modifiers of DM1 toxicity are known; to understand the mechanisms underlying RNA-repeat pathogenesis, I performed a genetic screen for DM1 toxicity using a *C. elegans* DM1 model. I identified the ubiquinone (CoQ) pathway as a suppressor of DM1 pathogenesis. Here we show that expression of RNAs with expanded repeats disrupt the CoQ biosynthetic pathway, and lead to mitochondrial dysfunction with changes in mitochondrial dynamics, morphology and respiration. Our data shows that mitochondrial dysfunction plays a key role in DM1 toxicity, resulting in loss of motility. Importantly, these toxic phenotypes can be rescued by CoQ supplementation, with reversal of both mitochondrial dysfunction and improvement in animal locomotion. Moreover, our data suggests that CoQ and mitochondrial dysregulation by toxic RNAs is dependent on the muscleblind-like splicing factor, MBL-1, homologue of mammalian MBNLs.

Together, our data identify the CoQ pathway and mitochondrial function as central contributors to DM1 pathogenesis and establish a link between MBL-1 dysfunction and disruption of these pathways in DM1 disorders. These studies further suggest a more critical role for mitochondrial dysfunction in RNA-based neuromuscular degenerative disorders.

1137C Influences on fat content, fat density and lipid droplets in *C. elegans* liquid culture

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C. elegans has become an emerging model system to investigate different toxicological endpoints. In particular, fundamental pathways regulating energy homeostasis are highly conserved between *C. elegans* and humans and thus, the worm provides an important model for the analysis of mechanisms leading to obesity. For example, the analysis of lipid homeostasis as well as fat accumulation in *C. elegans* can provide important insights into metabolic diseases and the activity of potential obesogenes. In addition, changes in lipid homeostasis by external factors are suspected to be transmitted to subsequent generations. Thus, lipid metabolism might also be a promising read-out in *C. elegans* to address inter- or transgenerational effects of xenobiotics.

In a first step, we studied the influence of different food regimes on worm development, fat content and changes on the level of lipid droplets (LD), the worm's major form of fat storage, to generate "low body fat" or "high body fat" phenotypes. We exposed *C. elegans* to different OP50 concentrations in liquid culture for three days (L1 to adulthood) and measured worm sizes, triacylglyceride (TAG) contents, LD sizes and density as well as fatty acid (FA) patterns.

After three days, worms fed with higher OP50 concentrations showed an increase in body size, higher TAG levels and significant increases in LD size and volume per μm^3 in the anterior part of the intestine. Moreover, GC-MS analysis revealed significant differences in FA pattern of adult worms and their eggs, when comparing the "fat" and "lean" phenotypes. Notably, cyclic FA and polyunsaturated fatty acids (PUFA) were affected in a diet-dependent manner. Interestingly, orlistat, an anti-obesogenic drug, could also influence fat density and FA patterns in adult worms as well as eggs.

Currently, we are testing effects of (anti-)obesogenes on fat content and FA composition and if those are transmitted to subsequent generations to assess the applicability of these read-outs for the analysis of inter- or transgenerational (adverse) effects.

1138A Decline of ribosomal proteins levels during L1 arrest

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Starvation of *C. elegans* upon hatching causes developmental arrest. Arrested animals can survive for more than to 2 weeks in the absence of food and restart growth when nutrition improves. However, upon re-feeding animals resume growth with a delay that increases proportionally with the duration of starvation. Survival during starvation requires autophagy, and the starvation response is under tight control by metabolic signaling. For example, we found that mutants with constitutively active mTOR signaling die within 1 week of starvation. The goal of this project is to characterize the dynamics of proteome turnover during starvation and during recovery, and to ask if specific proteins are preferentially degraded during starvation to maintain survival.

We hypothesize that under extended periods of starvation proteins needed for growth and biosynthesis, such as ribosomes and mitochondria, are preferentially turned over, as compared to structural proteins that are essential for survival, such as collagens or histones. Resuming growth upon feeding would consequently require re-synthesis of the biosynthesis proteins, and thereby delay onset of growth in proportion to the duration of starvation.

To test this hypothesis, we established an assay for protein quantification based on HiBit technology. HiBit relies on a split luciferase and allows for detection of specific proteins at high accuracy and sensitivity in a microplate-based readout. Using this assay, we find that indeed ribosomal proteins declined continuously during L1 arrest. We will now test if this decline relies on autophagy, and if the turnover rate of ribosomes can explain how survival is affected in mutants with perturbed autophagy and growth signalling. Finally, we will use proteomics to assess how the proteome composition globally changes during L1 arrest on a time scale of weeks.

1139B The impact of perceived and internal metabolic states on behavior and hypoxic responses in *C. elegans*

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Caenorhabditis elegans surveil their environment and respond through behavioral changes. One factor that influences behavior is the metabolic state. The energy status can influence worm locomotion behavior to increase chances of finding or staying on a food source. Mitochondria are metabolic hubs of the cell and use oxygen to produce energy. How mitochondrial function alters behavior and hypoxic signaling is unclear. We designed the optogenetic tools mitochondria-ON (mtON) and mitochondria-OFF (mtOFF) to mimic or suppress mitochondrial function. Upon activation with light, these tools can control

the energy state in select tissue through the use of tissue-selective promoters. Given that energy-sensing is driven by neurons, and the intestine is responsible for nutrient utilization, light activation of these animals can serve as a model of perceived or internal metabolic state, respectively. We characterized our model and observed a decrease in total ATP levels in response to mtOFF activation in the intestine, with no effect of activation in neurons. The metabolic state can lead to signaling that influences hypoxic responses. Hence, we next examined the response of *C. elegans* to short-term hypoxic exposures. The regulation of mitochondrial function in *C. elegans* led to alterations in the periods of recovery from 1-hour hypoxia. Overall, we demonstrate that the tissue-specific reduction of mitochondrial function by mtOFF in the intestine affects whole body ATP production compared to the activation of neuronal mtOFF, where there were no changes in ATP levels between worms with activated and deactivated mtOFF. In conclusion, our optogenetic model allows us to study the effects of modulating mitochondrial function on behavior and metabolic responses.

1140C Modular metabolites that connect bacterial growth-phase-dependent lipogenesis with *C. elegans'* peroxisomal β-oxidation and *N*-acyl ethanolamine metabolism

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Secondary metabolism in nematodes includes the biosynthesis of complex modular structures via the combinatorial assembly of diverse building blocks derived from primary metabolic pathways.

Using a combination of comparative HPLC-ESI-HR-MS^E analysis, enrichment by solid phase extraction, and preparative HPLC the dominating members of a novel class of modular metabolites were isolated from *Caenorhabditis wallacei* JU1904 and their β-glycosyl *N*-acyl ethanolamine phosphate structures carrying a diversity of homologous saturated, *iso*-branched, unsaturated, or cyclopropanated *N*-acyl moieties ranging from 9 to 15 carbons were identified by 1D and 2D NMR spectroscopy, along with HR-MS/MS and micro reactions.

This novel class of modular metabolites is widely distributed in *Caenorhabditis* spp. including the model organism *C. elegans*. In gonochoristic *Caenorhabditis* spp. the dominating β-sophorosyl derivatives are exclusively produced by males. The corresponding β-glucosyl derivatives are predominantly detected in females and are also abundant in hermaphroditic *C. elegans*, *C. briggsae*, and *C. tropicalis*. In *C. wallacei* the dominating male-derived β-sophorosyl *N*-acyl ethanolamine phosphate carrying a *cis*-5,6-methylenedodecanoyl (*cy*C13) moiety attracts females. Its biosynthesis depends on the nematode's diet and subsides upon starvation. Using mixed isotope labelling with natural abundance and [U-^{13}C]-*E. coli* the biosynthetic origin of the various building blocks was elucidated. Biosynthesis of the dominating components carrying *cy*C13 or C12:1 as *N*-acyl moiety depends on bacterial lipogenesis and thereby integrates bacterial food availability and developmental stage-dependent lipid cyclopropanation. Biosynthesis of the homologous series of *N*-acyl moieties further depends on chain shortening of dietary long chain fatty acids via β-oxidation by peroxisomal 3-ketoacyl-*S*-CoA thiolase *daf-22* but is independent of mitochondrial 3-ketoacyl-*S*-CoA thiolase *kat-1*. In contrast, biosynthesis of *de novo* produced *iso*-fatty acid building blocks (such as *iso*-C11) depends on the *iso*-fatty acid elongase *elo-5*, as well as peroxisomal β-oxidation by *daf-22* and proceeds via chain shortening of a long chain *iso*-fatty acid intermediate. The medium chain fatty acids are subsequently converted into the *N*-acyl ethanolamines, presumably via the *N*-acyl phosphatidylethanolamine (NAPE) pathway with glyceryl-phosphate *N*-acyl ethanolamines (GP-NAEs) as biosynthetic intermediates.

Characterization of the novel class of β-glycosyl *N*-acyl ethanolamine phosphates provides additional evidence for the structural diversity of nematode-derived modular metabolite and reveals a yet unidentified biosynthetic pathway that integrates bacterial and *de novo* lipogenesis along with *C. elegans'* peroxisomal β-oxidation and *N*-acyl ethanolamine metabolism.

1141A Modular metabolite assembly in *Caenorhabditis elegans* depedns on carboxylesterases and formation of lysosome-related organelles

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Signaling molecules derived from attachment of diverse metabolic building blocks to ascarosides play a central role in the life history of *C. elegans* and other nematodes; however, many aspects of their biogenesis remain unclear. Using comparative metabolomics, we show that a pathway mediating formation of intestinal lysosome-related organelles (LROs) is required for biosynthesis of most modular ascarosides as well as previously undescribed modular glucosides. Similar to modular ascarosides, the modular glucosides are derived from highly selective assembly of moieties from nucleoside, amino acid, neurotransmitter, and lipid metabolism, suggesting that modular glucosides, like the ascarosides, may serve signaling functions. We further show that carboxylesterases that localize to intestinal organelles are required for the assembly of both modular ascarosides and glucosides via ester and amide linkages. Further exploration of LRO function and carboxylesterase homologs in *C. elegans* and other animals may reveal additional new compound families and signaling paradigms.

1142B *Pediococcus acidilactici* CECT9879 reduces fat accumulation in *C. elegans* by affecting the insulin signaling pathway

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The increasing prevalence of metabolic syndrome-related diseases, such as obesity and type-2 diabetes, makes urgent to develop alternative therapies to prevent or reduce the progression of these pathologies. On the other hand, during the last years it has been demonstrated the important role that gut microbiota plays on human health, and the influence that alterations in this bacterial balance plays in the development of metabolic diseases. Thus, different bacterial strains have emerged as potential probiotics for the prevention of the complications characteristic of obesity, such as the excess of adiposity or the dysregulation of glycemia.

In this study, we have used *Caenorhabditis elegans* (*C. elegans*) as a model to examine the potential probiotic activities of the bacterium *Pediococcus acidilactici* (PA) CECT9879, together with the underlying mechanisms of action. Our work revealed that the treatment with PA reduced the fat accumulation of *C. elegans* in a 9% when grown in NGM medium, and a 16% in a glucose-loaded (10 mM) NGM medium, quantified by Nile Red staining, without affecting the development of the worm. The reduced fat content was also accompanied by an extension of the nematode median survival of two days in comparison with untreated control worms. Subsequent gene expression analyses demonstrated that the probiotic activities were mediated by modulation of the insulin/IGF-1 signaling (IIS) pathway through the overexpression of daf-16 and ins-6 mediators, but also by an increased expression of genes involved in the fatty acid peroxisomal β-oxidation. Taken together, our data suggest that PA CECT9879 could be considered a potential probiotic strain for the prevention of the metabolic syndrome-related disturbances, and highlight the use of *C. elegans* as an appropriate in vivo model for the study of the mechanisms underlying these diseases.

1143C A multi-modal biosensor for monitoring proteostasis in stress and aging

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Stress and aging can compromise cellular proteostasis and lead to the loss of proteome integrity, a hallmark of many degenerative diseases. Efforts to maintain the functional capacity of the proteostasis network hold promise for prolonging organismal health and reducing the burden of disease, however, the underlying basis for the decline are at present unknown. This is in part due to a lack of available methods for observing the consequence of stressful stimuli on proteome integrity in real time. Here we report a new genetically-encoded biosensor that allows quantitative assessment of proteostasis network capacity in living *Caenorhabditis elegans*. It is based on a metastable version of dihydrofolate reductase and a conditional proteasome-targeting signal, thereby linking conformational state to protein levels, and fused to a fluorescent protein tag for visualization inside cells. The sensor reveals systemic remodeling of the proteostasis network and identifies distinct cellular states in stress, early aging, and *C. elegans* models of human disease. Our results indicate that this multi-modal biosensor is a convenient tool for *in vivo* investigations into proteostasis network regulation in health and disease states.

1144A Force sensitive upconverting nanoparticles as a direct, noninvasive assay for force generation by muscles

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The digestive system relies heavily on muscles to breakdown, transport and expel material in order to provide the body the requisite nutrients to live. Additionally, activity of these muscles are key biomarkers of health status. Understanding the fundamental function of the digestive system and its dysfunction in aging and disease is hindered by a lack of suitable *in vivo* force sensors. Quantifying the forces generated in the alimentary lumen *in vivo* is currently not feasible through established mechanosensing platforms such as atomic force microscopy, traction force microscopy and Forster Resonant Energy Transfer (FRET)-based molecular strain sensors. To overcome that limitation, we are developing a microscale, biocompatible mechanosensing platform based on upconverting nanoparticles (UCNPs) that can rapidly and noninvasively readout micronewton scale forces within the intestinal and pharyngeal lumen of *C. elegans*.

We synthesize lanthanide-doped ceramic nanoparticles (SrLuF:Yb,Er) embedded in polystyrene microspheres for efficient delivery. When excited in the near-infrared, these mechanosensing UCNPs have an emission spectrum that depends on pressure [PMID: 29927609; PMID: 31592655] and applied force. The ratiometric emission (red/green) increases by 25% with applied forces of 2 micronewtons as measured using dual confocal microscopy and atomic force microscopy. These particles are bright enough to image even the most rapid luminal pressure cycle in the worm (pharyngeal pumping, 5 Hz). We demonstrate the efficacy of using UCNPs to map luminal forces associated with feeding and digestion in wild-type adults. These measurements provide a time-resolved and direct indication of coordinated muscle function. We apply mechanosensing UCNPs concurrently with electrophysiological measurement of pharyngeal activity (ScreenChip), enabling assessment of the relationship between electrical activity and muscle function. We describe how these tools may be further developed into a high throughput platform for muscle function during aging and in *C. elegans* disease models, as well as how our UCNPs can be adapted to perform analogous measurements in other animals.

1145B N-NOSE is an innovative, non-invasive and highly sensitive cancer screening method based on the chemotaxis of *C. elegans*

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A simple, affordable, non-invasive, and highly sensitive cancer detection method is the Holy Grail in health screening. Especially early diagnosis of cancer is critical to increase the likelihood of recovery.

N-NOSE (Nematode-NOSE) is a pioneering cancer screening method based on the chemotaxis of *C. elegans* which shows evasive behavior from the urine of healthy individuals while being attracted to the urine of cancer patients. N-NOSE has an average cancer-detection sensitivity of 86.3% in a clinical setting and detect the presence of cancer for 15 types at all stages: stomach, colorectal, lung, breast, uterine, liver, prostate, esophageal, ovarian, bile duct, gallbladder, bladder, kidney, pharyngeal, and pancreatic cancer. N-NOSE relies on a single drop of urine, is non-invasive and cheap, and is notably more sensitive than the blood tumor markers CEA, CA19-9 and CA15-3.

In this presentation, we detail the C. *elegans* olfaction and chemotaxis method behind N-NOSE. Next, we review and summarize our recently published clinical studies on N-NOSE performed in several hospitals in Japan and compare N-NOSE performance to other cancer screening methods.

Then, we briefly comment on the next generation of N-NOSE methods currently under development. Lastly, we outline our "World Consortium" R&D grant initiative to anyone in the world with the goal of contributing to the development of next generations of N-NOSE cancer-specific using nematodes.

N-NOSE by Hirotsu Bio Science has been launched commercially in 2020 in Japan and is quickly seeking to expand overseas.

1146C Applying the Q system in *Caenorhabditis elegans*: observed issues and challenges

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Gaining control over spatiotemporal features of gene expression is useful to improve our understanding of biological regulation. A common approach is to (ir)reversibly switch genes on or off via conditional expression systems. One such genetic toolkit is the Q system, a binary conditional expression system that was originally developed for mammalian cells and *D. melanogaster*, but has quickly been adapted for use in *C. elegans* as well.

The system consists of three components: QUAS, QF and QS. Expression of a sequence of interest can be controlled by placing it downstream of an enhancer sequence (QUAS) that can be recognized by a transcriptional activator (QF). Advantageous over

the canonical Gal4*UAS* system, the Q system permits to temporally control gene expression in a temperature-independent manner through the addition of quinic acid, which (reversibly) sequesters the transcriptional suppressor QS.

Because of this substantial benefit, we turned to the Q system to build a reporter strain to visualize the endocytic capacity of the *C. elegans* coelomocytes. The objective is to gain reversible temporal control (on and off) over the expression of a fluorescent reporter, mNeonGreen, to generate temporally resolved mNeonGreen secretion by source cells, of which the subsequent degradation by the coelomocytes could then be observed.

However, while the Q system has successfully been used to spatially restrict gene expression, there is little support for its performance in terms of temporal control. In an ongoing effort to validate the Q system for the research purpose described above, we encountered several points of attention which we here wish to share with the community. Especially at the level of the QF/QS ratio, there appears to be but a narrow window of opportunity that avoids leaky expression on one hand, vs inefficient de-repression of transcription by quinic acid on the other. We hope these results may engage others using conditional expression systems in a discussion balancing practical challenges and opportunities of these tools.

1147A Rethinking the worm pick: Alternative materials and sterilization methods

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The platinum worm pick, a fixture in *C. elegans* laboratories for decades, has two drawbacks: (1) the high cost of platinum, a significant problem in many educational settings, and (2) the reliance on an open flame for sterilization, which presents safety hazards.

To address the first drawback, we evaluated whether platinum could be replaced with an alternative metal. An ideal worm pick cools quickly after heating, withstands high temperature without degradation, can be flattened and shaped easily, and is inexpensive. With these criteria in mind, we compared 90% platinum, 10% iridium wire (PT9010) with 5 alternatives: stainless steel (SS), Nickel 200, two nickel chromium (Nichrome) alloys, and iron-chromium-aluminum (FeCrAl). To measure cooling rate we built a circuit to resistively heat wires (all 255 µm in diameter) to 800 ºC and measured the time it took them to cool to 25 ºC. We found that PT9010 and FeCrAl cooled more rapidly (6-7 s) than the other metals tested (8-9 s). To assay heat resistance we conducted a bending test after 3000 heating cycles of duration 4 s at 800 ºC. All materials except SS showed good heat resistance, withstanding >50 bends after 3000 heating cycles. SS exhibited poor heat resistance, breaking spontaneously after ~300 cycles. All materials could be easily flattened using standard tools. With regard to cost, all alternative materials were < 0.20 USD/m, as compared to 140 USD/m for PT9010. These results show that all metal alloys tested except for SS represent reasonable, economical alternatives for worm picks. The most promising is FeCrAl which cools as rapidly as platinum, exhibits good heat resistance, and is available at a fraction of the cost.

Next, to explore an alternative to flame sterilization, we designed an electric worm pick consisting of a loop of PT9010 or FeCrAl wire attached to a handle containing a rechargeable battery and circuit board. Depressing a button causes current to flow through the loop, heating it to about 800 ºC within 2 s. A battery charge lasts for ~500 sterilizations. Worm researchers who tested the device reported that the wire loop could be used similar to a worm pick and that electric sterilization promoted faster work since no movements to a flame were necessary. Our device represents a convenient and safer alternative to flame-sterilized worm picks. We are using a similar loop-based worm picking technique in our automated worm picking system (see abstract by Zihao Li et al).

1148B The OpenWorm Project: progress update, available resources and future plans

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The OpenWorm project (*http://openworm.org*) is a global, online collaboration of computational and experimental neuroscientists, software developers and interested volunteers with an ambitious long-term goal: creating a cell-by-cell computer model of *C. elegans* which reproduces the behaviour of the real animal in as much detail as possible. The project takes a unique Open Science approach to development, making all code, data and documentation publicly available at the time of production. This will provide a community resource which consolidates our anatomical and physiological knowledge of

the worm, and will allow investigators to examine the mechanistic underpinnings of how behaviour is generated by a complete nervous system.

The first concrete milestone for the project is an accurate simulation of locomotion including the motor system, with realistic electrophysiology of the muscle cells and connected neurons to reproduce the crawling gait. A prototype of this simulation has been released (*https://github.com/openworm/OpenWorm*), and the model is being validated by comparison with crawling behaviour from experimental recordings. Current work focuses on investigating the influence of gap junctions between adjacent muscles on synchronization of their activity, and incorporation of proprioceptive feedback from stretch sensitive motoneurons.

The public resources developed already by the project include: **Sibernetic**

: a simulation engine to model the environment and the 3D body of the worm, with the ability to simulate fluids of variable viscosities and contractible elastic matter with impermeable membranes; **Geppetto**

: a web based visualization and simulation engine which will help make the output of the project accessible through any browser; **c302**

: a framework for generating network models of *C. elegans* incorporating known connectomic data which can include model neurons of varying levels of biophysical detail; **owmeta**

: a framework for accessing and sharing anatomical and physiological data on *C. elegans*, facilitating their use in computational models. The **DevoWorm**

subproject focuses on embryogenesis and the study of comparative development between *C. elegans* and various vertebrate and invertebrate species.

These resources, which are being used for our own research as they are developed, are available for the wider community for simulation studies at multiple scales. Experiments can be trialled *in-silico* before planning *in-vivo* experiments. Additionally, having many experimentalists as users will encourage a feedback process, helping to improve the biological realism of the OpenWorm core model.

1149C Developing a high content, whole organism behavioral screening platform for specialized metabolites synthesized by plants

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Plants evolved complex strategies to influence animal behavior and humans have used plants for their medicinal properties for centuries. Both phenomena depend on specialized metabolites (SMs) synthesized by plants, many of which are potent chemical actuators of brain function. Prominent examples include nicotine, cannabinoids, and opiates. We are building on these evolutionary interactions to pair new SMs with their receptors in the nervous system based on a chemotaxis screen in *C. elegans*. In order to evaluate a large number of plant SMs, we developed a semi-automated, high-content screening pipeline. With the current version of the pipeline, we can test up to 96 compounds against wild-type (N2) animals with ~10 person-hours of screening time. The pipeline is not limited to testing a single genotype, but can easily test four genotypes in parallel at present. We continue to iteratively improve the pipeline and believe that we can improve throughput by at least 10-fold with adjustments to our workflow. Additionally, we envision extending the workflow to handle tens-to-hundreds of worm strains in parallel. To curate the SMs that we test, we use chemi-informatic tools and draw on knowledge of biosynthetic pathways in plants. Thus far, approximately 10% of the screened compounds are hits that either attract or repel worms. A key next step for evaluating hits will be to determine if chemotaxis responses depend on the *tax-4* or *osm-9* ion channels known to mediate chemosensory transduction in *C. elegans*. Given that other attractants and repellents act by binding to GPCRs or to receptor guanylate cyclases, we anticipate that SMs will also bind to these classes of receptors. Having developed this screening pipeline, we are poised to pair SMs from plants with their receptors in the *C. elegans* nervous system. These compounds and their receptors may enhance understanding of the chemical codes that link plants to nematodes while also providing entry points for drug discovery or new tools for research.

