8th Annual Midwest *C. elegans* Meeting

April 23, 2021

Organizers
Jamie Alan, Michigan State University
Kin Sing Stephen Lee, Michigan State University
Xantha Karp, Central Michigan University
Jennifer Schisa, Central Michigan University

Sponsors

Department of Pharmacology & Toxicology
Michigan State University

Department of Chemistry
Michigan State University

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9:15 am-9:30am	Log on, informal discussion	
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9:45am-10:45am	Session 1 (Long talks) 9:45-10:00 am Kiley Hughes. Long isoforms of mechanoreceptor pezo-1 control pharyngeal gland cell activity in the nematode Caenorhabditis elegans 10:00- 10:15 am Morteza Sarparast. Soluble