1150A Immobilization of C. elegans by thermoelectric cooling for high-throughput microscopy

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Microscopy, surgical techniques, and imaging in vivo are broadly utilized for model organisms. However, despite widespread usage, these strategies for multicellular organisms remain low-throughput and require significant manual involvement. Here, we report the implementation of a novel cooling stage to immobilize *Caenorhabditis elegans* on typical agar cultivation plates for these purposes. This device can effectively cool *C. elegans* to between 1-2 degrees Celsius for immobilization and maintain the temperature with minimal fluctuations. We demonstrate the ability to perform imaging and surgical techniques without classic anesthetic agents like sodium azide. This technique decreases animal processing time while maintaining organism viability and fecundity, using an intuitive device built with attainable materials. Our thermoelectric cooling stage, which is highly effective and built to combine with standard microscopy setups, can enable high-throughput microscopy and surgical techniques with decreased manual and chemical interventions.

1151B Optogenetic manipulation of individual or whole population *Caenorhabditis elegans* worms with an under hundred-dollar tool: the OptoArm

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Optogenetic tools have revolutionized the study of neuronal circuits in Caenorhabditis elegans. The expression of light-sensitive ion channels or pumps under specific promotors allows researchers to modify the behavior of excitable cells. Several optogenetic systems have been developed to spatially and temporally photoactivate light-sensitive actuators in C. elegans. Nevertheless, their high costs and low flexibility have limited access to optogenetics for a broad public. Here, we developed an inexpensive, easy-to-build and highly adjustable optogenetics device for use with different microscopes and wormtrackers, called the OptoArm. The OptoArm allows for single-and multiple-worm illumination during imaging and is adaptable in terms of light intensity, lighting profiles and light-color. We demonstrate the system's versatility by performing multi-parameter behavioral analysis upon cholinergic and GABAergic stimulation. Furthermore, we leveraged the OptoArm's power in a population-based study to dissect the contributions of motor circuit cells to age-related motility decline. We discovered that the functional decline of cholinergic neurons corresponds with motor decline, while GABAergic neurons and muscle cells appear relatively age-resilient. This would suggest a rate-limiting, cell type-specific vulnerability to ageing, which may underlie neuronal circuit aging.

1152C Burrrowing chip: A microfluidic platform to visualize and quantitate the burrowing behavior of *C. elegans*

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Caenorhabditis elegans has been widely used to study the genetics of behavior and neuromuscular function. Most of the behavioral studies in *C. elegans* are limited to two-dimensional (2D) assessment of animal locomotion on agar plates (crawling) or in liquid (swimming), and it was not until recently that *C. elegans* studies began to focus on 3D motion. 3D locomotion where animals burrow through a matrix is known to be physiologically distinct from 2D crawling and swimming (Bilbao et al. 2018) and 3D movement evokes different gene expression responses (Hewitt et al. 2020). We have previously deployed a Pluronic hydrogel environment to assess the burrowing performance of muscle mutants. The stiffness of the gel can be tuned to challenge the animals to burrow through a higher mechanical resistance environment to increase the assay sensitivity (Lesanpezeshki et al. 2019). However, due to the assay geometry being in a well-plate, the full burrowing trajectory was not observable, and the animals could be only visualized at the top surface, precluding evaluation of the burrowing behavior throughout the gel layers.

To address the above issues, we have developed a microfluidic platform called a burrowing chip, that enables visualization and assessment of burrowing behavior in the same Pluronic hydrogel environment. The microfluidic format provides excellent control of the chemotactic gradient and animal/gel loading. The shallow depth of the channel enables use of consumer cameras for visualizing and recording burrowing behavior. We show that the device provides a 3D environment to assess the animal's burrowing performance and the animal distribution along the channel during chemotaxis. Next, we identify two burrowing classes of behaviorally slow and fast movement, based on the time it takes animals to reach the attractant. Slow burrowers weakly maintain their direction toward the attractant. We have discovered pause intervals as a novel phenotype

for stimulated burrowing animals, as the burrowing animals exhibit long pauses. Finally, we show that the burrowing chip is capable of characterizing the muscle mutants based on their lower burrowing velocity and higher frequency of pauses compared to wild-type animals.

We suggest that the burrowing chip can be used to provide insights into the genetic basis of burrowing behavior and to assess the neuromuscular health in *C. elegans,* paving the road to identify therapeutic targets for age-related diseases and decline.

1153A Microfluidic-based platform for automated *C. elegans* culturing and phenotyping

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Caenorhabditis elegans (*C. elegans*) has been known for almost six decades as a powerful exploratory organism in fundamental research, helping to address biological questions in the fields of aging, developmental and cell biology, neurobiology and genomics. Nowadays *C. elegans* is also recognized as a valuable alternative to rodent and cellular models in drug discovery and toxicology. A lot of progress has been done over the last decade regarding the techniques employed to study *C. elegans*, particularly thanks to the introduction of microfluidic approaches in the field. However, there is still lack of an "all-in-one" solution providing full automation and standardization of all the main aspects of *C. elegans* experimentation, including culture, chemical/genetic treatment, observation and data recording/analysis. Moreover, often would be key to follow the dynamics of biological processes (e.g. gene, protein expression, development, etc.) over time, which is particularly challenging and limited using traditional *C. elegans* methods.

At Nagi Bioscience we have developed a "Worm-on-Chip" technology that allows fully automated *C. elegans* experimentation. Our microfluidic-based platform consists of three main components, altogether offering a possibility for long-term worm culturing and phenotyping:

- microfluidic chips: allowing standardized worm culture and testing of up to 16 different biological conditions in parallel

- robotic device: servicing the chips (up to 4 chips in parallel), which ensures automated worm distribution and maintenance of the optimal conditions for nematode culturing, and executes image acquisition

- data analysis and interpretation software, based on machine learning: allowing automated processing of the images acquired during an experiment, feature extraction, data analysis and interpretation

With our all-in-one platform we are capable to monitor development, growth, fertility, reproduction, aging and fitness of the worms. Here we show that this technology offers an unprecedented range of parameters that can be adapted for different research goals. An integrated incubator guarantees a fine-tuned temperature control at any moment of the experiment, approving the use of transgenic strains, which require a particular temperature regime for their maintenance. The images can be acquired in bright-field or fluorescent modes, allowing the user to take advantage of the large collection of existing reporter strains. Different dietary conditions can be mimicked by varying bacteria concentration and the experimental protocol can be adapted for various exposure schemes (e.g. exposure to compounds from a specific developmental stage).

1154B The auxin-inducible degron 2 (AID2) system provides sharp degradation control with low ligand concentrations and works at all developmental stages of *C. elegans*

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In cases where genetic mutants are not readily available, RNA interference has been used for many years for loss-of-function analyses. Recently, the genome-editing technology has made it possible to fuse a degron tag to endogenous proteins of interest, allowing us to induce their degradation for loss-of-function analyses . The auxin-inducible degron (AID) system is one of the major degron-based technologies that has been used in many research communities, including the *C. elegans* filed. The pair of a 44-amino acid degron tag (namely AID*) and the TIR1 E3 ligase subunit derived from Arabidopsis thaliana (AtTIR1) has been used in studies using *C. elegans*.

While the AID system is getting popular, two major drawbacks have been reported: 1) leaky degradation of AID-fused proteins in the absence of auxin and 2) the requirement for a high concentration of auxin. Recently, we developed an improved version, namely AID2, that worked in yeast, mammalian cells, and mice. In the AID2 system, we employed a TIR1 mutant derived from rice (OsTIR1(F74G)) and a new ligand, 5-phenyl-indole-3-acetic acid (5-Ph-IAA).

In this study, we applied AID2 to *C. elegans* and compared it with the original AID system. To this end, we inserted a transgene encoding AtTIR1(F79G) into the genome. First, we found that strain expressing AtTIR1(F79G) showed no significant basal degradation. Then, we found 5-Ph-IAA for AID2 induced rapid target degradation with a concentration 1,300 times lower than IAA for AID. Moreover, we developed a modified 5-Ph-IAA that has better permeability. Using this new ligand, we showed that AID2 worked effectively in the embryos surrounded by the eggshell. Our results indicate that the AID2 system is a better option for loss-of-function analyses of *C. elegans* in the future.

1155C easyXpress: an R package for processing high-throughput, image-based *C. elegans* phenotype data

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Developments in high-throughput imaging technologies have improved *C. elegans* studies by increasing experimental efficiency as well as both data quantity and quality. Existing systems for automated data collection, such as the Molecular Devices ImageXpress platforms, generate high-resolution images of nematodes that can be analyzed further by software like CellProfiler to extract phenotype information. Although this software is efficient at automating the extraction of phenotype data, the sheer quantity of generated data can be unwieldy and difficult to parse. We developed easyXpress, a novel software package, to move the processing and review of *C. elegans* phenotype data into the R environment. The package provides a logical workflow for the reading, processing, and visualization of data generated from CellProfiler's WormToolbox. Included are several powerful functions to customize the filtering of noise in data, specifically by identifying and removing objects that deviate in size and shape from expected nematode parameters. This flexibility in data filtering allows users to optimize their analysis pipeline to match their needs. In addition, easyXpress includes tools for generating compact yet detailed visualizations, allowing the user to visually observe and interactively compare summary statistics across wells and plates with ease. Researchers studying *C. elegans* will benefit from this streamlined and extensible R package, complementary to CellProfiler analysis software, that leverages the R environment to rapidly process and analyze large high-throughput imaging data sets.

1156A Testing the use of liposomes for drug delivery in *C. elegans*

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While *C. elegans* is an excellent model organism for studying the genetics of aging, for testing effects of drugs on aging it is problematic, for two reasons in particular. First, many compounds administered to *C. elegans* are not taken up (1). Second, increases in lifespan may reflect drug effects on the *E. coli* food source (2). Further issues are bioconversion of drugs by *E. coli* (3), and the high cost of some compounds. One possible solution to all these problems is the use of liposome-mediate drug delivery, which can increase uptake into the worm, as shown previously using the fluorescent dye uranine (4). This may reflect better carriage by the pharynx of particulate matter into the gut lumen and/or better passage from the lumen into intestinal cells. Liposomes should also reduce interactions between drug and *E. coli*, and enable delivery of drugs to worms using much smaller quantities than required when administering them via the agar.

With the ultimate aim of testing effects of anti-aging drugs on senescent pathology in *C. elegans*, we have been investigating the efficacy of liposome-mediated drug delivery, initially using fluorescent dyes. Our results thus far have confirmed that liposomes are an efficient means to deliver drugs into the intestinal lumen using very small quantities of compound. However, they appear not to improve passage across the apical intestinal membrane. We are currently testing effects on lifespan of liposome-mediated delivery of a range of putative anti-aging drugs, and will present the results at the meeting.

(1)*Nat. Chem. Biol*. 2010 6: 549. (2) *Cell* 2013 153: 228. (3) *Cell* 2017 169: 442. (4) *Mech. Ageing Dev*. 2009 130: 652.

1157B Simulated Microgravity Impairs *C. elegans* Gut Immunity

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Humanity had been fascinated with space exploration and habitation. However, weak gravity force in outer space, termed as microgravity, has been known to alter normal human biology and induce health risks, such as muscle atrophy and compromised immunity. The underlying processes on how gravity is sensed by the body and how it affects immunity are still unclear.

The nematode *Caenorhabditis elegans*, is a well-known model organism in the field of innate immunity and space research. Similar to humans, worm immune response signaling is regulating by MAPK and TGFβ pathways. Previous microgravity experiments in *C. elegans* have shown an increase in p38 MAPK *pmk-1* expression in simulated microgravity (Li, Wang, & Wang, 2018) and decreased expression of TGFβ ligand *dbl-1* in space (Harada et al., 2016).

In this study, we have utilized *Enterobacter cloacae* carrying tdTomato fluorescence reporter to observe gut colonization in *C. elegans* under simulated microgravity using a 3D clinostat. *E. cloacae* is a known commensal in *C. elegans'* gut but is invasive in immunocompromised *dbl-1* mutant worms (Berg et al., 2019).

Our preliminary results have shown that simulated microgravity can induce *E. cloacae* gut colonization in wild type *C. elegans* and aggravated colonization in *pmk-1* mutants, while there is no difference observed in *dbl-1* mutants which have robust colonization in both normal earth gravity and simulated microgravity, which demonstrates potential involvement of these pathways.

Our study aims to discover the underlying mechanism of gravity sensing and how it affects the immune signaling pathways. In addition, spaceflights are already scheduled to send *C. elegans* aboard the International Space Station (ISS) for actual space gravity experiments.

1158C Effects of a new bacterial pathogen, *Bordetella atroposiae*, on the genetic fitness of *Oschieus tipulae*

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Intracellular bacterial pathogens have long been known to cause infectious diseases within the human population. Obligate intracellular pathogens, like *Rickettsia parkeri* survive and reproduce solely within the host cell, whereas facultative pathogens, like *Listeria monocytogenes*, are able to exploit multiple host niches. To fully understand the microbial interactions taking place in the host epithelia, we sample for natural pathogens of wild rhabditid nematodes, like *Caenorhabditis elegans* and *Oscheius tipulae*. From ecological sampling in Finestere, France, we discovered a new facultative intracellular bacteria inside the intestinal cells of a wild isolate of *O. tipulae.* Characteristically, this bacterium, which we named *Bordetella atroposiae,* has a coccobacilli morphology and exhibits a unique filamentation mechanism for intracellular cell-to-cell spreading.

With the main purpose of understanding the effects of *B. atroposiae* on the genetic fitness of the cognate strain, we conducted lifespan and broodsize assays to determine any health and reproductive impacts of the bacterium. We found that *B. atroposiae* has a severe negative impact on the genetic fitness of the cognate *O. tipulae* (JU1501) strain. Animals placed on *B. atroposiae* lived on average for 3 days, compared to worms on OP50 which lived on average for 7 days. Broodsize assays to determine the number of progeny also showed that animals on *B. atroposiae* produced on average 2 progeny, compared to those grown on OP50 which produced on average 185 progeny. Aside from the cognate strain, we screened a small panel of wild isolates of *O. tipulae* and found a spectrum of resistance and susceptibility to *B. atroposiae.* Two strains that are naturally resistant to *B. atroposiae* were tested as above for fitness effects. We found that the lifespan of these resistant isolates, BA1009, and JU457, were increased compared to the susceptible strains.

Collectively, we discovered a new species of facultative intracellular pathogen, *B. atroposiae*, with high pathogenicity in the cognate and wild-type strains of *O. tipulae.* We have also found certain wild isolates of *O. tipulae* have natural resistance to *B. atroposiae*, indicating some level of coevolution occurring between this bacterium and *O. tipulae*. In the future, we will map the *O. tipulae* alleles leading to *B. atroposiae* resistance.

1159A Innate Immunity Promotes Sleep through Epidermal Antimicrobial Peptides

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Wounding and infection trigger a protective innate immune response that includes the production of antimicrobial peptides in the affected tissue as well as increased sleep. Little is known, however, how peripheral wounds or innate immunity signal to the nervous system to increase sleep. We found that, during *C. elegans* larval molting, an epidermal tolloid/bone morphogenic protein (BMP)-1-like protein called NAS-38 promotes sleep. NAS-38 is negatively regulated by its thrombospondin domain and acts through its astacin protease domain to activate p38 mitogen-activated protein (MAP)/PMK-1 kinase and transforming growth factor β (TGF-β)-SMAD/SMA-3-dependent innate immune pathways in the epidermis that cause STAT/STA-2 and SLC6 (solute carrier)/SNF-12-dependent expression of antimicrobial peptide (AMP) genes. We show that more than a dozen epidermal AMPs act as somnogens, signaling across tissues to promote sleep through the sleep-active RIS neuron. In the adult, epidermal injury activates innate immunity and turns up AMP production to trigger sleep, a process that requires epidermal growth factor receptor (EGFR) signaling that is known to promote sleep following cellular stress. We show for one AMP, neuropeptide-like protein (NLP)-29, that it acts through the neuropeptide receptor NPR-12 in locomotion-controlling neurons that are presynaptic to RIS and that depolarize this neuron to induce sleep. Sleep in turn increases the chance of surviving injury. Thus, we found a novel mechanism by which peripheral wounds signal to the nervous system to increase protective sleep. Such a cross-tissue somnogen-signaling function of AMPs might also boost sleep in other animals, including humans.

1160B The lipid biosynthesis master regulator *sbp-1* is critical for Orsay virus infection in *C. elegans*

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Many viruses utilize host lipids during the viral life cycle. We used the novel *C. elegans*-Orsay virus experimental model to define the impact of virus infection on lipid abundance as well as the dependency of virus infection on host lipids. Lipid staining with Lipidtox in live animals demonstrated that by 24 hpi there was a reduction in lipid abundance of around 60 %. To analyze the dependence of Orsay virus on lipids, we genetically depleted *sbp-1*, a transcription factor involved in the synthesis of lipids. RNAi knockdown of *sbp-1* reduced Orsay virus RNA levels by 200-fold. Likewise, mutant animals in this gene demonstrated a reduction of 1000-fold in the amount of viral RNA. *sbp-1* has been reported to regulate the expression of several enzymes involved in the synthesis of lipids. Mutants in *sbp-1* have reduced levels of oleic acid and increased accumulation of its precursor stearic acid. To analyze if we can recover the viral infection in *sbp-1* mutant animals, we supplemented the growth media with oleic acid. Orsay viral RNA levels were rescued to levels comparable to those found in wild type worms; addition of stearic acid had no effect. Likewise, to investigate what stage of virus infection was affected, an in vivo replicon system was generated in the *sbp-1*mutant animals. Our findings suggest that an early step of virus infection subsequent to viral entry was inhibited. These data demonstrate the critical role of host lipids for virus infection in an *in vivo* model, providing a novel route to further dissect the interactions between lipids and viruses.

1161C Characterization of a new model of Wilson disease to find innovative targets and pathways to attenuate Cu toxicity

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Copper is an essential nutrient that is required for many vitally important processes, such as respiration, connective tissue biogenesis and iron metabolism. However, excess of copper represents a serious danger, due to its ability to induce free radical-induced oxidative damage, as well as impairments of lipid metabolism and neuronal activity. Wilson disease (WD) represents an excellent system for Cu toxicity studies. WD is caused by mutations in ATP7B pump effluxing excess Cu from hepatocytes into the bile. Loss of ATP7B leads to toxic Cu overload in liver and then in brain, causing fatal hepatic and neurologic abnormalities.

Over the last years, *C. elegans* has emerged as an easy-to-use and highly responsive model of micronutrient metabolism. For this reason, we have generated and characterized a new *C. elegans* model of WD. CUA-1, the *C. elegans* ortholog of ATP7B, resides in lysosome-like organelles to sequester excess of copper and, therefore, represents a key component regulating copper supply and detoxification in order to maintain copper homeostasis.

Using Crispr-Cas9 technology a *cua-1(knu781 [H828Q])* strain was generated, that carries a substitution in conserved histidine corresponding to the most common H1069Q variant of ATP7B causing WD in European and North American population. In order to understand the effect of H828Q mutation on *C. elegans* phenotype we employed several assays that allow life span, motility, egg laying, larval development and mitochondria damage to be evaluated. Moreover, we are studying the

colocalization of the mutant CUA-1 protein with markers of different organelles, such as lysosome, Golgi and ER, as well as with the copper sensor CF4.

Our studies established that in absence of copper, *cua-1(knu781)* does not show any significant phenotypic aberrations. However, mutant worms exhibited very poor resistance to copper compared to the control strain. This manifested in a strong decrease in number of eggs, a delay in the larval development, a shorter lifespan, impaired motility and mitochondrial damage. Taken together our finding suggest that *cua-1(knu781)* represents an excellent model for Cu toxicity studies in WD. We further plan to use this model for identification and validation of the new therapeutic targets for WD. Moreover, *cua-1(knu781)* will be used for evaluation of the FDA-approved drugs that emerged from a high throughput screening for compounds reducing Cu toxicity in WD.

1162A Evaluating the effects of individual Orsay virus proteins on the *C. elegans* Intracellular Pathogen Response

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To successfully withstand a pathogen attack, organisms must first recognize the presence of an invader and then mount an immune response. *C. elegans* lack adaptive immunity and have no known professional immune cells, meaning that they rely exclusively on epithelial innate immunity for pathogen defense. The goal of this project is to better understand the interactions between viruses and host innate immune signaling. The Orsay virus, a natural pathogen of *C. elegans,* is a positive single-stranded RNA virus with an unusually small genome containing only four proteins (Félix et al., 2011). Orsay viral infection induces an innate immune response in *C. elegans* known as the Intracellular Pathogen Response (IPR) (Bakowski et al., 2014; Reddy et al., 2017, 2019). The IPR is a transcriptional innate immune response that provides defense against stress and intracellular pathogens (Reddy et al., 2017). The activity of the RNA-dependent RNA polymerase encoded by the Orsay virus RNA1 genome segment is known to trigger induction of the IPR through a mechanism dependent on the DRH-1 receptor, and expression of the RNA-dependent RNA polymerase alone is sufficient to turn on the IPR in the absence of infection (Sowa et al., 2020). However, the individual effects of the other three Orsay viral proteins on IPR induction have not yet been investigated. In this project, we will assess the effects of the Orsay viral capsid, delta, and capsid-delta fusion proteins on induction of the IPR. Thus far, molecular cloning has been used to construct plasmids for heat-shock-inducible overexpression of the capsid, delta, and capsid-delta fusion proteins. These constructs will be used to create transgenic lines overexpressing each of the three viral proteins. To visualize IPR activation levels in these strains, we will use the *pals-5p::GFP* IPR transcriptional reporter strain, in which the promoter for *pals-5* (a gene which is part of the IPR) drives the expression of GFP when the IPR is triggered (Bakowski et al., 2014). By assessing the degree of IPR activation in the viral protein overexpression strains vs non-transgenic siblings, under different stress conditions we will determine whether the Orsay viral capsid, delta, or capsid-delta fusion proteins affect IPR signaling.

1163B Regulation of the Intracellular Pathogen Response by purine metabolism in *C. elegans*

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The Intracellular Pathogen Response (IPR) is a host transcriptional response in *C. elegans* to two natural, yet distinct, intracellular pathogens: the microsporidian eukaryotic pathogen, *N. parisii,* and the positive-sense, single-stranded RNA virus, the Orsay virus. To better understand regulation of the IPR, our lab used a forward genetic screen to identify novel negative regulators of the IPR; and here, we found purine nucleoside phosphorylase *(pnp-1)*. *pnp-1* acts in the purine salvage pathway to convert the nucleoside inosine into the free base hypoxanthine, which suggests that perturbation of purine metabolism may activate the IPR.

Loss of *pnp-1* results in the constitutive expression of IPR genes and confers resistance to *N. parisii* and the Orsay virus. *pnp-1* mutants also have increased survival upon infection with *N. parisii*. These phenotypes are similar to the previously identified negative regulator of the IPR called *pals-22*. However, distinct from *pals-22* mutants, *pnp-1* mutants display increased resistance to the extracellular bacterial pathogen *Pseudomonas aeruginosa*. Interestingly, RNAseq analysis shows *pnp-1* mutants have broad upregulation of IPR genes, but distinct from *pals-22* mutants, they also have upregulation of genes induced upon bacterial infection and other immunity pathways, such as the p38 MAP kinase pathway.

To further understand the effects of purine metabolism on regulation of the IPR, we looked into other components of the purine salvage pathway. We used an RNAi screen and found that knock-down of only one other purine metabolism enzyme

gene in our screen, adenosine deaminase (*adah-1*), led to activation of the IPR. Interestingly, *adah-1* acts just upstream of *pnp-1* in the purine degradation pathway, converting the nucleoside adenosine into the nucleoside inosine. Full deletion of *adah-1* results in sterility, and so we are using RNAi knockdown of *adah-1* to investigate which genes are regulated by *adah-1* using RNAseq analysis. Here we expect to gain more insight to the genes regulated in common by *adah-1* and *pnp-1*. We also will investigate how *adah-1* regulates pathogen resistance to obtain a better picture of how purine metabolism regulates immunity.

1164C Determinants of Signaling Specificity for DBL-1/BMP in the Immune Response of the Nematode *Caenorhabditis elegans*

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When exposed to infection, the nematode *C. elegans* mounts an innate immune response through secretion of antimicrobial peptides (AMPs). Different signaling pathways in the worm regulate release of these AMPs. Our research aims to determine the way in which the *C. elegans* BMP like pathway - regulated by the ligand DBL-1 - interacts with other signaling pathways in order to confer immunity. The DBL-1 pathway plays a significant role in development and we are interested in discovering how it is able to differentiate a response specific to immunity, separate from its other functions in the worm. Through survival analysis we have shown that when exposed to pathogenic bacteria, expression of the DBL-1 effector SMA-3 in either the hypodermis or pharynx is capable of improving survival compared to *sma-3* mutants. These results suggest possible cross-talk between the pharynx and the intestinal site of infection, potentially through a non-canonical signaling pathway. Through the use of qRT-PCR we have found two immune-related genes with expression patterns that indicate regulation by DBL-1 signaling. We plan to use these data to further examine in which tissues DBL-1 signaling plays the largest role in the immune response of the worm and whether canonical or non-canonical DBL-1 activity is responsible for this function. We have also shown that mutation of three of the other *C. elegans* TGF- β ligands results in similarly impaired survival rates as our *dbl-1* mutants. This includes the BMP like ligand TIG-2 as well as TGF-β ligands DAF-7 and UNC-129. We plan to observe the survival patterns of double mutants for dbl-1 and these other TGF-β ligands in order to determine whether these are independent effects or due to involvement in the same pathway.

1165A *Commercial Citrus paradisi* and *Citrus reticulata* essential oils from Argentina rescue *Caenorhabditis elegans* from *Pseudomonas aeruginosa* infection

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Natural products with anti-quorum sensing (QS) properties are attractive therapeutic agents that could help fight antibiotic resistance. *C. paradisi* and *C. reticulata's* Essential Oils (EOs) have been shown to interfere with *Pseudomonas aeruginosa* QS signaling *in vitro* (on acyl-homoserine lactones AHLs production and elastase activity) and biofilm formation leading to an attenuation of the microbial virulence.

EOs from Argentina-grown *Citrus paradisi* (grapefruit) and *Citrus reticulata* (mandarin) were obtained by cold-pressing and cold-pressing followed by steam distillation. These EOs are complex mixtures of mainly monoterpene (94-98%) and sesquiterpene hydrocarbons and their oxygenated derivatives, with limonene as their main component (88-89.8%).

We assessed *C. paradisi* and *C. reticulata's* EOs in their ability to extend the survival of *Caenorhabditis elegans* infected with *P. aeruginosa* PA14 and PAO1 and their effect on bacterial growth and biofilm formation. Fast (phenazines-mediated), slow (QS-mediated) and HCN-mediated killing mechanisms of *C. elegans* were studied. Also, a food choice assay was used to study the effect of EOs on AHLs by PA14 *in vivo*. Mandarin and grapefruit EOs' toxicity was evaluated assessing *C. elegans* survival, brood size and body length.

Our results showed that mandarin and grapefruit EOs at 0.2 mg/mL were safe for the nematodes, significantly inhibited biofilm formation (up to 50%), and improved worm survival following infection in the three assays evaluated (40-74%) without affecting PA14 and PAO1 growth. The food choice assay revealed a clear preference for PA14 grown in the presence of citrus EOs. *C. paradisi* EOs were more effective in reducing the virulence of *P. aeruginosa* in comparison with *C. reticulata* EOs and limonene, their main component. In addition, we observed no difference between the *in vivo* activity of EOs of each citrus species obtained by the two methodologies described.

In conclusion, targeting the QS system is gaining much attention as an infection control strategy. Therefore *C. paradisi* and *C. reticulata* EOs could be proposed as sources of anti-QS agents for controlling human *P. aeruginosa* infections.

1166B Exposure to human microbiota isolates during development impacts *Caenorhabditis elegans* susceptibility to *Pseudomonas aeruginosa* infection

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Commensal microorganisms that colonize host surfaces can modulate susceptibility to infection. This can occur through direct inhibition of pathogen growth via competitive exclusion, or by modulation of the host environment to prevent pathogen dissemination and infection-mediated damage. However, understanding the mechanisms underlying these interactions is challenging due to the complexity of the microbiome. Here, we investigate the influence of human respiratory tract microbiota isolates on *C. elegans* susceptibility to *Pseudomonas aeruginosa* infection. Identifying isolates that differentially regulate resistance to infection will provide insight into how particular strains isolated from the human microbiome may improve or worsen infection outcomes.

In order to identify microbiotal isolates that can protect *C. elegans* from *P. aeruginosa* infection, a screen of bacterial isolates derived from the human respiratory tract was carried out using a liquid-based infection assay. Animals were first exposed to lawns of individual microbiotal isolates during development, prior to infection with a pathogenic strain of *P. aeruginosa*, PA14. We find that pre-exposure to two non-pathogenic isolates of *P. aeruginosa* is able to increase survival during PA14 liquid killing, compared to animals pre-exposed to *E. coli* OP50. This protective phenotype can also be induced by other mildly pathogenic strains of *P. aeruginosa*, including a laboratory strain with genetically attenuated virulence, PAO1Δ*vfr*. Current work focuses on identifying host pathways underlying this protective phenotype, as well as how previous exposure to other Gram-negative pathogens can regulate *P. aeruginosa* infection outcomes in *C. elegans.*

1167C Immunoglobulin light chain amyloidosis modelled in *C. elegans*

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Immunoglobulin light chains amyloidosis (AL) is caused by the systemic damage produced by amyloidogenic monoclonal immunoglobulin light chains (LCs), secreted by a bone marrow plasma cell clone and transported to target organs through blood. Cardiac involvement is the most frequent and is the main determinant of patients' survival because it results in the onset of a rapidly progressive and fatal cardiomyopathy. Notably, independently from the organs involved, cardiotoxicity is the main cause of death in AL patients since 80% die from heart failure or fatal arrhythmias. As it stands today, AL is still a mysterious disease: the mechanisms underlying the ability of LCs to exert tissue-specific cardiac targeting and damage remain still undetermined. Both soluble pre-fibrillar and fibrillar LCs were reported to exert toxic effects on tissues. Chronic extracellular fibril deposition contributes to organ damage, altering the tissue architecture, while soluble pre-fibrillar LCs induces oxidative stress reducing cell viability through mitochondria damage. The absence of an animal model recapitulating the main biochemical and clinical characteristics of disease makes difficult the elucidation of the molecular mechanisms underlining the different organ tropism of LCs.

Taking advantage of the *C. elegans*' "Ancestral Heart Theory" and based on the finding already obtained by our group on the ability of worm's pharynx to specifically recognize cardiotoxic LCs as toxic, we developed and characterized the first animal model of AL. Through the use of *mos1*-mediated Single Copy Insertion, we generated a transgenic strain constitutively expressing in body wall muscle cells an amyloidogenic LC whose sequence was deduced from an AL patient with cardiac involvement (MNH). As control, a strain expressing a non-amyloidogenic LC whose sequence was deduced from a patient with multiple myeloma (MNM) was developed as well as a strain expressing the empty vector only (MNV). MNH and MNM expressed comparable LC levels. No locomotor impairment was present in all worm' strains indicating that LC did not cause any cell-autonomous defect. The LCs produced by MNH and MNM worms were released, in a soluble form, in the extracellular

space but only the amyloidogenic LC induced in MNH a significant decrease of pharyngeal function accompanied by specific alterations of pharyngeal muscle ultrastructure such mitochondrial damage.

The development of these transgenic strains represents a significant advancement in the field of the AL amyloidosis for which so far, any attempt to produce an animal model of the disease had failed, and provides an unprecedented tool for investigating the physiological molecular basis of the disease and development of novel drugs.

1168A Quantifying Pathogen Load of Geographical Isolates of *Caenorhabditis elegans.*

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Organisms frequently encounter bacteria, some of which are pathogenic and threaten the survival of the organism. In their natural domain, *Caenorhabditis elegans* encounter various microbial pathogens that are capable of infecting their intestines. Such intestinal pathogens can infect the gastrointestinal tract of humans as well, prompting *C. elegans* as an excellent model to study host-pathogen relationships. Once pathogenic microbes have bypassed the epithelium, the organism relies on the innate immune system to detect and eliminate the pathogen. We were interested in determining whether natural isolates of *C. elegans* exhibited differences in susceptibility to microbial pathogen and whether these differences were correlated with the intestinal pathogen load in infected animals. To accomplish this, we obtained eight *C. elegans* isolates from diverse geographical areas and infected worms with the gram-negative pathogen *P. aeruginosa* before performing survival assays and measuring the intestinal pathogen load. Relative to N2, six of the eight isolates were more susceptible to *P. aeruginosa* infection, the exceptions being CB4856 and JU1171. The intestinal bacterial load was assessed in these six isolates to determine if susceptibility to *P. aeruginosa* was correlated with increased pathogen burden. We exposed *C. elegans* isolates to the non-pathogenic bacterium *E. coli* OP50 to establish a baseline measure of bacterial load. Relative to N2, the LKC34 isolate had a lower intestinal load of *E. coli* OP50, whereas there was no significant difference in bacterial load in the other isolates. Although all isolates possessed significantly greater amounts of *P. aeruginosa* compared to their *E. coli* OP50 bacterial loads, there was no significant difference in the amount of *P. aeruginosa* between natural isolates and the lab strain N2. However, the percent increase in intestinal bacterial load from the *E. coli* OP50 baseline was significantly higher in the LKC34 isolate following *P. aeruginosa* infection, suggesting that pathogen burden may be correlated with greater *P. aeruginosa* susceptibility for this isolate. Currently, we are examining the establishment and clearance of *P. aeruginosa* infection in *C. elegans* natural isolates using fluorescent bacterial strains. Ultimately, this study attempts to understand the implications of how intestinal pathogens infect and proliferate in *C. elegans*, which may provide additional knowledge regarding microbial infection of the human intestine.

1169B A novel pair of receptor tyrosine kinases are required for oomycete pathogen recognition by *C. elegans* and resistance to infection

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Over the past two decades, *C. elegans* has been used as a model to study innate immune responses to naturally infecting pathogens, such as viruses, bacteria, microsporidia and fungi. Studying natural *C. elegans* pathogens allows us to understand immune response pathways that have co-evolved alongside the pathogen, which may be pathogen-specific or more broadly conserved. We previously described a new natural oomycete infection of *C. elegans* by *Myzocytiopsis humicola.* Oomycetes are morphologically similar to fungi and cause disease in plants and animals, including humans. *Phytophthora infestans* is the most notorious oomycete, responsible for potato blight, which is thought to have resulted in the Irish Potato Famine. Animal oomycete infections are less well understood, leaving a niche that can be fulfilled by *C. elegans* as a tractable model host. We have found that *C. elegans* are able to detect an *M. humicola* extract and upregulate an immune response that appears to be unique to oomycete infection. Pathogen detection is likely to occur in sensory neurons, which in turn trigger a cross-tissue signalling cascade leading to upregulation of *chitinase-like* genes in the epidermis and structural changes in the cuticle that confer resistance to oomycete infection. Using a forward genetic approach, we have identified a pair of receptor tyrosine kinases expressed in the epidermis that are required for upregulation of *chitinase-like* genes. Loss of either of these RTKs leads to increased susceptibility to *M. humicola* infection, and overexpression of one results in constitutive activation of the downstream immune response pathway. Phosphoproteomic analysis upon extract treatment has identified RTK-dependent downstream phosphorylation events and highlighted genes that we are currently testing as to whether they play a role in mounting the immune response. By characterising the role and interactions of these RTKs, we can not only understand a novel

pathogen recognition response pathway in *C. elegans*, but also potentially assess its relevance for other animal oomycete infections that are largely understudied.

1170C *C. elegans* expressing human amyloidogenic proteins: a useful model for studying amyloidosis

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Systemic amyloidosis is a clinically heterogeneous type of protein misfolding diseases caused by the extracellular deposition of insoluble fibrillar aggregates that are known as amyloid.

The mechanisms for fibrils' formation have been extensively investigated *in vitro* leading to a better understanding of the pathogenesis of the disease. However, the pathway of amyloid toxicity *in vivo* remains an unmet challenging issue and animal models may provide crucial clues on the identification of the protein species causing tissue toxicity.

Choosing and creating a good animal model could be challenging. In some cases, transgenic mouse models have been successfully generated for the study of certain types of systemic amyloidosis but in other cases, those models were not capable of reproducing the clinical manifestations of the disease. Thus, the simpler multicellular organism *Caenorhabditis elegans* (*C. elegans*) served as model allowing ease of study and offered the chance to investigate and modulate the toxicity of specific protein conformers *in vivo*. Indeed, we have recently established transgenic strains expressing human wild-type and mutated β_2-microglobulin which are associated to two different types of amyloidosis. Using the INVertebrate Automated Phenotyping Platform (INVAPP) and an algorithm (Paragon), developed by Partridge and colleagues for high-throughput plate-based screening, we were able to detect a pathological phenotype in our transgenic strains. The phenotypic defect is strictly correlated to the expression and accumulation of the monomeric and soluble oligomeric species of the protein. Worms treated with doxycycline, a generic inhibitor of amyloid, ameliorated their phenotype, even if it was not possible to see a complete rescue.

It's almost certain that amyloid fibrils and prefibrillar oligomers directly induce the toxicity and the tissue damage in the patients through a mechanism which is still not completely clarified. However, a contribution to the toxicity could be also attributed to the presence of a hypoxic state as consequence of the amyloid accumulation.

Therefore, the nematode model could be useful also to evaluate the effect of oxygen deprivation, in enhancing the aggregation and toxicity of β_2-microglobulin *in vivo*. In particular, the observation of metabolic profiles of *C. elegans* models expressing amyloidogenic proteins in different environmental conditions, could contribute to identify signature biomarkers of the amyloid pathology.

1171A Understanding Adenylosuccinate Lyase Deficiency locomotion deficit using *C. elegans* as a model

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Adenylosuccinate Lyase Deficiency (ASLD) is a disorder at the intersection of genetic diseases, metabolic disorders, and nervous system diseases. ASLD is caused by decreased function of adenylosuccinate lyase (ADSL), which converts SAICAR to AICAR and S-AMP to AMP in purine metabolism. Symptoms range from mild, such as slower intellectual development to severe, such as neonatal fatality. Severe motor delay and low muscle tone are common symptoms of this disorder with SAICAR accumulation being suggested as a key player in muscle dysfunction.

I use *C. elegans* to model ASLD. *adsl-1* loss of function mutant *C. elegans* are slower and uncoordinated when moving. I hypothesize abnormal crawling and swimming locomotion in *adsl-1* deficient *C. elegans* has distinct pathogenesis due to usage of *adsl-1* in neuronal and muscular tissues. We use knockout allele engineered tissues specific knockdowns and overexpressing animals to study the movement of normal and *adsl-1* deficit animals. Using WormLab software we were then able to quantify speed, bending angle, and thrashing of these animals during crawling and swimming locomotion. Crawling data from whole-body loss of *adsl-1* results in an increased preference to commit tighter bending patterns and slower moving speed. Knockout of *adsl-1* in neuronal tissue results in similar crawling patterns as whole-body loss of function animals which is not ameliorated

when *adsl-1* is overexpressed in the muscle alone and *adsl-1* is not expressed in the neuron. Swimming data from whole-body loss of *adsl-1* results in loss of a bending pattern and decreased thrashing. Knockout of *adsl-1* in muscular tissue results in altered control of bending angle while swimming, whereas neuronal knockout of *adsl-1* with expression remaining in muscle is unimpacted. In addition to using mutant animals, we use RNAi of *adsl-1* for metabolic studies. Liquid chromatography paired with mass spectrometry allows for the quantification and identification of metabolites within purine metabolism as well as other pathways of interest. Metabolic analysis shows *adsl-1* RNAi animals have a distinct metabolic profile. SAICAR is increased, while S-AMP and other metabolites remain relatively unchanged. Further studies of muscle dysfunction amelioration and metabolic restoration using drug treatments are to be completed. In conclusion, our work suggests *adsl-1* has specific phenotypic outcomes depending on location of expression.

1172B Analysis of PALS-25 as an activator of the Intracellular Pathogen Response in *C. elegans*

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Tight regulation of immune responses is important for overall fitness, but the mechanisms underlying many of these pathways are incompletely characterized. One example is the recently described “Intracellular Pathogen Response” (IPR) in *C. elegans*. Our lab has defined the IPR as a set of ~80 genes that are upregulated in response to several triggers including intracellular pathogen infection, proteasome inhibition and heat stress. The IPR is also genetically regulated by the species-specific antagonistic paralogs *pals-22* and *pals-25*. *pals-22* represses the IPR; loss-of-function mutations in *pals-22* promote IPR gene expression, increase pathogen resistance and improve tolerance of proteotoxic stress. *pals-25* acts downstream of *pals-22* to activate the IPR; loss-of-function mutations in *pals-25* suppress the phenotypes of *pals-22* mutants.

Recently, we identified a gain-of-function mutation of *pals-25* that truncates the C-terminus of PALS-25 by 13 amino acids, or ~5% of the total protein. Unlike previously characterized mutations of *pals-25*, this *pals-25(gf)* allele results in constitutive expression of IPR genes in both wild type and *pals-22* mutant backgrounds. However, only a subset of IPR genes appear to be upregulated in *pals-25(gf)* mutants when compared to *pals-22* mutants. *pals-25(gf)* animals are similar to *pals-22* mutants in that they are resistant to infection by *Nematocida parisii* but are dissimilar in that they display wild type tolerance of heat stress. Together, these observations may allow for the identification of IPR-related genes that are specifically important for pathogen resistance phenotypes.

Previous co-IP/MS studies determined that PALS-22 and PALS-25 are physically associated. Here we show that FLAG-IP of PALS-22::GFP::3xFLAG identifies PALS-25 as a binding partner, but this interaction is no longer detected for the C-terminally truncated version of PALS-25 encoded by the *pals-25(gf)* allele. Yeast two-hybrid analysis of full-length PALS-25 also revealed PALS-22 as a binding partner, but the truncated version of PALS-25 did not identify PALS-22 as a binding partner. Together, our results suggest a model where PALS-22 physically represses the ability of PALS-25 to activate the IPR and the interaction of the two proteins requires the C-terminus of PALS-25. Ongoing studies will explore the mechanism of IPR activation by PALS-25 after it is released from repression by PALS-22.

1173C Ubiquitin-related modifying enzymes in the regulation of HLH-30 signaling during *S. aureus* infection

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Ubiquitination and ubiquitination-like modifications are conserved post-translational modifications that influence virtually all physiological processes, including innate immunity. They are controlled by hundreds of ubiquitin (Ub) and Ub-like modifying enzymes that regulate their targets by modulating their stability, localization or activity. In recent years, the importance of these enzymes in *C. elegans*’ innate immunity has been revealed, with the identification of key Ub-related regulators of innate immune pathways, as well as Ub-related effectors in the host response (Garcia-Sanchez *et al,* 2021). Little is known, however, about the role of these processes in the immune response to the intestinal bacterium *Staphylococcus aureus*, which is coordinated by the transcription factor TFEB/HLH-30 (Visvikis *et* al, 2014; Najibi *et al*, 2016). Our research focuses on deciphering the importance of Ub-modifying enzymes in regulating this immune pathway, as well as their role as effectors in the host response.

Firstly, we undertook an RNAi-based screen to identify new immune regulatory enzymes. We generated a fluorescent reporter strain which allowed us to screen the effect of 216 enzymes on the activity of HLH-30 during *S. aureus* infection. We identified 22 genes whose downregulation reduced reporter fluorescence. To distinguish between specific and more general regulators, we performed a parallel screen using the model of epidermal infection by the fungus *Drechmeria coniospora,* which triggers an immune response mainly controlled by the p38 MAPK/PMK-1 pathway (Zugasti *et al*, 2014; 2016). We found 6 enzymes that are common to both screens; the remaining 16 enzymes are potentially specific regulators of the HLH-30-dependent immune response to *S. aureus*.

Secondly, to identify Ub-related immune effectors important for the response to *S. aureus* infection, we analyzed previously published RNA-seq data (Visvikis *et* al, 2014) and determined that 16 out 19 Ub-related enzymes induced during infection are controlled by HLH-30. Focusing on 3 conserved E3 Ub-ligases, we confirmed their HLH-30 dependent induction by qPCR. Interestingly, using survival assays, we could demonstrate their importance in *C. elegans*' host defense against *S. aureus* infection suggesting they play a specific effector role in infection.

Altogether, these results demonstrate the importance of Ub-related enzymes in HLH-30 dependent host response to *S. aureus* infection and have revealed specific and general Ub-related mechanisms in the regulation of immune pathways.

1174A Defining the microbiota host defense response in *C. elegans*

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The intestine is in constant contact with the external environment that predisposes it to harmful microorganisms. Therefore, there is an urgent need to understand the fundamental mechanisms that are used by a host to induce a defense response in intestinal epithelial cells. Given the inherent complexity of the mammalian intestine, it is challenging to parse out host-pathogen interactions in a whole-animal model. The intestine of the nematode *C. elegans* shares extensive morphological and functional similarities with the mammalian intestine, making it a powerful whole-animal model to dissect interactions between intestinal epithelial cells and microbes *in vivo.* Research done over the last several years has identified conserved innate host defense pathways in *C. elegans*, including the p38 mitogen-activated protein kinase (MAPK), Wingless-Integration site mutant (Wnt), and transcription factor EB (TFEB), among others. What is not known is the extent to which these innate host defense pathways are critical for the survival of *C. elegans* in its natural habitat. To approach this question, we asked which of the host defense pathways are activated by the natural microbiota of *C. elegans*. We identified specific microbiota that are able to elicit Wnt and HLH-30/TFEB activation. In addition, we identified distinct expression of FMO-2, a flavin-containing monooxygenase that is required for host defense, by Gram-positive microbiota but not by Gram-negative microbiota. Future directions are to define the distinct effector function of innate immune pathways in the context of microbial communities of *C. elegans*.

1175B Nuclear receptors downstream of HLH-30/TFEB modulate host defense responses.

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Human pathogen *Staphylococcus aureus* colonizes 30% of the population and causes diverse infections. Recent studies identified a novel helix-loop-helix TF(HLH-30)/Transcription factor EB (TFEB) is activated upon *S. aureus* infection in *Caenorhabditis elegans* and murine macrophages respectively. HLH-30 activation leads to upregulation of antimicrobial peptides, lysozymes, together with autophagy genes in *C. elegans.* This host defense response is conserved in macrophages. Although the signaling cascade leading to TFEB activation has been characterized in both systems, very little is known about mediators downstream of TFEB. Interestingly, 70% of host defense genes regulated by HLH-30 do not possess the promoter sequence required for its binding, indicating a role for other downstream TFs and interactions. RNA-sequencing in WT vs TFEB KO macrophages and *C. elegans* revealed upregulation of several nuclear receptors (NR) upon *S. aureus* infection, in a TFEB dependent manner. We identified 17 nuclear hormone receptors (NHR) in *C. elegans* and 5 NRs in macrophages that are highly upregulated upon infection. NR are involved in regulating a multitude of biological processes including metabolism, development, and immune responses. Our studies found *nhr-55, nhr-42* in *C. elegans* and NR1D1 in macrophages, are involved in regulating a host defense response. Animals lacking *nhr-42* are more resistant to *S. aureus* and *E. faecalis* infection compared to WT animals. We found that *nhr-42* functions in multiple tissues to confer this resistance. In contrast, animals lacking *nhr-55* are hypersusceptible to infection compared to WT. Moreover, *nhr-55* functions only in the intestine, the site of infection. Importantly, both *nhr-42* and *nhr-55* mutants had no defects in lifespan or fecundity compared to WT.

We performed RNA-seq on uninfected and infected animals, comparing the transcriptomic profile of animals lacking NHRs to WT. We found genes involved in host defense and detoxification of xenobiotics downregulated in *nhr-55* mutant animals,

compared to WT upon infection. Conversely, uninfected *nhr-42* mutants had a higher expression of around 300 genes when compared to WT. GO analysis showed overrepresentation of innate immune genes. Several antimicrobials including antibacterial factor related peptides, c-type lectins, caenacins, and saposins were upregulated in *nhr-42* mutants. Furthermore, *nhr-42* mutants showed a lower bacterial burden after 24h of infection, compared to WT. Also, infected animals lacking *nhr-42* showed lower expression of lipid metabolism genes compared to infected WT animals. We performed lipid staining on infected and uninfected worms, and found *nhr-42* mutants retain more lipids upon infection as compared to WT. We have found two novel NHRs, *nhr-55* an activator and *nhr-42* a repressor, functioning downstream of an evolutionarily conserved positive regulator of immunity in HLH-30.

1176C Neuronal C-type lectin receptors mediate recognition of oomycete pathogens in *C. elegans*

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Innate immune responses rely on signaling events triggered by the detection of pathogen-associated molecular patterns or damage related host biomolecules by specific host receptors in specialized immune cells, such as macrophages and dendritic cells. While such cells are missing in *C. elegans*, there is some evidence of pathogen recognition, which points towards existence of detection strategies that are yet unknown. We have previously shown that *C. elegans* can respond to an innocuous extract made from oomycete infected animals by triggering a recognition response characterized by the induction of chitinase-like (*chil*) genes in the hypodermis. Through a forward genetic screen aimed at identifying suppressors of *chil* gene induction upon treatment with this extract, we recovered loss-of-function mutations in *ceh-37*, *clec-27* and *clec-35* genes which led to complete loss of oomycete recognition response in *C. elegans* and made animals more susceptible to infection by the oomycete *Myzocytiopsis humicola*. Using smFISH and fluorescent reporters, we found *clec-27* and *clec-35* to be expressed in neurons while RNA-seq analysis revealed changes in the expression of these genes in a *ceh-37* mutant background. Furthermore, neuronal rescue of *clec-27* function specifically in *ceh-37* expressing neurons, and particularly in AWA, was sufficient to restore *chil* gene induction in *clec-27* mutants upon exposure to oomycete extract. Interestingly, *clec-27* and *clec-35* are neighboring genes sharing a bidirectional promoter, an organization which indicates coregulation of the two genes and a possible requirement to produce the two proteins in stoichiometrically equal amounts. These observations suggest that CLEC-27 and CLEC-35 could be forming a heterodimeric receptor involved in oomycete recognition in AWA neurons. Additionally, when these mutants were exposed to the phylogenetically distinct oomycete *Haptoglossa zoospora*, both *clec-27* and *clec-35* mutant animals showed *chil* gene induction, whereas *ceh-37* mutants did not. This suggests that CLEC-27 and CLEC-35 are receptors specifically involved in the detection of *M. humicola* and a different receptor(s) in *ceh-37* expressing neurons mediates detection of *H. zoospora.* Overall, our study provides evidence for neuronally expressed C-type lectins as pathogen recognition receptors in *C. elegans* which mediate detection of a newly identified class of natural pathogens of *C. elegans* in a pathogen-specific way.

1177A Investigating organophosphate intoxication and mitigation using Pharyngeal Pumping: a novel bio-assay to probe poisoning

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Organophosphate poisoning remains an important issue associated with bioterrorism and global health exposure pertaining to their use in the agrochemical industry. Organophosphate poisoning results from inhibition of acetylcholinesterase at cholinergic synapses including the neuromuscular junction. The resulting effect is the accumulation of acetylcholine, overstimulation of acetylcholine receptors and powerful neurotoxic effects. Atropine acts as the primary symptomatic treatment while oximes mitigate poisoning by reactivating the OP-inhibited acetylcholinesterase. The incomplete efficacy of current oxime treatments promotes the need for continued development of better drugs or distinct approaches to mitigation.

Caenorhabditis elegans promises a route to quantify and investigate the intoxication at cholinergic neuromuscular junctions. This is because the integrity of cholinergic transmission that controls muscle contraction and relaxation is dependent on acetylcholinesterase function. In recent work we have shown that the dose-dependent inhibition of pharyngeal pumping observed in whole worms exposed to OPs is a direct correlate of the levels of intoxicated acetylcholinesterase activity (2021 Neurotoxicology 82:50-62). Moreover, we have shown that inhibition of pumping is susceptible to oxime mitigation. We have analysed genetic determinants of the OP dependent inhibition of pharyngeal pumping and made the surprising observation that it is largely driven by cholinergic determinants expressed at the body wall rather that the pharyngeal muscle.

A comparison of mutants that enable us to discriminate different classes of nicotinic acetylcholine receptor has highlighted sub-types of receptors that generate the receptor hyperactivity that defines the effects on pharyngeal pumping. Interestingly, modulation at the level of these receptors defines a behavioural plasticity that might provide novel routes to poisoning mitigation. Overall, this work highlights how C. elegans can be used to model OP therapeutics and highlight unexpected routes for inter tissue toxicity and routes to mitigation of intoxication.

1178B Identification and characterization of *C. elegans* genes that *S. maltophilia* targets to evade host insulin-like DAF-2/16 pathway defenses

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The bacterivorous nematode *Caenorhabditis elegans* is an excellent model to study host innate immune responses to various bacterial pathogens, including the emerging nosocomial pathogen *Stenotrophomonas maltophilia*. Members of the *Stenotrophomonas* genus are components of the *C. elegans* natural habitat and native microbiome (Dirksen *et al.,* 2016; Samuel *et al.,* 2017). Thus, the study of this interaction has both medical and ecological relevance. We have 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 suggests that pathogenic stains of *S. maltophilia* evade the pathogen resistance conferred by activation of the DAF-2/16 pathway. In an effort to understand how pathogenic *S. maltophilia* JCMS and JV3 are able to bypass the nematode's DAF-2/16 pathway defenses, we used transcriptional profiling in *wild-type* and *daf-2* mutants to identify candidate *C. elegans* innate immunity genes that may be targeted by *S. maltophilia* 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 pathogenic *S. maltophilia*. We hypothesize that pathogenic *S. maltophilia* may block the function of expressed genes and candidate target genes will be contained within this subset. We also hypothesize that *S. maltophilia* may prevent the expression 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 and mutant allele availability to select candidate target genes for functional analyses. We expect that candidate *S. maltophilia* target genes should be required for *daf-2*-mediated lifespan extension and are using RNA interference to test this prediction. 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.

1179C The role of the gut microbiome in host adaptation to environmental xenobiotics

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The use of *C. elegans* in microbiome research has opened new venues for addressing fundamental questions about host-microbiome interactions. Among those are questions regarding the role of gut bacteria in host adaptation to a changing environment. Gut bacterial communities offer huge biochemical diversity that has been shown to alter concentrations, efficacy (of drugs), or toxicity of various xenobiotics. However, to what extent and how frequently do bacteria enable host adaptation, and what determines their acquisition from the environment is not well understood. Using *C. elegans* raised on the CeMBio synthetic community, we examined the ability of environmentally acquired gut bacteria to enable host adaptation to xenobiotics, comparing antibiotics exposure that affected bacteria alone, versus a similar exposure which was also toxic to the host. Antibiotic resistance among individual members of the bacterial community varied considerably, and relative resistance correlated well with their ability to protect worms from toxic effects, as well as with their relative abundance in the environment following antibiotic exposure, as determined with 16S rRNA next generation sequencing. However, similarly characterized community composition inside worms was not determined solely by environmental availability, and its configuration was further determined by host-associated factors and inter-bacterial interactions. Beyond the immediate benefit of toxin resistance gut bacteria appear to provide to the host, current work is underway to determine long-term consequences of altered gut microbiomes constrained by the exposure to xenobiotics.

1180A A Survey of the Kinome Pharmacopeia Reveals Multiple Scaffolds and Targets for the Development of Novel Anthelmintics

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Over one billion people are currently infected with a parasitic nematode. Symptoms can include anemia, malnutrition, developmental delay, and in severe cases, death. Resistance is emerging to the anthelmintics currently used to treat nematode infection, prompting the need to develop new anthelmintic drugs. Towards this end, we identified a set of kinases that may be targeted in a nematode-selective manner. We first screened 2040 inhibitors of vertebrate kinases for those that impair the model nematode *Caenorhabditis elegans*. By determining whether the terminal phenotype induced by each kinase inhibitor matched that of the predicted target mutant in *C. elegans*, we identified 17 druggable nematode kinase targets. Of these, we found that nematode EGFR, MEK1, and PLK1 kinase orthologs have diverged from vertebrates in key residues within their drug-binding pocket. For each of these targets, we identified small molecule scaffolds that may be further modified to develop nematode-selective inhibitors. Nematode EGFR, MEK1, and PLK1 therefore represent important targets for the development of new anthelmintic medicines.

1181B Transcriptomic Profiling of *Caenorhabditis elegans* Wild Isolates Reveals Gene Expression Differences in Response to Microbial Infection

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Infection from microbial pathogens is a constant threat to organismal survival. In its natural environment the microbivorous nematode, *Caenorhabditis elegans*, frequently encounters pathogenic bacteria. Although *C. elegans* possess physical barriers and exhibit behavioral responses to decrease the likelihood of infection, they must also recognize and respond to pathogens that have bypassed these defenses. This response is modulated through the innate immune system, a defense mechanism comprised of evolutionarily ancient components that are highly conserved across phyla. Yet, *C. elegans* do not exhibit obvious conservation of microbial defense pathways found in arthropods and mammals. Rather, pathogen detection occurs via many different systems that converge upon a core set of physiological responses as well as a set of pathogen-specific responses. Geographical isolation has enabled natural isolates of *C. elegans* to adapt to different microbial ecologies, making them an excellent model to examine the evolutionary basis of innate immunity. We assessed survival of two *C. elegans* isolates, N2 and CB4856, infected with pathogenic bacteria (*Enterococcus faecalis, Providencia rettgeri, Pseudomonas aeruginosa*, or *Staphylococcus aureus*) and found isolate-specific responses to the gram-positive pathogen, *E. faecalis*. To identify molecular changes that may be responsible for the intraspecific difference in immunity, we performed RNA sequencing of whole animals following 24 hours of exposure to *E. faecalis*. In N2 and CB4856 animals, 2384 and 1776 genes were differentially expressed, respectively. A minority of genes, 410 in total, were differentially expressed in the same direction in both genotypes, suggesting that *E. faecalis* infection has elicited dissimilar transcriptomic responses in N2 and CB4856 isolates. Geneset enrichment analysis of DEGs unique to N2 or CB4856 or common to both genotypes identified a handful of biological processes, most of which are involved in stress response, metabolism, or the extracellular matrix. Currently we are identifying transcription factor binding sites present in genes that are part of an enriched GO term category with the aim of identifying trans-acting regulatory elements that may be involved in the transcriptomic responses of N2 and CB4856 to *E. faecalis*. Ultimately, our study seeks to shed light on the evolutionary origins of innate immunity as well as reveal uncharacterized aspects of mammalian defenses against infection.

1182C The role of the bZIP transcription factor ZIP-1 in the Intracellular Pathogen Response of *C. elegans*

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How does the nematode *Caenorhabditis elegans* respond to natural pathogen infections with non-professional immune cells? To answer this question, we are studying the Intracellular Pathogen Response (IPR) – a host transcriptional response induced by infection with molecularly diverse natural pathogens – the Orsay virus and fungal-related microsporidia *Nematocida parisii*. In addition to intracellular pathogens, the IPR can be activated upon exposure to heat stress and following proteasome inhibition. The IPR is genetically regulated by two antagonistic paralogs of unknown function, PALS-22 and PALS-25, as well as by PNP-1, a protein involved in regulation of purine metabolism. Loss-of-function mutations in *pals-22* and *pnp-1* cause constitutive IPR activation with increased intracellular pathogen resistance, indicating that the IPR confers a protective response.

The IPR involves transcriptional activation of about 80 genes, some of which are predicted to encode ubiquitin ligase components. One of the most highly induced IPR genes is *pals-5*, which serves as a robust readout for the IPR activation. Using *pals-5* transcriptional and translational GFP reporters, we identified the predicted bZIP transcription factor ZIP-1 as a positive regulator of the IPR in two reverse genetic screens. We found that ZIP-1::GFP is expressed in intestinal and epidermal nuclei following IPR activation. Our qRT-PCR and smFISH studies demonstrated that ZIP-1 controls *pals-5* mRNA expression early after proteasome blockade. At later time points, however, *pals-5* transcription does not require ZIP-1. Interestingly, our data suggest that the majority of translated *pals-5* mRNA belongs to the early, ZIP-1 dependent fraction. We also performed RNA-seq analysis, which revealed that ZIP-1 is required for mRNA expression of multiple IPR genes. Based on these results, we have identified three distinct types of IPR genes: 1) completely ZIP-1 dependent, 2) early ZIP-1 dependent and 3) ZIP-1 independent. Importantly, we found that ZIP-1 is required for the increased resistance to Orsay virus and microsporidia infections in *pnp-1(-)* mutants, suggesting that ZIP-1-dependent genes play an important role in innate immunity.

1183A Oxidative stress is important for triggering avoidance of pathogenic *Pseudomonas aeruginosa*

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Epithelial cells of most animals are constantly exposed to a large range of microbes; many are benign, but some have the potential to be pathogenic. Thus, epithelia must have recognition mechanisms that distinguish between commensal and pathogenic microbes. Rather than detecting the presence of bacteria, epithelial immune systems are thought to monitor for signs of damage or distress that might indicate an infection. These are known as patterns of pathogenesis, which, when detected, activate host immune defenses. We asked whether oxidative stress might serve as a pattern of pathogenesis to trigger immune defenses in *C. elegans* infected with the bacteria *Pseudomonas aeruginosa*. In this host-pathogen model, one measure of the immune response is the ability of worms to avoid pathogenic bacteria. Our preliminary data suggest that oxidative stress caused by a Ce-Duox/BLI-3- mediated Reactive Oxygen Species burst during infection with *P. aeruginosa* is necessary for normal pathogen avoidance behavior. The G-Protein Coupled Receptor *fshr-1* has also been previously shown to be necessary for pathogen avoidance; however, *fshr-1* may mediate a pathway parallel to oxidative stress. In addition, we found that mock infecting worms with oxidizing agents in the absence of infection is not sufficient to trigger avoidance behavior. Together, we suggest that oxidative stress is one of several important factors in pathogen recognition and avoidance behavior against *P. aeruginosa.*

1184B Looking for a possible treatment for type III galactosemia

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Galactose metabolism is universally conserved in all living organisms, including *Caenorhabditis elegans* and humans. Galactose is metabolized by three conserved enzymes that constitute the Leloir pathway. GALE participates in the third step of the galactose metabolism pathway, catalyzing the interconversion of UDP-galactose (UDP-gal) and UDP-glucose (UDP-glu) and in some species, including *C.elegans* and humans, the interconversion of UDP-N-acetylgalactosamine (UDP-galNAc) and UDP-N-acetylglucosamine (UDP-gluNAc). All of these UDP-sugars are required for the glycosylation of proteins and lipids. Type III galactosemia is a rare disorder caused by mutations in GALE, homologue to the *C. elegans gale-1* gen. The symptoms are due to the accumulation of intermediary galactose metabolites and the reduction of UDP-galNAc, because differently to the others, this sugar is only synthesized by GALE. Therefore, patients with type III galactosemia are recommended to follow a galactose-restrictive diet and take UDP-galNAc to avoid UDP-galNAc deficiency. This treatment is not able to improve the psychomotor retardation of these patients; it only slightly improves some of the symptoms. Our team has isolated a *Caenorhabditis elegans* mutant in the GALE homologue gene (*gale-1(pv18)*) which can be used as a model for type III galactosemia. Like humans, this mutant has an increase of UDP-gal and reduction of UDP-galNAc under regular diet. They are also sensitive to galactose rich diet and exhibit multiple developmental defects. We have found that the phenotype of the mutant and expression of *gale-1* gene can be modified by diet. We also observed that treatment with some sugars improves the phenotype of the type III galactose model. The result observed, if conserved in humans, could be of interest to improve the quality of life of patients with galactosemia type III.

1185C DBL-1/TGF-β signaling pathway regulates pathogen-specific innate immune responses in *C. elegans*

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Innate immunity in animals is orchestrated by multiple cell signaling pathways, including the TGF-β superfamily pathway. While the role of TGF-β signaling in innate immunity has been clearly identified, the requirement for this pathway in generating specific, robust responses to different bacterial challenges has not been characterized. Here, we address the role of DBL-1/TGF-β in regulating signature host defense responses to a wide range of bacteria in *C. elegans*. This work reveals a role of DBL-1 in animal survival, organismal behaviors, and molecular responses in different environments. Additionally, we identify a novel role for SMA-4/Smad that suggests both DBL-1/TGF-β-dependent and -independent functions in host avoidance responses. RNA-seq analyses and immunity reporter studies indicate DBL-1/TGF-β differentially regulates target gene expression upon exposure to different bacteria. Furthermore, the DBL-1/TGF-β pathway is itself differentially affected by the bacteria exposure. Collectively, these findings demonstrate bacteria-specific host immune responses regulated by the DBL-1/TGF-β signaling pathway.

1186A The kynurenine pathway is a major modulator of *E. faecalis* infection in *C. elegans*

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Key words

Kynurenine pathway, *E. faecalis*, gut infection, microbiota, lysosome-related organelles autofluorescence.

Abstract

The kynurenine pathway (KP), main catabolic route for the essential amino-acid tryptophan, is well-known for its immunomodulatory role in mammals. While investigating death fluorescence in *C. elegans*, anthranilic acid (AA)-loaded lysosome-related organelles (LROs) were previously found responsible for the blue auto-fluorescence seen in the worm gut (Coburn et al. PLOS Biol. 2013). Given the bacteriostatic potential of AA and other kynurenine pathway compounds, we hypothesised that LROs and the KP play a key role in *C. elegans* gut microbial control. To test this idea, we exposed *C. elegans* to a worm-pathogenic strain of *E. faecalis* (OG1RF) and observed changes in gut morphology and autofluorescence dynamics upon infection. Transcriptomics and targeted metabolomics analyses further showed that KP activity is modulated upon *E. faecalis* infection. Using a combination of KP mutants from the Nollen lab (Van Der Goot et al. PNAS 2012), we observed that inhibition of various KP enzymes differentially affect *C. elegans* resistance to *E. faecalis* infection. *E. faecalis* growth on KP mutant worm extracts confirmed that resistant mutants produce bacteriostatic compounds, which we measured by HPLC. This was verified by the delayed or reduced gut colonisation of OG1RF-GFP (gifted by D. Garsin), and the ability for some mutants to thrive on OG1RF loans. We are currently investigating a broader role for the KP in *C. elegans* gut microbiota control, notably using newly generated CeMBio fluorescent strains.

1187B Dramatic and reversible developmental slowing of *C. elegans* by a bacterial pathogen

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Caenorhabditis elegans is widely used for studying host-pathogen interactions. In addition to its easy maintenance on a wide variety of bacteria and genetic tractability, it is susceptible to important human pathogens and it has conserved innate immunity pathways. *C. elegans* is also an excellent tool to study development. While the *C. elegans* larval development is fast and robust under controlled laboratory conditions, its development is also plastic. The developmental rate and the trajectory are adjusted in response to environmental cues such as nutrients and pathogen stress. We took advantage of these qualities of *C. elegans* to explore the effects of pathogen stress on a developing host.

By screening 35 *Pseudomonas aeruginosa* strains, we found that *C. elegans* exhibit three developmental phenotypes: normal, slow and very slow. Among these strains, the *P. aeruginosa* strain CF18 (CF18) causes the most extreme developmental slowing. CF18 fed larvae are only able to reach L2 stage in three days. Yet, this developmental slowing is reversible; larvae can recover and resume its development when transferred to non-pathogenic bacteria.

We found that CF18 causes high reactive oxygen species (ROS) and mitochondrial damage in L1 larvae exposed to CF18 for just four hours. However, the protective mitochondrial unfolded protein response is not activated in CF18 fed larvae. Consistent with these observations, antioxidants or iron supplementation rescues high ROS, mitochondrial damage, and developmental rate. We further characterized the larval response to CF18 by RNA-sequencing and found that mitophagy, detoxification and immune response genes are upregulated in CF18 fed larvae.

Unexpectedly, genetic deletion of the biosynthesis of phenazines - well-known mitochondrial toxins - does not alleviate mitochondrial damage or rescue growth rate, suggesting non-phenazine bacterial effectors are responsible for the observed phenotypes. To determine the bacterial effectors causing developmental slowing, we generated a transposon insertion library in the CF18 background and screened for bacterial mutants that support normal development. We identified 42 bacterial genes, including the expected quorum sensing and two-component system genes, that allow larvae to overcome developmental slowing when inactivated. We also conducted RNA sequencing in wild-type CF18 and a CF18 *gacA* mutant to characterize toxic bacterial gene expression in CF18.

1188C Isolating a non-culturable, microbiome bacterium that adheres to the intestinal lumen of *Caenorhabditis* nematodes

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From sampling of wild nematodes, we have found that they are naturally associated with a diverse array of microbes such as bacteria, viruses, fungi, and microsporidia. In Bangalore, India, a wild *C. briggsae* strain (LUAb1) was discovered with adhering bacteria that colonized within the intestinal lumen, causing the morphology of the worm to change and potentially affect its health and growth. Phenotypically, this microbe appears to attach perpendicular to epithelial cells in the intestinal lumen, giving it a bristle-like appearance. This microbe may be pathogenic, as colonized animals appear to grow slower and have severely reduced intestinal cell sizes. Similar phenotypes were seen when LUAb1 was transferred to *C. elegans* N2 via co-culture with the wild cognate *C. briggsae* strain. Given that the NGM plates containing these nematodes are visibly contaminated, it is not possible to associate pathogenic phenotypes with LUAb1. Therefore, to determine if LUAb1 is causing pathogenic phenotypes in *C. elegans,* we aimed to eliminate any associated microbes to focus on the interactions between LUAb1 and the host.

We have created a protocol that reduces the presence of microbes that cause visible contamination on the plates of the worms and enrich for our non-culturable LUAb1. This protocol involved a series of antibiotic and SDS washes of dauer animals to preserve live bacteria in the lumen while eliminating external contamination. After this decontaminating procedure, plates containing LUAb1 colonized animals showed no visible contamination, and comparative FISH found no other bacteria in the lumen except for LUAb1. However, to identify any potential contaminating bacteria in the lumen, we will use 16S amplicon sequencing to compare LUAb1 colonized and uncolonized *C. elegans*. This procedure involves PCR amplification of bacterial variable regions of the 16S sequence using nested PCR and universal bacterial probes.

Altogether, we have discovered and identified a new species of *Enterobacteriaceae* bacteria that can bind to the apical side of intestinal epithelia cells in *C. briggsae* and *C. elegans.* Our objective is to conduct whole genome analysis to inform our attempts to grow LUAb1 in vitro and to discover host and bacterial factors necessary for bacterial binding to the intestinal cells of the lumen. In fact, an intestinal GFP *C. elegans* strain allows for indirect visualization of bacteria colonization which can be used in a forward genetic screen.

1189A Identification of genes which regulate SMN-1 levels to identify putative treatment for Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is a progressive neuromuscular disease characterized by loss of motor neurons and atrophy of skeletal muscles. SMA is caused by recessive mutation of the SMN1 gene, involved in the assembly of small nuclear ribonucleoproteins (snRNPs). A second SMN gene, SMN2, is 5 nucleotides different from SMN1, however is able to produce 10% of functional mRNA like SMN1. The promoter sequences of both genes are identical (Boda et al., 2004), so it is expected they could share the same mechanisms that regulate their expression or protein levels. Then, a therapeutic option may be to search for regulatory elements that increase SMN2 expression both transcriptionally and at the protein level. The SMN complex is highly conserved, even in invertebrate organisms (Lanfranco et al., 2017). We have generated a strain in *C. elegans* containing *smn-1* fused to mCherry and have inactivated by RNAi 10 genes which have been identified with the bioinformatic

program ASACO, as underexpressed which in turn correlates to increased SMN levels. One gene shows increased expression of smn-1::mCherry in neurons and we are testing drugs inhibiting this gene as a potential treatment for SMA.

1190B High-Throughput Drug Screen Reveals Novel Inhibitors of Microsporidia Infection in *C. elegans*

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Microsporidia are a large and diverse group of fungal-related spore-forming obligate intracellular parasites known to infect hosts in most major animal phyla. Despite the emerging threat that microsporidia have become, few reliable treatment options exist. To address this, we developed a high-throughput drug screening protocol using *C. elegans* and the microsporidia species *Nematocida parisii* in order to identify novel chemical inhibitors of microsporidia infection. Using this protocol, we screened through the Spectrum Collection of 2,560 FDA-approved compounds and natural products. We developed a semi-automated method for quantifying progeny number in liquid culture, confirming 11 candidate microsporidia inhibitors. We show that 6 of these compounds, including the known serine protease inhibitor ZPCK, are likely able to prevent microsporidia infection by inhibiting spore firing. We also show that 1 compound, Dexrazoxane, along with the known microsporidia inhibitor Fumagilin, are able to slow infection progression. Given the established role of Dexrazoxane as an iron chelator, it may act by depriving microsporidia of its essential iron resource, consequently putting the brakes on replication within the host. Together, our results demonstrate the effectiveness of *C. elegans* as a model host for drug discovery against intracellular pathogens, and provide a scalable high-throughput system for the identification and characterization of additional microsporidia inhibitors in the future.

1191C Toxicological Evaluation of AZT derivates-with organic chalcogens in C.elegans as SARs-CoV-2 therapy candidates

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The pandemic caused by the new coronavirus (SARS-CoV-2) started in 2019 in China being the cause of many deaths around the world. In this perspective the development of effective drugs capable of inhibiting the virus replication or the cytokine storm to reduce deaths caused by the coronavirus is essential. One strategy is the drug repurposing. For example, Zidovudine (AZT) has been used against the HIV-virus and has been also investigated against Sars-CoV-2. In addition, modifications in AZT structure, such as the insertion of organic chalcogens like Selenium, Tellurium or Sulfur to improve its activity are promising. That because ebselen, a selenium derivate molecule, has demonstrated the capacity to strongly inhibit the virus protein Mpro. Therefore, the insertion of chalcogens can be a great strategy. *C.elegans* is a great model to evaluate the safety of these molecules due to advantages such as: Low cost of maintenance, mutant strains, short life circle and genetic homology with mammals. Then, the objective of this work was the safety assessment of six AZT- derivative molecules containing chalcogens. N2 wildtype worms were exposed at L1 stage for 30 min to six AZT-derivative molecules in a concentration range of 1,10,50,100 and 500µM (in DMSO 2.5%). Toxicological endpoints were assessed 48h after the end of exposure. We observed that none of the molecules caused toxicity at any concentration in any toxicological parameter tested (survival rate, body length and brood size). Then with these promising results the next step of the study is to evaluate the modulation of the redox system capacity of these molecules, considering that the formation of ROS in patients with SARS-CoV-2 is dangerous and avoiding oxidative stress may be beneficial for recovering patients. Also, parallel studies with these molecules are made *in silico* to evaluate the antiviral effect of them, by the evaluation of the interaction with important proteins in the virus like Mpro.

1192A Acid sphingomyelinase mutants show increased resistance to infections with *Staphylococcus aureus* and an accumulation of electron dense multilamellar bodies

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Staphylococcus aureus exerts its pathogenicity partly by the secreted pore-forming α-toxin. The formation of pores in the plasma membrane of the host cell leads to lysosome exocytosis and thus the trafficking of the lysosomal-resident acid sphingomyelinase (ASM) to the plasma membrane. ASM catalyzes the conversion of sphingomyelin on the outer membrane layer to ceramide, which induces rearrangement of lipid microdomains and the internalization of the damaged membrane in small endocytic vesicles. Whether α-toxin or the whole pathogen is engulfed during this process can vary among the infection

models and it is not clear whether this effort to repair the membrane is contributing to the cell toxicity of *S. aureus in vivo*. *Caenorhabditis elegans* encodes three ASM: ASM-1, ASM-2 and ASM-3 that are all homologues of the human ASM. Here, we use deletion alleles of the three *asm* genes to investigate the role of sphingomyelinases and their product ceramide during *S. aureus* infection in the model host *C. elegans*.

We infected control worms as well as single or double *asm* mutants with *S. aureus* and compared their survival rate using Kaplan Maier plots. After 72 hours of feeding on *S. aureus*, the survival rate of the control strain was 0%. In contrast, survival of the *asm* mutants ranged between 40% to 60%, implying that ASM activity or their product ceramide is required for the pathogenicity. Searching for the cellular basis of the observed resistance of the *asm* mutants, we prepared non infected young adult hermaphrodites for Transmission Electron Microscopy by high pressure freezing and freeze substitution. Looking for altered cell or organelle morphology, we observed highly electron dense membranous organelles that resemble the multilamellar bodies described in various tissues in human Niemann Pick Disease, a sphingolipid-storage disease. While such organelles were rarely observed in N2 controls, various cell types in *asm* mutants contained these structures with *asm-1*/*asm*-3 double mutants showing the highest count. Based on the high electron density, we predict that this phenotype is caused by accumulation of osmophilic unsaturated lipids, including sphingomyelin, that are inefficiently metabolized.

Our findings show that the host ASM is required for full *S. aureus* virulence. Since the increase in multilamellar organelles in the *asm* mutants is also described in human Niemann Pick Disease, molecular mechanisms of ASM function may be conserved in nematodes and mammals. In the next steps we aim to reveal the mechanisms of increased survival of the *asm* mutants after *S. aureus* infection.

1193B *C. elegans* natural microbiota-mediated protection against pathogens

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It has become increasingly clear how important gut bacteria are for the protection of the host against invading pathogens. The underlying mechanisms of microbiota-mediated protection, however, are largely unknown. In recent years *C. elegans* has been established as a model system to study host-microbiota interactions and microbiota-mediated protective effects against pathogens. Our work focuses on two *Pseudomonas* natural microbiota isolates, *Pseudomonas lurida* (MYb11) and *Pseudomonas fluorescens* (MYb115), which have previously been shown to protect *C. elegans* from infections with *Bacillus thuringiensis* (MYBt18247) and *Pseudomonas aeruginosa* (PA14). We are currently characterizing the influence of these microbiota isolates on the lifespan, reproduction, and most importantly, immune defense and aim to understand on how exactly microbiota-mediated protection is induced on both the microbiota and the host side. On the microbiota side, we have identified a biosynthetic gene cluster encoding a type I polyketide synthase (PKS), the resulting natural product induces the protective effect of MYb115 when worms are infected with Bt247. On the host side, we are currently using a reporter gene approach to identify genes and defense pathways involved in the worm response to infection. We will discuss our most recent results at the conference.

1194C Sensory Neurons Regulate Innate Immune Responses in *Caenorhabditis elegans*

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Caenorhabditis elegans, a bacterivorous forager, often encounters pathogenic microbes in its habitat. Upon encountering pathogens, the nematode either avoids them to minimise chances of infection, or induces an immune response to combat infection. Despite being equipped with only an innate immune system, recent studies have shown that the worm can induce selective responses against specific pathogens. To mount such responses, the worm has to recognise and differentiate among these pathogens. We hypothesised that the recognition of pathogens, induction of an immune response and its subsequent regulation is likely performed by the sensory neurons, since they are the primary site of sensory perception in the worm. We created stable genetic neuronal ablation lines of the amphid sensory neurons and tested for their roles during infections with *Pseudomonas aeruginosa* and *Enterococcus faecalis*. 9 out of the 11 neuron pairs tested showed involvement in regulating immune responses against these pathogens. We found 6 of these pairs to either have a *P. aeruginosa* or an *E. faecalis* specific function, while the remaining 3 pairs were involved in mounting an immune response against both pathogens. Only one

of these 3 pairs showed a very strong involvement in mounting an effective immune response; we found this neuron to negatively regulate innate immunity in *C. elegans*. This neuron also suppresses immune responses against *Salmonella enterica*, *Staphylococcus aureus* and *Cryptococcus neoformans*, and might thus have a broader role in immune-homeostasis. RNASeq analysis of worms lacking this neuron showed an upregulation of several immune-related genes even at the basal level, along with a set of infection-inducible genes. In particular, we found a number of bZIP family transcription factors to be upregulated in these worms upon infection. Knockdown of three of the bZIP family transcription factors neutralised the enhanced resitance displayed by these neuron ablated worms against all tested pathogens. In conclusion, our study illustrates the roles of a chemosensory neuron that negatively regulates immune responses by downregulating bZIP family transcription factors, to maintain immune-homeostasis. With increasing evidences suggesting roles for the nervous system in immune regulation in humans and other higher vertebrates, the findings of our study could help us better understand neuroimmune regulation in higher animals.

1195A Network analysis reveal novel genes involved in the *P. aeruginosa PA14* pathogen response during *C. elegans* infection.

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The study of host–pathogen interactions (HPI) is essential to understanding bacterial infection and adaptation within the host system. Recently, network biology through applications of mathematical and graph theory methods has offered insights into complex biological systems such as HPI. It has the capability to integrate biological omics data (e.g., transcriptome, proteome, and metabolome) and biological interactome data (e.g., gene-gene relations, protein-protein interactions) and to reveal informative patterns within these systems.

In our preliminary experiments, we found that *P. aeruginosa PA14* exhibits distinctive host-genotype-specific colonization and infection trajectories. To determine what strategies *P. aeruginosa PA14* employs to infect different *C. elegans* hosts, we took a systems biology approach, which enables unbiased analysis of transcriptomic changes in pathogen during the infection. Here, we used *P. aeruginosa PA14* exome-sequencing to compare bacterial gene expression changes in three hosts of different immunocompetence (immunocompromised [*pmk-1(km25)*], immunoresistant [*hsp-12.6(gk156)*], and control [N2]) to that grown on SK growth media. Our analysis recapitulated several genes previously identified using a forward genetics screen to be differentially regulated during the infection. Additionally, we found several novel genes differentially expressed (DE) in a host-genotype dependent and independent manner.

Following, instead of focusing on investigating the role of the individual DE gene, we mapped the DE genes to a custom-built *P. aeruginosa PA14* gene network and identified 26 genetic modules of pathogen's response to the host during the infection. While 22 modules were enriched for three host-specific genotypic categories, four modules showed genotype specific enrichment. Next, we applied network principles (e.g., high centrality measures (betweenness, closeness, etc.)) to this module system to discover putative genes that are critical to pathogenesis. Subsequently, we performed host survival analysis on mutants of these genes and founds that they play distinct roles (enhanced or suppressed) during pathogenesis in different hosts. Furthermore, using dual host-specific RNA-Seq, we identified the host genetic response to mutants of these genes.

Our study provides a paradigm for a new discovery route of network biology to understand hidden functions of pathogenic genes and generate novel hypotheses, or to characterize the effects of specific perturbations across an entire genetic network. This previously unknown, emerging comprehensive map of pathogenic response and regulatory function during *P. aeruginosa PA14* infection in *C. elegans* offers a powerful resource for systems biologists studying host-pathogen interactions.

1196B Modeling Rare Genetic Diseases in *C. elegans*: Neuromuscular Junction Involvement in Multiple Mitochondrial Dysfunctions Syndrome 1

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A disease is defined as rare if there are fewer than 200,000 affected people in the United States. There are approximately 7,000 rare human diseases, the majority of which are thought to be genetic. Model organisms give hope for improved ability to diagnose, understand, and treat these diseases. The nematode *Caenorhabditis elegans* has about 20,000 protein-coding genes, an estimated 40% of which have human homologs. *C. elegans* as a research tool has many benefits, including being a complex, multicellular system and having a short lifecycle. This, along with the power of more accessible CRISPR-Cas9 editing strategies, allow researchers to make pathogenic mutation lines, as well as full length deletions of disease-causing genes, and more easily observe phenotypic outcomes.

Multiple Mitochondrial Dysfunctions Syndrome (MMDS) is a class of six autosomal recessive diseases caused by mutations in various nuclear-encoded mitochondrial genes, but with similar disease outcomes. Multiple Mitochondrial Dysfunctions Syndrome 1 (MMDS1), one of these syndromes, is caused by pathogenic mutations in NFU1. NFU1 is an iron-sulfur cluster transfer protein that primarily delivers 4Fe-4S clusters to target apoproteins. The *C. elegans nfu-1* gene produces the protein NFU-1, which is orthologous to human NFU1, so five CRISPR/Cas9 alleles were made with pathogenic mutations, as well as a full-length deletion of *nfu-1*. Presently, the phenotypes of the mutant animals are being characterized in order to better understand the function of the protein and identify the mechanisms and molecular pathways by which the protein is regulated. It was previously shown that three of the MMDS1 patient alleles made in *C. elegans* (G148R, G166C, and C168F) showed reduced thrashing when placed in liquid. This leads to the question: was the reduced thrashing caused by a problem with neurons, muscles, or both? To determine whether neuronal and/or muscle cells have impaired function, a series of drug assays were used to pinpoint neuromuscular junction issues. It is now hypothesized that mutations in the disease-causing gene of MMDS1 affect the availability and/or binding of neurotransmitters. These results create strong ties between the known genetic mutations and disease symptoms.

1197C A *C. elegans* Motor-Centric Screening Pipeline Yields Novel and Selective Nematicidal Scaffolds

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Nematode parasites of humans, livestock and crops pose a significant burden to human health and welfare. Alarmingly, our arsenal of effective nematocidal compounds is being depleted. Parasitic nematodes of animals have rapidly evolved resistance to anthelmintic drugs, and traditional nematicides used for crop protection have been restricted or banned because of poor phylum-selectivity. Here, we present our *C. elegans*-based discovery pipeline focused on lethal molecules that also induce motor defects. This pipeline yielded multiple new and selective nematicidal small molecule scaffolds (i.e., structurally-related families of molecules). We show that one of these scaffolds, tentatively called the APPs, stimulates neurotransmitter release and immobilizes larvae of multiple nematode parasites of plants and mammals *in vitro*. At similar concentrations, APP-1 does little to model flies, fish, plants or human cells. Forward genetic screens of 100,000s of mutant genomes failed to yield resistant animals that resist the lethal effects of APP-1, suggesting that resistance to the APPs in the field may not be easily generated. Hence, the APPs represent a novel, selective and potentially useful addition to our nematocidal armament.

1198A Disruption of mitochondrial calcium homeostasis by loss of presenilin promotes mTORC1 signaling to drive neurodegeneration

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Metabolic dysfunction and protein aggregation are associated with neurodegenerative disorders such as Alzheimer's disease (AD). However, the mechanisms underlying these abnormalities remain poorly understood. Mutations in the presenilin encoding genes are the primary cause of early onset familial AD, but their role in the disease is unclear. The presenilins are primarily known to function as the catalytic component of the γ-secretase complex, which is involved in the cleavage of the amyloid precursor protein (APP) to produce amyloid beta (Aβ) peptides, whose aggregation into Aβ plaques is considered the hallmark of AD. However, many studies have demonstrated a γ-secretase independent role for presenilins in calcium homeostasis that is critical for neuronal health. Previously, we showed that loss of the *Caenorhabditis elegans* presenilin ortholog SEL-12 elevates mitochondrial calcium signaling, which increases mitochondrial metabolism to drive

neurodegeneration and loss of protein homeostasis. Here, we demonstrate that this elevated mitochondrial calcium and concomitant mitochondrial hyperactivity promotes activation of the mechanistic Target of Rapamycin Complex 1 (mTORC1) pathway. We utilize several models of protein homeostasis to show mTORC1 hyperactivity contributes to protein homeostasis defects. Reducing mTORC1 activity improves neurodegenerative phenotypes associated with loss of SEL-12/presenilin function. We also show mTORC1 plays a cell-autonomous role in neurodegeneration and that rescue of neuronal mTORC1 signaling is sufficient to abrogate improvements to neuronal function caused by global mTORC1 inhibition. Consistent with high mTORC1 activity, we find that SEL-12/presenilin loss reduces autophagy, and this reduction is prevented by limiting mitochondrial calcium uptake. Furthermore, the improvements to protein homeostasis and neuronal function in *sel-12* mutants due to mTORC1 inhibition require the induction of autophagy. Collectively, these results indicate that mTORC1 hyperactivation exacerbates the defects in protein homeostasis, autophagy, and neuronal function in *sel-12* mutants and suggest a potential therapeutic target for treating AD.

1199B Innate immune responses of *Caenorhabditis elegans* to the emerging pathogen *Elizabethkingia anophelis*

Emilio Ramirez-Fajardo[1], Wesam Albayati[1], Sarah Grossen[1], Skylar List[1], Matthew Siderhurst[1], *Kristopher Schmidt*[1] 1) Eastern Mennonite University.

Elizabethkingia anophelis (Ea) is an emerging pathogen that causes sepsis, meningitis, and death in people with comorbidities. While usually innocuous, a recent community outbreak resulted in at least 20 deaths and 66 laboratory confirmed cases. The outbreak stemmed from the rapid formation of a novel and more virulent Ea sublineage created by a conjugative element that disrupted the *muty* DNA repair gene. The virulence associated factors, pathogenic mechanisms, and ecology of Ea are not known. *C. elegans* is a well-established model for the characterization of pathogen-host interactions. When we exposed mixed stage wild-type *C. elegans* to Ea (Ag1 strain), the animals exhibited decreased movement, slowed development, early larval death, and an overall decreased fecundity compared to animals exposed to *E. coli* OP50 (Ec). We also made the striking observation that worms exposed to Ea actively avoided Ea lawns. To understand the aversive response, we conducted two-choice assays between Ea and Ec in experiments using L1 and L4 animals. L1s not previously exposed to a food source initially chose Ea over Ec (-0.75 CI, Choice Index [CI] of -1.0 represents Ea selection) but actively selected Ec over time (+0.41 CI at 96hrs). L4 animals first reared on Ec chose Ec over Ea in two-choice assays (+0.49 CI). Importantly, we found that staged adults placed on Ea bacteria without another bacterial food source adopted a lawn avoidance phenotype, suggesting that they are repulsed from Ea even in the absence of Ec. These avoidance responses depend on an intact odorant detection system because *lim-4(ky403)* and *tax-2(p671); tax-4(p678)* mutant animals failed to avoid Ea lawns. We used solid-phase microextraction (SPME) gas chromatography mass spectrometry (GC-MS) to identify volatile odorant differences between Ea and Ec and observed a marked increase in the amount of indole in Ec compared to Ea. Indole is an attractive compound recently shown to modulate predator-prey interactions between *C. elegans* and bacteria. To examine the pathogenic mechanisms of Ea in *C. elegans*, we exposed N2, *sek-1 (km4)*, *tol-1(nr2033)*, *dbl-1(nk3)* and *daf-2(e1370)* animals to Ea and Ec. Wild-type animals displayed several pathological changes when grown on Ea and died faster than their Ec exposed counterparts. Decreased lifespan and increased pathologies were found in *sek-1* while *daf-2* animals appeared immune to Ea. The *tol-1* and *dbl-1* alleles did not influence Ea pathogenicity. To complement these immunity assays we also examined the antimicrobial activity of *dod-24*, *irg-1* and T24B8.5 in *C. elegans* exposed to Ea using transcriptional reporter GFP strains. We observed decreases in the expression of T24B8.5 and increased but more variable changes in *irg-1*. Taken together these experiments suggest that Ea is pathogenic to *C. elegans*, opening new opportunities to investigate pathogen and host factors influencing Ea related disease.

1200C Investigation of the relationship between manganese exposure and the development of Huntington's like-disease in *Caenorhabditis elegans*

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Metals may influence the development of neurogenerative diseases. Particularly, Manganese (Mn) increases the risk of developing Parkinson's Disease after occupational exposure. However, the role of this metal in other neurodegenerative diseases such as Huntington's disease (HD) is still unclear. HD is characterized by the impairment of the cognitive functions due to the accumulation of polyglutamine (PoliQ). Several changes in response to metal exposure have been reported using the *Caenorhabditis elegans* nematode, an experimental model of simple maintenance, easy genetic manipulation and feasible for *in vivo* toxicity analysis. Remarkably, there are many transgenic strains available that express the human poliQ in different tissues, allowing the study of factors that exert influence in this disease. Therefore, the aim of this study was to evaluate the effects of acute Mn exposure on HD development through the *C. elegans* model. Strains N2 (wild type), AM101 [F25B3.3p ::

Q40 :: YFP] and AM141 rmIs133 [unc-54p :: Q40 :: YFP] were submitted to acute treatment (30 minutes) with $MnCl_2$ (2.5mM-100mM). 48 hours after the end of the treatment, survival rate and polyQ aggregates in the muscles and neurons of the worms were analyzed. ferroportin (*fpt*) expression following a sub-lethal concentration exposure (25mM) was assessed by polymerase chain reaction-PCR. Mn (25mM) was able to reduce the longevity of muscle polyQ expressing animals (AM141) and curiously reduced the formation of protein aggregates. The formation of polyQ aggregates in neurons (AM101) was not significantly altered with the treatment, neither their longevity. In these mutants, there was an increase of *fpt* expression after Mn exposure, indicating an increase in divalent metal export from the cells. In conclusion, animals expressing polyQ unexpectedly showed higher resistance to Mn. In addition, Mn did not increase polyQ aggregation in both strains, demonstrating low metal toxicity in this model. This effect may have been caused by increased cell expression of ferroportin, a Mn exporter, in these HD strains but not in wildtype, blocking Mn accumulation in cells and preventing Mn toxic effect in polyQ aggregation.

1201A T14E8.4 limits bacterial colonization but assists microsporidia invasion in *C. elegans*

Hala Tamim El Jarkass[1], Aaron Reinke[1] 1) University of Toronto.

Animals have evolved extensive immune pathways to combat the myriad of pathogenic microbes they encounter. Conversely, pathogens have evolved many mechanisms to exploit their hosts. To understand how *Nematocida parisii*, a natural microsporidian pathogen of *C. elegans*, infects its host, we performed a forward genetic screen to identify mutant animals that had a Fitness Advantage with *Nematocida* (fawn). All three fawn isolates produce progeny at high levels, are less infected than wild-type animals, and contain mutations in *T14E8.4*. This signal peptide containing gene is expressed in the pharynx and intestine. Expression of T14E8.4 in the intestine of *T14E8.4* animals restores *N. parisii* infectivity, which is dependant upon secretion. Resistance to *N. parisii* infection in *T14E8.4* mutants is developmentally restricted to the L1 stage and results in decreased parasite invasion. *N. parisii* spores in *T14E8.4* animals display improper orientation in the intestinal lumen, indicating spores are firing incorrectly. Interestingly, *T14E8.4* expression is upregulated by both *N. parisii* and *Pseudomonas aeruginosa* infection. *T14E8.4* mutants display both increased susceptibility and colonization from *P. aeruginosa* and over expression of *T14E8.4* reduces *P. aeruginosa* colonization. Competitive fitness assays show that *T14E8.4* mutants are favoured in the presence of *N. parisii* but disadvantaged on *P. aeruginosa.* Furthermore, *C. elegans* wild isolates don't possess predicted loss of function mutations in *T14E8.4*. Together, this work demonstrates how microsporidia exploits an antibacterial immune protein to facilitate host invasion. The opposing fates of *T14E8.4* mutants on different pathogens highlights a central role in infection and immunity.

1202B An intracellular bacterial pathogen of *Oscheius tipulae* uses filamentation as a novel mechanism for cell-to-cell spreading

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Intracellular bacteria are highly adapted to use resources inside host cells for their growth and replication. To maximize this utilization, some bacteria have evolved specialized mechanisms to spread from the initial infected cells to uninfected neighbors. To date, the known mechanisms of cell-to-cell spreading fall within one of the three main paradigms: exit-reentry, hijacking of host actin for protrusion to adjacent cells, and cell fusion. We have discovered a new Bordetella bacterial species infecting intestinal cells of an *Oscheius tipulae* isolate that appears to use a unique mechanism for cell-to-cell spreading. Upon invasion, the bacteria convert from a coccobacillus to a filamentous morphology that appear to invade multiple neighboring cells, a phenotype that prompted us to name it *Bordetella atroposiae* after the Greek Fate Atropos who cuts the thread of life.

To elucidate the role of filamentation during infection, we isolated a mutant bacterial isolate deficient in filamentation. This mutant showed a similar growth rate compared to wild type in vitro, but significant reduction in anterior-posterior spreading in vivo. Strikingly, infection by the mutant was restricted to an average of 1-2 intestinal cells (measured by infection to an average of 2 consecutive DAPI stained intestinal nuclei), while the wild-type bacterial filaments spread to 4-6 host cells (an average of 6 DAPI stained nuclei). This data suggests that filamentation is required for cell-to-cell spreading. Sequencing of the mutant revealed a missense mutation in a member of a putative glucose-sensing pathway which inhibits bacterial cytokinesis under rich conditions to increase cell size. Complementation restores in vivo filamentation and spreading capacity of the mutant at a level comparable to that of the wild type. Additionally, knockout of another downstream member in the same pathway phenocopied the mutated gene, suggesting the involvement of this glucose-sensing pathway in *B. atroposiae* filamentation. Altogether, we have discovered the first intracellular bacterial pathogen of the nematodes *O. tipulae* that appears to employ filamentation as a spreading mechanism. We propose that *B. atroposiae* evolved a novel mechanism for cell-to-cell spreading

by coopting a highly conserved glucose-sensing pathway that regulates bacterial cell size in order to trigger filamentation inside host cells.

1203C Toxicological evaluation of curcumin nanocapsules in *Caenorhabditis elegans*

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Curcumin is the main bioactive polyphenolic compound present in the turmeric rhizomes and has become the target of studies in several areas of knowledge due to the variety of its biological properties that it presents, as an antioxidant, anti-inflammatory and neuroprotective activity. Its low bioavailability, insolubility in water and its instability limit its application, hindering its action and absorption. Various techniques can be used to improve the dissolution and bioavailability of bioactives. Curcumin nanoencapsulation has been used to increase its bioavailability, being an excellent tool for controlled release and vectoring for tissues of interest. However, little is known about its safety. Recently, there is an increasing number of studies on nanotoxicology in Caenorhabditis elegans. C. elegans is an animal model with potential advantages for studies of nutraceutical activity such as relatively short life, easy maintenance, low cost, litter size and several mutant strains. In this context, our objective was to evaluate the interaction effects of curcumin nanoparticles encapsulated by the poly capr - caprolactone polymer (PCL) in C. elegans in order to obtain information about the potential for toxicity. The worms in the first larval stage (L1), were obtained by a process of synchronization. After 14 hours of the synchronization process, the newly hatched larvae were treated with different concentrations (10 µM, 30 µM and 100 µM) of nanoparticles containing curcumin, empty nanoparticles and free curcumin, for 30 minutes, in liquid medium. Soon after, all this medium containing the treatment was placed in Petri dishes containing NGM and E. coli for 48 h. After 48 h (chronic exposure), the worms were analyzed. For survival tests, they were counted and compared with the control group in order to draw a survival curve. Reproduction was assessed by the size of the litter. To determine the size of the animals, photos were taken with the aid of a microscope with a camera attached. All experiments were done in duplicate and repeated at least 3 times. Data were expressed as mean ± standard error, and were statistically analyzed using one-way ANOVA and Tukey's post-hoc. It can be seen that both nanoparticles with curcumin, white nanoparticles and free curcumin did not cause a significant difference in the survival rate of the worms compared to the control. In determining the size of the litter and the size of the worms, it was observed that only the free curcumin significantly decreased these parameters in the concentration of 30 µM in relation to the control. The formulation of PCL nanoparticles carrying curcumin can be considered safe, and these results indicate that C. elegans can be a good alternative in vivo for nanotoxicology studies providing accurate and fast results.

1204A Impairment of *C. elegans* ribosome integrity by *Pseudomonas aeruginosa*

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Translation is an essential cellular process that is commonly targeted for inhibition in antagonistic organismal interactions. Pathogenic bacteria hamper translation in their eukaryotic hosts to fend off the host immune response and promote host damage. To dysregulate translation, bacteria produce virulence factors that elicit deleterious modifications to the host ribosome or translation factors.

Pseudomonas aeruginosa inhibits translation in many hosts. For insects and mammalian hosts, a main virulence effector is the exotoxin A protein, which ADP-rybosilates the translation elongation factor 2. However, for the infection of *Caenorhabditis elegans* by *P. aeruginosa*, the precise pathways and mechanism(s) of translational inhibition are not well understood. We found that upon exposure to *P. aeruginosa* PA14, *C. elegans* undergoes a rapid loss of intact ribosomes accompanied by the accumulation of ribosomes cleaved at helix 69 (H69) of the 26S ribosomal RNA (rRNA), a key part of ribosome decoding center. H69 cleavage is elicited by certain virulent *P. aeruginosa* isolates in a quorum sensing (QS)-dependent manner and independently of exotoxin A-mediated translational repression. Among *P. aeruginosa* strains, the bacteria's capacity to induce H69 cleavage strongly correlates with the presence of R-bodies, a multi-protein bacterial virulence effector that promotes H69 cleavage. Consistent with H69 cleavage resulting from an extracellular bacterial virulence factor that is transferred to the worm's tissues, the H69 cleavage is predominantly localized in the worm›s intestinal cells, increases with time of exposure of live bacteria to the intestinal lumen, and requires the activity of the worm's endocytic uptake machinery.

Genetic and genomic analysis suggests that H69 cleavage leads to the activation of the worm's *zip-2*-mediated defense response pathway, consistent with translational inhibition. Indeed, H69 cleavage is antagonized by the *zip-2* pathway, as well as by the worm's two other major host defense pathways defined by *pmk-1* and *fshr-1*. Taken together, our observations suggest

that *P. aeruginosa* deploys a novel virulence mechanism to cleave the host's large ribosomal subunit RNA at H69, and induce ribosome degradation, thereby impairing host translation and hence blocking antibacterial responses.

1205B *C. elegans* offers a unique window into the early pathophysiology of Duchenne muscular dystrophy

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Duchenne muscular dystrophy is an x-linked disorder that claims the lives of one in every 3,500 males. The disease is caused by mutations that result in the absence of the dystrophin protein from the membrane of muscle cells where it plays important structural and signaling roles. Without dystrophin, muscle-generated forces are unable to safely exit the cell. This results in cellular injuries, most consequentially to the plasma membrane. Individuals become wheelchair bound between seven to twelve years of age, and typically die in their late teens to twenties. While there is some variability in the rate and the severity of the degeneration, patients suffer a plethora of debilitating symptoms associated with progressive muscle degeneration and loss.

There is no cure for Duchenne muscular dystrophy. Lack of progress and understanding of this disorder is largely the result of animal models failing to recapitulate both the genetic and phenotypic severity observed in humans. However, understanding the molecular events that link loss of dystrophin to muscle death remains of paramount importance. This knowledge will help us identify targets amenable to intervention and offer hope to more than one million individuals suffering this disease across the world.

Our lab uses dystrophic nematodes to generate insights into the pathophysiology of Duchenne muscular dystrophy, and to identify novel therapeutic avenues. By harnessing the natural burrowing behavior of *C. elegans* we demonstrated that dystrophic worms recapitulate key aspects of the disease to a greater extent than those observed in most research models currently used. We report a study of the embryonic development of dystrophic animals. Here we show that sarcoplasmic calcium dysregulation associated with dystrophic muscles starts early during development. Furthermore, a suppressor screen, and a comparison of dystrophic strains displaying different severity, both point to muscle calmodulin as a potential therapeutic target. Dystrophic worms provide a unique opportunity for studying the mechanism by which dystrophic cells degenerate, and to identify intervention avenues. To validate findings obtained using our dystrophic animals we are presently developing a human muscle culture system.

1206C The neuropeptide receptor NMUR-1 regulates the specificity of *C. elegans* innate immunity against pathogen infection

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Increasing evidence indicates that the innate immune system can generate high levels of specificity. However, the underlying molecular basis for such specificity is not well understood. Like other invertebrates, *Caenorhabditis elegans* does not have the adaptive immune system and only has innate immunity, and yet it can differentiate different pathogen attacks and launch proportionate innate immune responses. We found that functional loss of NMUR-1, a neuronal GPCR homologous to the mammalian receptors for the neuropeptide neuromedin U, had diverse effects on *C. elegans* survival against various bacterial pathogens. Further investigation revealed that NMUR-1 modulates *C. elegans* transcription activity by regulating the expression of transcription factors, which, in turn, controls the expression of distinct immune genes in response to different pathogens. Our study uncovered a molecular basis for the specificity of *C. elegans* innate immunity and could provide mechanistic insights into understanding the specificity of vertebrate innate immunity.

1207A Multi-species nematode screening uncovers a new broad-spectrum class of anthelmintic compounds targeting mitochondrial lipid metabolism

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Parasitic worms infect more than 1.5 billion people and cause significant losses in livestock and crops. New anthelmintic drugs are urgently needed, as resistance to the existing drugs is emerging. We built a high-throughput (HTP) 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 platform is capable of screening compound collections and genetic libraries in model organisms and mammalian cells in a fully integrated manner. To find novel anthelmintics we screened a ~2300 compound library containing most of the FDA-approved approved drugs, plus natural products and bioactive compounds for broad spectrum toxic effects on two distantly related free living nematodes *C. elegans* and *P. pacificus* as model animals while having low toxicity on human cell lines. The screen confirmed the effects of most known anthelmintics on these nematode models. Additionally, among a set of new compounds, we found six natural lipid molecules that exhibit broad-spectrum anthelmintic activity. These novel compounds kill *C. elegans* and *P. pacificus* when applied at various developmental stages, including the dauer and embryonic stages, in a dose-dependent manner. Importantly, these compounds cause mortality in all three veterinary parasite species we tested: the hookworm *Haemonchus contortus* (ruminants), *Teladorsagia circumcincta* (sheep and goat) and *Heligmosomoides polygyrus* (rodents). Further characterization of their mode of action using *C. elegans* revealed defects associated with mitochondrial function and lipid metabolism. The lethality phenotype in *C. elegans* is partially rescued by knocking down the enzymes *acs-2*, *cpt-4*, *dif-1* and *cpt-2*, which facilitates mitochondrial lipid transfer, while drug inhibitors of CPT1 and CPT2 enhance the phenotype, suggesting that these compounds perturb specifically fatty acid oxidation in nematodes. These compounds constitute a new class of broad-spectrum anthelmintics targeting lipid metabolism.

1208B Solving host-microbe interactions and gut dysbiosis

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Gut host-microbe interactions critically impact *C. elegans* health and ageing. Multiple pathways and interactions have already been characterised in probiotic and dysbiotic contexts. However, we are still to unravel the complex host-microbe core or pathogen-specific genetic networks that orchestrate these interactions. We recently observed that the kynurenine pathway, main catabolic route for tryptophan, is majorly involved in regulating host-microbe interactions in worms, while insulin/IGF1 signalling (IIS) is a major upstream determinant of *C. elegans* gut biology and reproduction, immunity and longevity trade-offs. To solve part of this picture, we are now performing microbial and host transcriptomics, proteomics, and Tryptophan metabolite measurements in *daf-2(e1370)* and wild-type worms exposed to *E. faecalis* or *P. aeruginosa* for up to 12h, combined with host genome-wide RNAi infection screens relying on Label-Free Survival Assays (Benedetto et al. Aging 2019). In this poster we present the result of our bioinformatic investigations, main experimental pipelines and project strategy.

1209C Safaty evaluation of nanoparticles prepared with differents polymers in *Caenorhabditis elegans*

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Nano-sized drug delivery systems have been the subject of intense research in recent years because polymeric materials allow the absorption and release of active substances in a controlled manner. The use of polymeric nanoparticles as a drug carrier has emerged as an alternative. Despite the benefits, the safety of nanoparticulate systems is an aspect to be understood, particularly in *in vivo* systems. *Caenorhabditis elegans* is a very useful alternative model for nanotoxicology and has been recently applied in this field. The aim of this study was to evaluate toxicological endpoints in worms exposed to nanomaterials prepared with different polymers: polyethylene glycol (PEG), chitosan (CH), eudragit (EU) and polysorbate 80 (P80) in *C. elegans*. First larval staged worms were obtained by a synchronization process and treated with nanomaterials at concentrations of 0.015, 0.225 and 0.45 mg/mL for 30 minutes in liquid medium (acute exposure). Soon after, they were washed to remove the treatments and transferred to Petri dishes containing NGM and *E. coli* OP50 for 48 h. Survival rate, brood size and worms length were determined. Data were expressed as mean ± standard error, and statistical analysis were done by one-way ANOVA followed by Tukey post-hoc test. We observed that the EU nanoparticles did not cause any significant change in toxicological endpoints when compared to control. On the other hand, exposure to CH, PEG and P80 nanoparticles decreased worms survival, reduced their progeny and significantly altered worms size. This work demonstrates the toxicological differences between polymers and a potential for EU to be used in new formulations for future drug vectoring and targeting systems.

1210A Using genetic code expansion to develop a photo-activatable FLP recombinase.

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Sequence-specific DNA recombinases such as FLP and Cre are powerful and widely used tools for controlling gene expression. However, their application is currently limited by the availability of specific promoters to target their expression to cells of interest. Our lab has recently developed a photo-activatable version of Cre which allows for spatiotemporal control of gene expression with single-cell precision.

Here we present a method for generating a photo-activatable version of FLP recombinase using genetic code expansion. The photo-activatable FLP can be used as an alternative to, or in combination with, the previously developed photo-activatable Cre, further expanding the set of tools for controlling gene expression in *C. elegans*.

Genetic code expansion refers to a method of incorporating non-canonical amino acids into proteins *in vivo* in a site-specific manner. We use photocaged amino acids, which contain a 'caging' group attached to the side-chain of an otherwise canonical amino acid. This caging group can render the protein containing the photocaged amino acid inactive by blocking its active site. The caging group can be rapidly removed by illumination with 365 nm light, which restores the canonical amino acid and allows for the photo-activation of the protein.

We have generated photo-caged variants of FLP by replacing either a catalytic lysine or a catalytic tyrosine in the active site with their photo-caged counterparts. We demonstrate that we can use the photo-caged FLP in several tissues, including neurons and body wall muscle cells. We can uncage and activate the FLP recombinase to drive gene expression either globally, by illuminating the entire animal, or in individual cells by using a 365 nm laser. Our system thus provides a valuable tool for the spatiotemporal control of gene expression in *C. elegans*, and can be used to study functions of cells, or combinations of cells, which otherwise could not be genetically targeted by other methods.

1211B Thimerosal toxicity in the reproductive system in *C. elegans*

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Mercury (Hg) is a heavy metal with no biological function, it can be found worldwide, and is presented in organic and inorganic forms. Among the Hg organic compounds is Thimerosal (THIM), which is a vaccine preservative that is used in Brazil. One of the targets of this organomercurial is the dopaminergic system, however it is necessary to know which other systems could be affected by this compound. The objective of the study was to evaluate the toxicity of THIM on the reproductive system of *Caenorhabditis elegans.* For this study we have used MD 701 [lim-7p::ced-1::GFP + lin-15(+)] transgenic, which were exposed to THIM from the L4 stage for 24H (vehicle, 10, 25, 50 and 75µM). The worms were maintained in NGM (Nematode Growth Medium) plates with UV-inactivated *Escherichia coli* OP50. After exposure the survival, brood size, egg laying – levamisole induced, egg production and number of germline apoptotic cells were assessed. Our results demonstrate that THIM does not increase mortality rate in *C. elegans*, at the concentrations tested. However, THIM exposure caused a decrease in brood size and egg laying at all concentrations. We have found an increase in the number of apoptotic cells and in the egg production at the highest concentrations. Therefore, our results suggest that THIM exposure causes reprotoxicity, since there was an impairment on brood size and increase of germline cells apoptosis. In addition, the reduction in egg laying induced by levamisole and the increase in the number of eggs inside the worms indicate a neuromuscular damage. Resulted increased of eggs inside of worms. However, further studies are needed to understand the mechanism by which THIM exerts its toxicity on *C. elegans* reproductive system.

1212C Developing *Steinernema hermaphroditum* as a model system to study symbiosis

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Entomopathogenic nematodes (EPNs, which include *Steinernema* and *Heterorhabditis*) seek out insect hosts that they infect and rapidly kill with the assistance of their mutualistic symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*), with which they exist in species-specific partnerships. The development of genetic tools in *Xenorhabdus* and *Photorhabdus* bacteria have proved these symbiotic systems to be highly valuable for the study of both mutualism and parasitism. However, hopes that EPNs could be genetic models have been frustrated for decades by inconsistent *in vitro* growths, variable success in cryopreservation, and low mating efficiency in the laboratory. We obtained a recently described Indian strain of *Steinernema hermaphroditum* (Bhat *et al.,* 2019) and optimized its *in vitro* growth with a generation time of three days on a thin layer of its native symbiotic bacteria *Xenorhabdus griffiniae* on transparent growth media (NGM). We developed a simple and efficient

cryopreservation method by freezing mixed juvenile stages of nematodes. Previously, *S. hermaphroditum* was described as first generation hermaphroditic and second generation gonochoristic (male-female) when isolated from its insect hosts. We discovered that at least when growing *in vitro,* this species produced exclusively self-reproducing hermaphrodites for consecutive generations, except for rare spontaneous males. We performed EMS mutagenesis screens in *S. hermaphroditum* which generated multiple mutant lines with visible phenotypes. Mating of recessive mutant hermaphrodites with wild-type males produced wild-type hermaphrodite F1 cross-progeny that produced mutant and wild-type F2 self-progeny with Mendelian ratios, consistent with self-fertilization. We also acquired X-linked recessive mutants that showed sex determination is chromosomal. We report *S. hermaphroditum*-India as the first case of a true consistently hermaphroditic species of EPN known to date, and find it to be highly tractable in the laboratory. We will present our ongoing progress in developing this species into a genetic model for symbiosis research.

1213A Local compression mechanosensing by DVA proprioceptors curbs body bends during locomotion

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Coordinated cell shape changes is the basis for a myriad of dynamic processes in animals, from limb movement to locomotion, but also for visceral morphodynamics such as lung expansion. These processes are supervised by specialized, proprioceptive mechanosensors, which are capable to accurately transduce mechanical information from organ deformation into biochemical signals. However, we still have little knowledge about the physiologically relevant mechanical stresses and deformations that lead to the activation of mechanosensitive neurons during proprioception or visceral mechanosensation.

The stereotypical locomotion phenotype of *C elegans* can help us elucidate several mechanotransduction pathways coming into play for proprioception. We used a conditional knock-out strategy and found that *unc-70 β-spectrin* has cell-specific roles in the DVA neuron in limiting body bending amplitudes during locomotion. By means of calcium imaging, microfluidic manipulation and genetically encoded tension sensors we found that the spectrin cytoskeleton is able to bear compressive stresses that are transduced by the *trp-4* ion channel of the TRPN/NOMPC family. In conjunction with *optical tweezer* based force spectroscopy assays of cultured DVA neurons, we found that the potassium leak channel *twk-16* is responsible for inhibiting depolarization during axonal stretch, which adjusts the proprioceptive mechanism of DVA within compression. Finally, we formulate our observations within a neuromechanical framework that shows that compartmentalized, compressive mechanosensitivity triggers local muscle contraction that is critical for animal locomotion.

1214B Identification of Novel Uric Acid Gluconucleosides in *C. elegans* regulated by insulin signaling

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Few nucleoside-derived natural products have been identified from animals, despite the ubiquity of nucleosides in living organisms. Our recent metabolomic analyses of *C. elegans* revealed a variety of novel nucleoside derivatives, some of which incorporate additional moieties, for example ascarosides. Using a combination of chemical synthesis and the emerging electron microscopy technique microcrystal electron diffraction (MicroED) we elucidated the structures of two unusual uric acid nucleosides named uglas#1 and uglas#11, which are based on attachment of the purine base to glucose instead of ribose as in canonical nucleosides. These noncanonical gluconucleosides integrate the ascaroside pheromone ascr#1 as a building block.

Via comparative metabolomics we showed that an enzyme from the carboxylesterase (CEST) family, CEST-1.1, is specifically required for the production of uglas#1 and its phosphorylated derivative, uglas#11, but not for the biosynthesis of structural isomers or other related gluconucleosides. Biosynthesis of the phosphorylated uglas#11 is starkly upregulated in long-lived *daf-2 (e1368)* mutants, suggesting a possible intersection of nucleoside metabolism and insulin signaling. The highly specific biosynthesis of uglas#1 and uglas#11 and their regulation by insulin signaling suggests that gluconucleosides may represent a novel class of signaling molecules.

1215C Fe_3O_4@Ag nanoparticles synthesized by biogenic route cause reprotoxicity *in Caenorhabditis elegans*

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Metal nanoparticles (NPs) are the most studied nanomaterials today. From a biomedical point of view, hybrid metallic NPs are very promising when compared to monofunctional NPs, as they combine characteristics and functions that alone cannot be achieved, such as Fe_3O_4@Ag-NPs (magnetite NPs). In addition, green synthesis has been pursued in order to reduce environmental impacts due to solvents used in the reactions. However, little is known about Fe_3O_4@Ag synthesized in a biogenic manner using the extract of *Camelia sinensis* (green tea), requiring reliable and reproducible studies that certify the biosafety of these nanomaterials and guarantee the safety for users and for the environment. A promising model for nanotoxicological analysis is *Caenorhabditis elegans*, as it is invertebrate, transparent, has a short life cycle and is easy to manipulate and genetically manipulate. In this sense, the present work aimed to evaluate the toxicity of Fe_3O_4@Ag-NPs synthesized in a biogenic way in *C. elegans.* N2 (wild type), CL2166 [(pAF15*) gst-4*p :: GFP :: NLS] and TJ356 [daf-16p :: *daf-16*a / b :: GFP + rol-6 (su1006)] were used. The animals were submitted to treatment at the first larval stage (L1) in an acute manner (30 minutes) at concentrations of 1, 5, 10, 50 and 100 µg / mL. The survival rate, progeny size, egg laying, number of eggs inside the worm, translocation of the DAF-16 transcription factor and glutathione-S-transferase (GST-4) expression were analyzed. We observed that the acute exposure to Fe_3O_4@Ag-NPs decreased the survival rate of *C. elegans*, in addition to decreasing reproductive parameters such as progeny size and egg laying. In addition, the treated worms showed greater translocation of DAF-16 from the cytoplasm to the cell nucleus, indicating possible activation of antioxidant enzymes in response to damage. An increase in GST-4 expression was also observed, which suggests a detoxification mechanism against xenobiotics. This study indicates that Fe_3O_4@Ag-NPs induces reprotoxicity in *C. elegans* and that there is a modulation of the transcription factor DAF-16 with its translocation into the cell nucleus and the consequent activation of detoxification processes, as observed in this study, with increased expression of the target enzyme GST-4.

1216A Uncovering microbiome-mitochondrion interactions in the worm-bug model

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Microbiomes can have a big impact on the general health and wellbeing of their hosts. Both metagenomic-sequencing and experimental studies have shown that the microbiome can alter host immune function, cognitive function, and the pathogenesis of neurodegenerative diseases.

The mitochondria, cousins of the free-living bacteria which populate the microbiome, may be crucial for mediating these effects by interacting with bacterial products. Investigating such microbe-mitochondria interactions is a relatively new area of study, but already researchers have shown that mitochondrial components are capable of binding bacterial siderophores to facilitate iron uptake, perceiving bacterial quorum-sensing signals, and interacting with many other bacterial products. Furthermore, *C. elegans* electron transport chain mutants display constitutive activation of p38-mediated innate immunity, highlighting the role of mitochondrial dynamics in immune signalling.

Our lab has established a model system to study host-microbe interactions using *C. elegans* in conjunction with an experimental microbiome consisting of 11 bacterial strains derived from the microbiota of wild *C. elegans* isolates. Members of the microbiome differ in their propensity to colonise the *C. elegans* gut, resulting in the formation of a gut microbiome which diverges in composition from that of the bacterial lawn. Using the open-source program GapSeq, we have generated genome-scale metabolic models using genomic assemblies of all our bacterial strains. By examining these models and subsequently performing flux-balance analysis, we have demonstrated that our experimental microbiome is metabolically diverse and potentially able to provide its host with many beneficial compounds, including thiamine, butyrate, nicotinamide, and GABA. We have also demonstrated that worms grown on our experimental microbiome display a disordered, globular mitochondrial network. Moreover, worms grown on our experimental microbiome show reductions in mtDNA copy number as early as L3-L4, while whole-body ATP levels are elevated in early adulthood. Together, these results illustrate the metabolic capacity of our experimental microbiome, and demonstrate that it can induce significant changes in host mitochondrial dynamics.

1217B Insecticide resistance and toxicity mechanisms in malaria vectors and *C. elegans*

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Malaria continues to claim more than 400.000 lives every year, and represents one of the biggest health issues for humanity worldwide. Deltamethrin (DM) is one of the most potent insecticides used to eradicate malaria and belongs to type II pyrethroids. DM targets voltage-gated sodium channels (VGSCs) expressed in neuronal cells of target species. However, the off-target effects of chronic exposure to DM are poorly characterized. We use *C. elegans* as a non-target species, and the malaria vector *Anopheles gambie*, to identify molecular pathways that mediate insecticide toxicity and/or resistance to DM. Proteomic analysis of resistant mosquitoes compared to susceptible counterparts shows increased levels of OXPHOS and proteasomal proteins, and decreased levels of aminoacyl-tRNA biosynthesis and ribosomal proteins, suggesting reduction in protein translation. The proteomic profile of DM resistant mosquitoes resembles the profile of low insulin/IGF1 signaling (IIS) *C. elegans* mutants and indicates that resistant mosquitoes could experience low IIS. We tested the effects of DM on nematodes treated throughout their post-embryonic development. DM treated worms can develop to adulthood and produce viable progeny. We found that DM does not induce *sod-3* expression in wt animals, rather it attenuates *sod-3* induction in *daf-2* mutants and starved wt animals. In addition, DM significantly induces ER stress and antioxidant response in a dose dependent manner, while it alters the exploratory behavior of wild type worms in the presence of food. Our findings implicate IIS in deltamethrin-induced toxicity, in non-target species and in the development of mosquito resistance mechanisms. We are currently investigating whether modulation of IIS could serve as a putative toxicity and resistance management strategy.

1218C *C. elegans* as a model for studying *Cannabis sativa* extracts

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The *Cannabis* genus includes a variety of plants that are a valuable source of secondary bioactive metabolites, including flavonoids, terpenes, and cannabinoids. These metabolites are responsible for a wide variety of biological activities such as anti-inflammatory, anti-oxidant, psychotropic and others. The long term objective of this project is to develop *C. elegans* as a model for studying the bioactive potential of different extract types from cannabis plants.

Previous studies showed that aqueous extracts from *Cannabis sativa* leaves exhibit nematicidal activity on plant parasitic nematodes, and suggested that phytocannabinoids present in the extract are responsible for this effect. However, little is known about the nematicidal activity from extracts of other parts of cannabis plants, including female flowers where phytocannabinoids accumulate in highest amounts.

C.elegans has proven to be an excellent model for the discovery of new anthelmintic drugs and its mechanism of action. In this sense extracts from flowers of two Cannabis plant varieties which differ in its ratio of tetrahydrocannabinolic acid (THCA)/ cannabidiolic acid (CBDA) content were prepared and its activity on *C. elegans* motility assessed. The phytocannabinoids profile of the extracts was studied and the main phytocannabinoids were identified and quantified by HPLC-DAD.

Automated motility assays were conducted with N2 worms at 10 µM and 100 µM in the main cannabinoid (CBDA or THCA). Failure to see a motility effect in this background prompted us to use mutants with a compromised cuticle. *C.elegans* has a robust cuticle that functions as a physical barrier against chemicals which may represents a problem for compounds testing, such as acidic phytocannabinoids. In this sense, motility assays were performed using mutants in the *bus-8* gene, which encodes a predicted glycosyltransferase required for cuticle integrity. Cannabis flower extracts caused severe reduction in motility in *bus-8* mutants at 50 µM and 100 µM in the main cannabinoid. We are currently analyzing purified compounds to dissect the complex effect of these extracts and using mutant analysis to understand its mechanism of action.

1219A Synergistic Neuroprotective Effects of Mix Extract from Biosearch Life Product Against AD-hallmarks and Cognitive Decline in *Caenorhabditis elegans* and SAMP8 Mice Model

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Neurodegenerative diseases such as Alzheimer's disease (AD) are well recognized as major public health problems in the aged population. Recognized events in AD include cognitive impairment, oxidative stress (OS), Aß plaques accumulation in brain tissue. The current drugs to treat AD no cure or reduce the progression of the disease. Thus, the study of different extracts can provide a wealth of bioactive compounds and their combination, which can exert a new strategy for several neurodegenerative diseases, including AD. Among the natural extracts, Ginkgo biloba is one of the most investigated herbal remedies for cognitive disorders and AD. Moreover, docosahexaenoic acid (DHA), and Pinitol and Ursolic acid (UA) are associated to a prophylactic role in certain age-related diseases with particular emphasis on some of the effects of certain degenerative diseases. This study aimed to investigate the synergistic neuroprotective effects of mixed extract composed by different concentrations of DHA (150 µM), Ginkgo (120 µM) Pinitol (105 µM), and UA (35 µM) from Biosearch Life product, including OS tolerance, Thioflavin-S staining Aß plaques, and lifespan in several transgenic *Caenorhabditis elegans (C. elegans)* as well as cognitive performance in *C. elegans* and senescence-accelerated prone mice 8 (SAMP8) model. Firstly, we found a significantly higher survival percentage in *C. elegans* treated with Mix extract group in comparison with the single extract treated groups (DHA 150 µM group, Ginkgo 120 µM group, Pinitol 105 µM group, UA 35 µM group), reaching the Vitamin C group. Likewise, we found a significantly increased the lifespan in C. elegans Mix extract-treated group compared to the other groups, suggesting the synergistic effects. Remarkably, we determined a significant reduction in Aß plaques accumulation in C. elegans strain CL2006 Mix extract group compared to other groups, including all treated groups, confirming the synergistic effect again. Finally, we demonstrated better cognitive performance in the Mix extract group in both AD models (neuronal Aß *C. elegans* strain CL2355 and SAMP8 mice model), confirming the molecular result and demonstrating the synergist effects of this Mix extract. Taken together, our results demonstrated the potential therapeutic strategy for AD of this new Mix extract product from Biosearch Life.

1220B Investigating the metabolic impact of inhibiting bacterial folate synthesis: A novel method to measure amino acids in agar beneath the bacterial lawn.

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The microbiome has a strong impact on host health but the molecular mechanisms of this relationship are hard to elucidate in the complex human gut microbiome. *C. elegans* provides a simplified model for investigating the gut microbiota-host system whereby the growth media (diet), worm (host) and bacteria (microbiota) are specifically and easily manipulated to control variables. Our group has shown that inhibiting E. coli folate synthesis increases worm lifespan independently of worm folate status with no adverse effects on bacterial growth or host health. We have shown that inhibiting bacterial folate synthesis removes a factor or activity from the bacteria that accelerates ageing but the identify and mechanism of that factor are unknown. One possibility is that bacterial metabolism is altered and/or there is a change in molecules secreted from the bacteria. In our lab we have replaced peptone in our agar media with defined amino acids to provide a more controllable nutrient environment. To develop a method to monitor molecular changes in the agar, our aim was to measure the difference in amino acid concentration in the agar after growth of the bacteria. To the best of our knowledge there has not been a protocol developed to metabolites from solid agar inhabited by a bacterial lawn. Here we present a simple but robust and easily reproducible method for amino acid extraction and HPLC-MS analysis that could be applied to a larger study of the bacterial exo-metabolome from a solid agar source. With the exception of Lys, Arg and His we could recover and measure amino acids within an RSD threshold of <5%. We will use this method to understand how inhibiting bacterial folate synthesis alters bacterial metabolism in the context of a solid bacterial lawn, to inform our studies on how this intervention slows ageing.

1221C Honey bee (*Apis mellifera*) venom toxicity in breast cancer cells and the nematode *Caenorhabditis elegans*

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Bee venom, also known as apitoxin, is produced by honey bees (*Apis mellifera*) and comprises a complex mixture of substances with reported therapeutics and pharmacological properties. However, this natural product can cause severe allergic reactions, and further toxicological studies on animal models are necessary to ensure safe use. Bee venom obtained from Apis mellifera and collected in Northeast Brazil was used to investigate its effects in normal and breast cancer cells and the nematode *Caenorhabditis elegans.* In the present study, we employed the acute exposure assay system of *C. elegans* to evaluate bee venom›s toxicity *in vivo*. Synchronized L4 larval stage worms (N2-Bristol) were exposed for three hours in M9 buffer to bee venom. Behavioral parameters, including reproduction, survival, DAF-16 transcription factor location (zIs356 [daf-16p::daf-16a/b::GFP + rol-6(su1006)]), and superoxide dismutase-3 (SOD-3; muIs84 [(pAD76) sod-3p::GFP + rol-6(su1006)]) expression, were analyzed. Bee venom cytotoxic impacts on MDA-MB-231 and J774 A.1 cells were evaluated by the MTT assay until 72 hours of exposure. Acute exposure to bee venom resulted in a decrease in *C. elegans* survival, feeding behavior ($p<0.0001$), movement ($p<0.0001$) while induced an increase in the gaps between the cycles of defecation ($p<0.001$). Bee venom has also decreased nematode reproduction by reducing both egg-production ($p<0.0001$) and egg-laying ($p<0.05$). This toxin enhanced DAF-16 translocation from the cytoplasm to the nucleus, which did not affect the SOD-3 expression. Bee venom significantly inhibited the proliferation of MDA-MB-231 cells and caused a cytotoxic effect on macrophages. Our results show that exposure to bee venom produced significant toxic effects on the cells and animal model studied. *C. elegans* can provide information about the molecular and cellular mechanisms of bee venom toxicity and serve as a model organism to study the toxic effects of this natural product on human health.

1222A Deciphering the molecular mechanisms underlying the anthelmintic effect of essential oils evaluated in *Caenorhabditis elegans*

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Helminths consist of a diverse group of parasitic worms including nematodes, which cause diseases of major socio-economic importance globally. Control of infections in both human and veterinary medicine currently relies mainly on chemotherapy, but acquisition of resistance is an increasing problem, so there is an urgent need for discovery of novel drugs. As parasitic nematodes are not ideal laboratory animals, *C. elegans* has demonstrated to be a model system for the discovery of new anthelmintics and for characterizing their mechanisms of action and resistance. Essential oils (EOs) are natural products produced by aromatic plants. We here perform paralysis assays of wild-type and mutant *C. elegans* strain to identify EOs with potential anthelmintic activities, reveal the active components, their target sites and mechanisms of action. We found that EOs belonging to different orders produced rapid paralysis of *C. elegans* showing EC50 values between 0.02-2 % of EOs. All EOs tested also inhibited egg hatching, a property related to anthelmintic ability. Thus, EOs mediate both rapid and long-term anthelmintic effects. Terpenoids are terpenes with added oxygen molecules, thymol and carvacrol are the most common and well-known terpenoids present in EOs. Phenylpropenes, such as eugenol and trans-cinnamaldehyde (TC), are named as such because they contain a six-carbon aromatic phenol group and a three-carbon propene tail from cinnamic acid. We determined that TC, produces both paralysis and egg-hatching inhibition. By testing mutant worms, we identified the muscle L-AChR and GABA receptors as EOs and TC targets. Thus, by modulating two receptors with key roles in worm motility, these EOs emerge as novel sources of anthelmintic compounds. To unequivocally confirm that these receptors are targets of TC and to describe the mechanism by which they affect these receptors, we performed whole-cell and single-channel recordings from L1 *C. elegans* muscle cells. Electrophysiological recordings revealed that thymol, eugenol and carvacrol are not capable of eliciting macroscopic currents but they significantly reduce ACh- and GABA-elicited responses. At the single-channel level, we found that the activity of L-AChRs is significantly reduced in the presence of different terpenoids or phenylpropenes, without changes in channel properties. The results are compatible with the action of these drugs as allosteric inhibitors. Current studies are being carried out to determine if TC shows a similar action and to determine structure-activity relationships of the active compounds. It is hoped that this work can update the recent progress on natural nematicide discoveries and provide new ideas for the design and mechanism of action studies of anthelmintics. In addition, our study increases our knowledge related to the molecular function and pharmacology of the two main receptors involved in *C. elegans* locomotion.

1223B Worm Developmental Dynamics Database 2 – an open database with visualization for biological dynamics of large-scale RNAi experiments on *C. elegans* embryos

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Collections of images and quantitative, numerical information about morphological dynamics support understanding the molecular mechanisms of development.

Recently we conducted RNAi experiments targeting all 351 essential embryonic genes reported in the previous genome-wide RNAi screening for *C. elegans*. We recorded three-dimensional time-lapse, i.e., 4D differential interference contrast (DIC) microscopy images of developing embryos at 66 consecutive focal planes spaced at 0.5 um for 2 hours at 20 seconds intervals. We obtained a collection that includes 33 sets of quantitative data, such as 3D coordinate values of nuclear regions and their dynamics from 1 to 8-cell stage, for wild-type embryos and 1,142 sets of quantitative data for RNAi embryos corresponding to 263 essential embryonic genes. We detected over 26,000 phenotypic alterations for 421 phenotypic characters, such as cell division timing and division axis orientation from the collection (Kyoda et al., bioRxiv 2020).

To make the collection openly available, we have developed Worm Developmental Dynamics Database 2 (WDDD2; https://wddd.riken.jp/), as the successor of Worm Developmental Dynamics Database (WDDD; http://so.qbic.riken.jp/wddd/), with a completely new implementation using modern web frameworks such as Django and Three.js. WDDD consists of images and quantitative data of 50 sets of wild-type embryos and 136 sets of RNAi embryos corresponding to 72 of the 97 essential embryonic genes on chromosome III, with sparser time intervals, but WDDD2 needs to handle ten times more or over.

The database shows nuclear division dynamics information interactively as a combination of 4D DIC images and 4D visualization of quantitative data. The system displays the region of the nuclei and the center of gravity of them as wireframes in 3D space, and the user can select to view the data at a specific time or all time points simultaneously. The related cell images are displayed next to the wireframe. The image and wireframe's viewpoint can be manipulated synchronized but freely by pointing device movements to compare them to each other.

The 4D DIC images in the original microscopy manufacturer's format are openly available by download from the database. The quantitative data are also openly downloadable in the unified format for biological dynamics, BDML/BD5 (Kyoda et al., 2015; 2020). We plan to develop Web APIs in the future to use images, quantitative data, and phenotypic characters for analysis without download.

1224C UPS modulation and autophagy.

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The ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway (ALP) are the two main eukaryotic intracellular proteolytic systems involved in maintaining proteostasis. Autophagy is responsible for degrading defective cellular organelles and long-lived proteins in the cytosol and is involved in cell growth, survival, development and death. UPS mostly degrades soluble and short-lived proteins in the nucleus and cytosol and affects various cellular processes. These two proteolytic systems are essential components of the cellular protein quality control system. Several studies have reported an interplay between the UPS and ALP, however it still remains largely unknown how these systems communicate in a multicellular organism. We have recently shown that downregulation of autophagy genes elicits tissue-specific effects on UPS function in *C. elegans*[1]. Here, we address how genetic or pharmacological modulation of the proteasome and the proteasome-associated DUBs affects autophagy. We use transgenic *C. elegans* expressing the autophagy reporters GFP::LGG-1[2] or mCherry::GFP::LGG-1[3] and analyze the accumulation of LGG-1 puncta and autophagic flux. Our preliminary data show that downregulation of the proteasome-associated DUBs *ubh-4*, *usp-14* and *rpn-11* as well as some proteasomal subunits affect the number of puncta positive for autophagosomes in hypodermal seam cells, intestinal cells as well as in pharynx. To nvestigate a conserved function of the UPS on autophagy in human cells, we are utilizing a HeLa cell line expressing the fluorescence probe GFP::LC3::RFP::LC3ΔG[4]. A better understanding of the multilayered crosstalk between UPS and ALP *in vivo* may facilitate development of therapeutic options for various disorders linked to dysfunction in proteostasis.

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1225A Histone demethylase AMX-1 provides sensitivity to interstrand crosslink DNA damage

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Histone methylation is dynamically regulated to shape the epigenome and adjust central nuclear processes. Here, we investigated the histone demethylase LSD2 homolog AMX-1 in *C. elegans* and uncovered a potential link between H3K4me2 modulation and DNA interstrand crosslink (ICL) repair. AMX-1 is an uncharacterized histone demethylase and mainly localizes to embryos, the mitotic gut and sheath cells. AMX-1 expression compensates for the lack of the histone H3K4 demethylase SPR-5 and the mutants show increased H3K4me2 levels in the germline, indicating that AMX-1 and SPR-5 regulate H3K4me2 demethylation. *amx-1* mutant activates the CHK-1 kinase acting downstream of ATR and leads to the accumulation of RAD-51 foci and increased DNA damage-dependent apoptosis in the germline. AMX-1 is required for the proper expression of mismatch repair component MutL/MLH-1 and sensitivity against ICLs. Interestingly, the formation of ICLs lead to ubiquitination-dependent subcellular relocalization of AMX-1. Taken together, our data suggest that AMX-1 functions in ICL repair in the germline.

1226B Using *C. elegans* in prognosis, diagnosis, and drug screens for splicing-related retinitis pigmentosa

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Retinitis pigmentosa (RP) is a rare, heterogenic, and hereditary disease that produces gradual loss of the visual field and can cause blindness. Mutations causing the disease are still unknown in about 50% of the cases. By CRISPR, we mimicked a few RP mutations in splicing-related genes such as PRPF8/*prp-8* and SRNPN200/*snpr-200* (Kukhtar et al, 2020). One of the alleles displaying a strong phenotype was used in a small-scale drug screen to identify small molecules capable of alleviating the phenotype. Unexpectedly, we found an FDA-approved drug having a detrimental effect on some of the mutant strains.

Since RP onset and progression are highly variable due to environmental or genetic modifiers, *C. elegans* could help RP prognosis by identifying such modifiers. We performed an RNAi screen on RP mutants with no overt phenotypes and found synthetic interactions with other splicing-related genes. Thus, secondary mutations in these genetic interactors could act as modifiers of the course of the disease.

We are taking steps towards establishing *C. elegans* as an RP diagnosis model by evaluating the functional impact of potential RP mutations, or variants of unknown significance (VUS), in worms. For that purpose, we are setting a panel of features associated with splicing-related RP mutations, including a genetic interaction with a CRISPR-edited Slow Polymerase II mutant (*ama-1(cer135[R743H])*), mortal germline, or aberrant splicing events at specific transcripts. We are also humanizing the sequence encoding the splicing factors *prp-3* in the endogenous locus to investigate if such humanization is beneficial for functional studies of VUS.

Therefore, our RP research line in RP demonstrates the value of *C. elegans* for investigating rare diseases and for providing valuable information in the search of drugs, diagnosis, and prognosis.

1227C A lysosomally-localized protease inhibitor is necessary for *C. elegans* molting

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How do gene regulatory networks in the nucleus promote remodeling of the extracellular matrix (ECM)? We are addressing this question through studying the nuclear hormone receptor transcription factor NHR-23. NHR-23 directly regulates a set of collagens, ECM components, signaling factors, proteases and protease inhibitors which are necessary for molting. MLT-11 is a large protein with 9 Kunitz protease inhibitor domains and is regulated by NHR-23, potentially through a number of NHR-23 binding sites in its promoter region as detected by ChIP-seq. Reduction of *mlt-11* levels through RNA interference (RNAi) causes animals to become trapped in cuticles during ecdysis and the formation of large vacuoles. MLT-11 is required

for integrity of the cuticle barrier, as *mlt-11(RNAi)* animals are sensitive to hypo-osmotic stress and cannot exclude Hoechst 33258 dye. We generated an *mScarlet::3xMYC* knock-in that labels all *mlt-11* isoforms. Unlike previously described proteases and protease inhibitors, MLT-11::mScarlet was not localized to the extracellular matrix. Rather, MLT-11::mScarlet localized to punctate and tubular structures under the surface of the hypodermal plasma membrane. Using markers of different endocytic compartments, we found MLT-11::mScarlet co-localizes with lysosomes, late endosomes/multivesicular bodies, and recycling endosomes. Inactivation of the GTPase *rab-5*, which regulates early endosomes, lead to an accumulation of MLT-11::mScarlet outside the hypodermis, under the old cuticle. This data suggests that MLT-11 is secreted and then endocytosed in order to reach the lysosome. Together, our work provides mechanistic insight into how NHR-23 promotes *C. elegans* ECM remodeling and the emerging role of lysosomes in this process.

Funding: This work was supported by the National Institutes of Health (NIH) grants R00 GM107345 and R01 GM138701.

1228A Lutein rescues a *nlg-1*-mediated synaptic defect in a *C. elegans* mitochondrial complex I deficiency model

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Complex I deficiency represents the most frequent pathogenetic cause of human mitochondria-associated diseases (HMAD). Therapeutic options for these devastating, life-threating disorders, which in most cases present with neurodevelopmental defects, do not exist, in part due to the scarcity of appropriate model systems to study them. Caenorhabditis elegans is a powerful, genetically tractable model organism widely used to investigate neuronal development and degenerative pathologies. Here, we generated new *C. elegans* models for HMAD and we focused on two complex I disease models associated with Leigh Syndrome, *nuo-5*/NDUFS1- and *lpd-5*/NDUFS4-depleted animals, which nicely recapitulated biochemical, cellular and neurodevelopmental defects of the human diseases. The two models were exploited for a suppressor screening that identified lutein, among a library of natural compounds, for its ability to rescue the developmental arrest and neuronal deficits observed upon *nuo-5* and *lpd-5* depletion. We specifically found that lutein exerts its beneficial activity by rescuing a neuroligin-mediated synaptic defect we disclosed for the first time upon *nuo-5* depletion, thus pointing to possible novel therapeutic targets for the human disease.

1229B Evaluación del efecto del extracto etanólico de *Witheringia coccoloboides* sobre agregados de *α- sinucleína* en la cepa NL5901 de *Caenorhabditis elegans*

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Neurodegenerative diseases (ND) are a public health problem, cause permanent disability and a high cost in health systems. People affected with Parkinson disease have protein deposits of *α-synuclein* (α-syn) in brain cells. The use of biological models such as *Caenorhabditis elegans (C. elegans)* can answer questions that arise day by day for the development of new therapeutic targets for ND. The transgenic strain NL5901 of *C. elegans* is characterized to have α-syn aggregates labeled with yellow fluorescent protein (YFP), which allows to evaluate compounds effect on the physiological characteristics and / or the reduction of protein aggregates α-syn in this model. On the other hand, *Witheringia coccoloboides (W. coccoloboides)* has been described as a promising plant in the palliative treatment of ND and therefore of interest in ethnopharmacology due to its possible neuroprotective action. Therefore, the main objective of this study was to evaluate the effect of the ethanolic extract of leaves of *W. coccoloboides* on the physiological characteristics and protein aggregates of α-syn in the mutant strain NL5901 of *C. elegans*. The results of the present work show that the ethanolic extract of leaves of *W. coccoloboides* has a reducing effect of protein aggregates α-syn, improving the physiological characteristics of reproduction and motility, as well as, decreasing the levels of Reactive Oxygen Species (ROS) in strain NL5901 of *C. elegans.* Therefore, it is possible to suggest that the ethanolic extract of leaves of W. coccoloboides has a protective effect on strain NL5901 of *C. elegans*, probably attributed to the presence of sterols and / or terpenes, flavonoids and alkaloids, which in turn, it is generate a recovery of muscular

activity in the egg lay and locomotion, probably all linked, to synergistic interaction of the phytocompounds present in the ethanolic extract of leaves of *W. Coccoloboides.*

Keywords: *Witheringia coccoloboides*, *α-synuclein*, Parkinson disease, *Caenorhabditis elegans*

1230C Dichlorvos exposure aggravates behavioral toxicity in high glucose fed *C. elegans*

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Glucose is one of the most abundant monosaccharide and an essential carbohydrate for our daily routine life. It has been also reported as one of the major causative agents of obesity that ultimately causes diabetes and cardiovascular diseases. Several studies have shown that high glucose intake results in reduced lifespan, increased oxidative stress, reduced fecundity, accelerated ageing in exposed *C. elegans* and high glucose fed worms are more vulnerable for having behavioral alteration and neurodegeneration. Thus, in the present study we aimed to investigate the effects of dichlorvos (DDVP) exposure (10, 20, 40µM) on behavior of *C. elegans*, fed on high glucose diet. In our study, we have measured the behavioral responses in terms of pharyngeal pumping (feeding), locomotion behavior including head thrashing, speed and body bending behavior. We examined that worms exposed to high glucose diet from embryonic to late larval development, showed enhanced toxicity to DDVP in terms of behavioral alterations. Our studies strongly advocate the use of *C. elegans* as an alternate model to study the glucose-insecticide interactive neurotoxicity as all the parameters can be easily studied in the worm and study can be well extrapolated to those expected in higher model organism. Collectively, our studies suggested that high intake of glucose aggravates DDVP toxicity and provides an experimental paradigm to explore the possible relationship between insecticide exposures associated behavioral alterations in humans under hyperglycemic conditions.

1231A Genotoxicity and *C. elegans* – using the worm for DNA damage and DNA repair/ damage response research

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Most DNA repair pathways that are known through studies of bacteria, yeast, mammals, and human cell lines, are also highly conserved in *C. elegans*, which makes the worm an experimental model greatly suited for research on processes required in genomic stability and mutational change. Even though it is known that most major repair pathways are conserved in the nematode, the potential of this model organism in genotoxicity is still not used to its full potential. By adapting various assays from cell culture or rodent experiments, we are able to provide a test strategy for this research field according to the 3R (replace, reduce, and refine experiments in vertebrates) concept.

Due to the widely recognized difficulty in quantifying oxidative stress and DNA damage markers, multiple endpoints were investigated in the nematode. A major cause of (oxidative) DNA damage is oxidative stress via excessive reactive oxygen species. We determined various oxidative stress markers including glutathione levels, oxidised cardiolipins, and the intracellular reactive oxygen species themselves in nematodes and found time- and dose-dependent increases of these markers after treatment with established *in vitro* positive controls. Moreover, we were able to quantify 8-oxodG increase, which is the most common oxidative damage on a DNA base, using an ELISA system or immunofluorescence staining in the worms. To assess the cellular DNA damage response, we quantified poly(ADP-ribosyl)ation via LC-MS/MS. The amount of DNA strand breaks within a sample can be determined utilizing the alkaline unwinding assay, which is an established genotoxicity test in *in vitro* and *in vivo* models. Additional to the detection of DNA damage and DNA damage response, gene expression studies can point out the relevant DNA repair pathways and using transgenic DNA repair mutants may identify sensitivity towards specific compounds. Using this assembly of assays it is possible to analyse the genotoxic potential of a compound – from cause to consequence.

Classical genotoxicity testing still relies mainly on expensive and time-consuming animal experiments or less transferrable cell culture systems. Utilizing the many known advantages of *C. elegans,* we were able to assemble a complete model for genotoxicity testing from oxidative stress endpoints, to activation of the DNA damage response and DNA repair to measuring the DNA damage itself. Thus creating a modern, 3R-conform approach for genotoxicity testing.

1232B Biophysical models of *C.elegans* neurons: the case of AWC^{ON} and RMD neurons.

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Caenorhabditis elegans nervous system constitutes an ideal framework for computational neuroscience studies. Its nervous system has been fully reconstructed in neuron numbers and connections. However, the mechanisms at the basis of neuronal signals generation in single neurons are still largely unexplored due to experimental difficulties related to the small size of the neurons. In this context, biophysical models of single neurons could help elucidate the single-neuron dynamics and guide future experiments. In this work, we present biophysical models of the ionic currents found in the nematode neurons and muscles, based on the application of the Hodgkin-Huxley model to the *C. elegans* case. The models of single ionic currents are combined to describe the dynamics of the AWC^{ON} sensory neurons and RMD motor neurons [1]. Our models properly replicate experimental voltage-clamp recordings on AWC^{ON} neurons and current-clamp recordings on RMD neurons. The role of single ionic currents in the whole-neuron dynamics is investigated by analyzing the conductance and the responses of *in silico* knockout neurons. These analyses highlighted the importance of T-type calcium currents and leakage currents in the peculiar bistable behavior observed in RMD neurons. Moreover, our analysis highlighted different dynamical regimes in *C. elegans* neurons, including bistable and sustained oscillatory regimes. Furthermore, we study the chemosensory responses of AWC^{ON} neurons in a wide range of odor concentrations and exposure times by coupling our model of the electrical responses [1] with the model of chemosensory responses developed by Usuyama et al. [2,3]. In conclusion, our work constitutes the basis for extensive biophysical modeling of the C. elegans nervous system from the single-cell up to network scale.

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1233C Engineering photo-inducible GFP-binding nanobodies for *in vivo* applications using genetic-code expansion and computational alanine scanning

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Nanobodies are fragments of camelid heavy chain only antibodies that have high antigen specificity and are easily expressed *in vivo*. They are increasingly used as *in vivo* tools to specifically target proteins of interest and control protein function. The ability to control nanobody/antigen binding using light would add precise spatiotemporal control over nanobody based tools.

Here, we present a general method for engineering photo-activatable nanobodies using genetic code expansion and computational alanine scanning. The photo-activatable nanobodies can be optically controlled using 365 nm light, which makes them compatible with other optogenetic tools and imaging approaches using visible wavelengths.

We used genetic code expansion to introduce a photocaged amino acid to the nanobody's antigen binding domain. Genetic code expansion allows the site specific incorporation of non-canonical amino acids (such as photocaged amino acids) into proteins, and has been established in several model systems, including *C. elegans*. Photocaged amino acids are a type of non-canonical amino acid with sterically interfering aromatic groups that can be removed by illumination with 365nm light.

The introduction of a photo-caged amino acid to an anti-GFP nanobody has been shown to reduce nanobody/GFP binding *in vitro*. However, we found that this was not sufficient to abolish *in vivo* binding. To design photocaged anti-GFP nanobodies that display no binding to GFP before illumination with 365nm light, we used computational alanine scanning (CAS). CAS utilizes 3D structures of binding complexes to predict the change in binding strength that results from mutating each residue to alanine. We introduced such alanine mutations, predicted to reduce GFP binding, into nanobodies that contain photocaged amino acids, and validated their binding properties experimentally.

Using this approach, we created photo-inducible variants of two anti-GFP nanobodies. The improved variants display no GFP binding in the caged form, and binding is restored after illumination with 365nm light. We apply our photo-inducible nanobodies to photocontrol subcellular protein localisation *in vivo* in *C. elegans*.

1234A Reprotoxicity induced by Acute exposure to Aqueous Root Extract of Peruvian Maca (*Lepidium meyenii*) in *Caenorhabditis elegans*

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Ethnopharmacological relevance: Maca (*Lepidium meyenii*) has been used in folk medicine to treat fertility disturbances. However, there are few scientific evidences validating this use or assuring extract safety. Aim of the study: Hence, in this study we tested the reproduction safety of this extract in *Caenorhabditis elegans*. Materials and Methods: Root *maca* powder, obtained from local commerce, was used to prepare the aqueous extract. Worms were acutely exposed to *maca* extracts (40, 120, 240 and 330 µg/µl), based on human consumption of 1g capsules of the same powder. 48h after treatments, assays were conducted. Results: Maca extract caused a significant decrease in total number of eggs and in the number of eggs per worm from. These effects were associated to increased lipid peroxidation, reduced triacylglycerol levels and also vitellogenin-2 expression, besides increase in the number of apoptotic germline cells. We have detected and quantified alkaloids in this *maca* extract, which presence could be related to this toxicity. Conclusions: Collectively, our data suggest that *maca* extract exposure causes reproductive toxicity to worms which could be, at least in part, associated to both an increase in apoptosis of germline cells and also to a decrease in vitellogenin expression, needed for egg yolk production, and consequently, successful reproduction.

1235B Mutability of mononucleotide repeats explains the discrepancy between lab-accumulated mutations and the natural allele frequency spectrum of *C. elegans*

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Mutation is the fuel of evolution, and is of fundamental importance in evolutionary biology. A usual and efficient way to estimate the properties of spontaneous mutation divorced from the influence of natural selection is by means of mutation accumulation (MA) experiments, in which the efficacy of selection is minimized. However, MA experiments potentially come with their own biases. Previous studies have consistently shown a discrepancy between the mutation spectrum observed in MA lines and the site frequency spectrum (SFS) of wild isolates. By applying MA experiments and whole genome sequencing to three strains of *C. elegans*, (N2, PB306, and an N2-derived strain carrying a defective allele at the *mev-1* gene), we investigated the property of spontaneous mutations in different part of the genome. We find that the mutational properties of mononucleotide repeats differ in both rate and spectrum from non-mononucleotide sequences, both for base-substitutions and insertion/deletion (indel) mutations. Comparison of the MA spectrum to that of segregating “private” alleles (which have presumably arisen recently as new mutations) reveals that the spectra at non-mononucleotides are similar between MA lines and wild isolates, whereas the mononucleotide spectra are very different, both for base-substitutions and indels. In addition, we compared the mutational properties of highly divergent regions of the *C. elegans* genome to those of weakly diverged regions. Our preliminary analysis suggests that the mutation rate is slightly higher in divergent regions, but the difference in mutation rate is not nearly large enough to explain the difference in nucleotide diversity.

1236C The Mitochondria-targeted hydrogen sulfide delivery improves health and mitochondrial function in a C. elegans primary mitochondrial disease model

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Introduction:

Primary

mitochondrial diseases (PMD) are genetically inherited disorders culminating in poor health and premature death. *C. elegans* possess vast orthologous genes associated with PMD, allowing high throughput analysis of the functional consequences of mutant genes. The endogenous gaseous mediator hydrogen sulfide (H_2S) is an evolutionary conserved mitochondrial electron source and post-translational regulator across species. Since H_2S augments cellular bioenergetics in conditions where mitochondrial dysfunction is a consequence of disease, we investigated whether mitochondria-targeted H_2S using novel

compounds we have developed (mtH_2S) could restore cellular bioenergetics where mitochondrial dysfunction is a direct cause of the disease state (e.g. PMD).

Methods:

We characterised life/healthspan of

40 *C. elegans* homologues of human PMD mutations to identify PMD genes associated with poor health using a high-throughput microfluidic device. PMD strains displaying reduced life and/or health were assessed for improved health/longevity in the presence of our novel mtH_2S molecules. Strains showing mtH_2S -induced improvements were further assessed for ATP production, mitochondrial ΔΨm, oxygen consumption and total sulfide.

Results:

Seven PMD mutants were characterised with 5 showing reduced survival/locomotion. Of these, 2 strains displayed significant improvement with mtH_2SD (100nM). Animals with widespread electron transport chain deficiency (*gfm-1* and *mrsp-16*) were not responsive to mtH_2S. *abtm-1* mutants showed no improvement in life/healthspan despite significant preservation of mitochondrial ΔΨm on day 2 of adulthood. However, in a complex I mutant (*nuo-4*; a Leigh syndrome orthologue), mtH_2SD significantly reversed the decline in ATP levels, preserved mitochondrial ΔΨm and increased survival/movement. Furthermore, mtH_2S compounds significantly increased global sulfide levels in a complex IV mutant (*sco-1*), preserving mitochondrial ΔΨm and delaying severe movement decline (untreated *sco-1:* 32% of population active vs 68% in mtH_2S *sco-1* at day 6).

Conclusion:

Our data shows for the first time that mtH_2S are potent molecules in PMD, effective in the nM range, and present a novel approach to at least delay mitochondrial perturbations in a variety of PMD disorders. Complete characterisation of all known PMD orthologues will significantly contribute to the precise disease mechanisms of PMD and potential therapeutics.

1237A *Ce*SnAP: Machine-learning based snapshot analysis platform toward high-throughput Caenorhabditis elegans behavioral screen of Parkinson's disease

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Parkinson's disease (PD) is a movement disorder and the mechanisms that induce neurodegeneration in PD are still poorly understood. We recently found that RNAi-mediated knockdown of neuronal branched-chain amino acid transferase 1 (BCAT-1) in nematode *C. elegans* cause age-dependent spasm-like 'curling' phenotype mirroring PD clinical symptoms. Manual quantification of curling is labor-intensive making large-scale drug and genetic screens challenging. Here, we report the development of a machine learning-based automated workflow for *C. elegans* image analysis, and its application to the discovery of potential compounds that may be repurposed as late-in-life interventions for PD. This high-throughput workflow is 40X faster than the manual assay and constitutes a major advance in the efficiency and precision with which Parkinson's-like curling behavior in *C. elegans* can be quantified. In a screen of 50 FDA-approved drugs, we have identified four drugs (enasidenib, ethosuximide, metformin, and nitisinone) that reduced curling to <50% of vehicle-treated levels. These findings point to the utility of our high-throughput platform for screening for modifiers of the disease phenotypes, including but not limited to chemicals or genetic manipulations that may ameliorate or worsen motor function in the worms.

1238B Quantitative cell shape analysis in the *C. elegans* embryo

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Cell shapes in developing embryos hold crucial information on the processes that give rise to cellular self-organization, such as cellular contacts, adhesion and transmission of forces. However, automatically extracting cell shapes from microscopy images remains challenging. Many existing segmentation packages are often optimal only for a specific imaging protocol, are difficult to parameterize for data with different characteristics, or require additional information such as nuclei markers.

We have developed a novel segmentation pipeline for accurate 3D cell segmentation. We use the method for segmenting *C. elegans* embryos with fluorescently labeled membranes imaged with confocal microscopy. Initially, a tracking algorithm takes a region clustering approach, allowing for an adaptive segmentation threshold that varies across space, while still permitting fast processing. After identifying the cells and their rough shapes, a Discrete Element Method mechanical simulation evolves 3D

cell meshes constrained by a biophysical model of cell shapes to achieve a precise and physically sound segmentation. The cell shape model captures adhesion and cortical tension forces that act on a biological cell and pixels from the microscopy image serve as an additional attractive force.

We apply the reconstructed cell shapes to characterize and quantitatively compare cellular morphologies during early development, which can yield important clues with respect to the underlying processes. For example, we search for systematic morphology differences between cell types reflecting differential cellular activities as well as the effect of cell polarisation and asymmetric divisions. The morphological characterization can also help to identify novel and specific phenotypes during perturbation experiments such as gene knock downs. For example, by applying our method for embryos treated with *dsh-2/mig-5* RNAi we showed that sphericity of the E-cell is signifficantly lower compared to wild-type, reflecting lower cortical tension following the loss of asymmetry in F-actin distribution between E and MS in these mutants. Volumetric asymmetry is however maintained, and increases 12% to an E/MS ratio of 0.78. Besides phenotyping cell geometry, our method opens up ways to link shape with cellular mechanics, such as cortical tensions, adhesion and active motions that can eventually facilitate mechanical modelling of embryogenic events.

1239C Combining engineering with machine learning to automatically and reliably measure the *C. elegans* brood size.

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Brood size evaluations are routinely used in *C. elegans* to characterize new alleles, the number of which has exploded following the implementation of efficient CRISPR/Cas9 technology. Manual brood size measurements (number of eggs laid, hatched larvae, adult progeny) are labour intensive, error-prone, and may also be disruptive for animals. Indeed, the experimenter must take individuals outside of the incubator for the long and tedious microscopic analysis of their progeny, during which larvae move constantly and L1 are especially hard to visualize. This creates variability between independent assessments. Manipulations also inevitably cause thermal[1,2], mechanical[3] as well as photooxidative[4] stresses to the sample. Even though such classical evaluations of brood size are somewhat imprecise, that method has been used for the past few decades because no alternative has yet been proposed. Here, we present a novel machine learning-based method that fully automates *C. elegans* brood size measurements. All that the experimenter will have to do is to pick 1 animal per plate into a 24-well plate, place it in the incubator, and wait that the numbers come out. Although still in development, this project has passed the proof-of-concept stage. We are thus confident that it will be able to acquire, and subsequently analyze, images of entire 15.6mm wells up to once every minute. Our system will allow to measure embryonic lethality, as well as larval lethality at each larval stage. Variations in the environmental parameters will be minimal since the device fits on an incubator shelf, while light exposure, temperature, pressure and vibrations will be recorded. Beyond these basic features, we are working on expanding our device to record other parameters. For example, the neural network used in machine learning could be trained to recognize notable phenotypes (*e.g.* Dpy, Unc, Rol) which could be useful for segregation analyses. We also aim to couple this system to an additional high-magnification microscopic lense, in order to characterize features (*e.g.* length, width, turns, and velocity) of the parent during the first days of brooding. As such, we may be able to define adult features that would be predictive of an animal's brood size. Using both macroscopic and microscopic lenses along with a controllable moving platform, we expect to characterize brood size and features of hundreds of strains, with replicas easily exceeding a sample size of 50. We estimate that the cost to build the entire system will be under 500 USD.

1240A Identification of novel ivermectin resistance and hypersensitivity associated genes in a primary *C. elegans* mutant screen

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In 2016, nearly 800 million tablets of ivermectin were distributed to countries for use in elimination programs for human filarial diseases. Despite its widespread use, the mode of action of ivermectin against filarial nematodes is not well understood, and its *in vivo* potency cannot be replicated *in vitro*. To better understand how ivermectin affects filarial worms, our lab previously performed a transcriptomics study to identify differently expressed genes (DEG) in *Brugia malayi* adults and microfilariae after treatment of infected gerbils. Forty-four of these DEG had *C. elegans* orthologs available as mutant strains through the *C. elegans* Genetics Center. We have assayed these mutant strains for differential sensitivity to ivermectin by measuring three phenotypes affected by ivermectin: egg production, development, and motility. We have identified several resistant and hypersensitive strains of *C. elegans* as well as differences between responses to the three assays. Mutations conferring resistance included those in che-12 (e1812), a gene involved in chemotaxis, cilium assembly, and

hyperosmotic response; and inx-14 (ag17), which is predicted to have gap junction hemi-channel activity and is expressed in the muscular, nervous, and reproductive systems. The che-12 mutants are additionally resistant to ivermectin's effect on pharyngeal pumping, while inx-14 mutants are not different from control. Overall, twenty-three genes, with eleven strong candidate genes, have been identified as altering ivermectin sensitivity in at least one assay, supporting the validity of the overall approach. These may give insight into how ivermectin acts against filarial parasites as well as potential mechanisms of resistance. These results are currently being used as the basis of an RNAi screen in *B. malayi* to identify the effect of these candidate genes on ivermectin sensitivity in filarial parasites.

1241B Signals from the germline act systemically to regulate cytosolic protein oxidation in somatic cells in *C. elegans*

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The reversible formation of disulfide bonds between cysteine residues is an important post-translational modification that modulates the activity of hundreds of cytosolic proteins involved in a wide variety of cellular processes. Little is known about the intercellular signaling pathways that can sway the oxidation of these proteins in a concerted manner. The primary determinant of cysteine oxidation across the proteome is the glutathione redox potential (E_{GSH}). The mechanisms that regulate this potential *in vivo* remained largely unexplored until the development of the E_{GSH} -specific, reduction–oxidation-sensitive Green Fluorescent Protein (roGFP) family of genetically-encoded biosensors. These GFP-derived biosensors include two cysteines that form a (reversible) intramolecular disulfide bond upon oxidation, resulting in spectral changes that can be quantified via fluorescence-ratio microscopy. Using these biosensors, we determined the extent to which the germline regulates protein oxidation in different somatic tissues *C. elegans*. We found that ablation of the germline lowers cytosolic protein oxidation in multiple tissues, including the intestine, pharyngeal muscles, and vulval muscles. Within the pharynx, germline ablation affects both the overall level of cytosolic protein oxidation and the spatial pattern of protein oxidation within the tissue. The effects of germline ablation on pharyngeal muscle protein oxidation are mediated in part by the partially redundant action of the DAF-16/FOXO and SKN-1/NRF transcription factors. We have begun identifying the specific germline cell types that modulate protein oxidation in different tissues. Our finding suggest that the germline sends signals that act systemically to promote protein oxidation in somatic cells in *C. elegans*.

1245C MitoSegNet: Easy-to-use Deep Learning Segmentation for Analysing Mitochondrial Morphology

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While the analysis of mitochondrial morphology has emerged as an important tool in the study of mitochondrial function, efficient quantification of mitochondrial microscopy images presents a difficult task and bottleneck for statistically robust conclusions. Here, we present the Mitochondrial Segmentation Network (MitoSegNet), a pretrained deep learning segmentation model that enables researchers to easily exploit the power of deep learning for the quantification of mitochondrial morphology (Fischer, Besora-Casals et al. 2020). The MitoSegNet was generated by training a modified fully convolutional neural network with fluorescent microscopy, maximum-intensity projection images, depicting mitochondria in body wall muscle cells of adult *C. elegans* worms. We tested the performance of MitoSegNet against three feature-based segmentation algorithms and the machine-learning segmentation tool Ilastik. MitoSegNet outperformed all other methods in both pixelwise and morphological segmentation accuracy. We successfully applied MitoSegNet to unseen fluorescence microscopy images of mitoGFP expressing mitochondria in wild-type and *catp-6*^{ATP13A2} mutant *C. elegans* adults. Additionally, MitoSegNet was capable of accurately segmenting mitochondria in HeLa cells treated with fragmentation inducing reagents. We provide MitoSegNet for all operating systems as an easy-to-use graphical user interface tool that combines segmentation with morphological analysis.

Reference

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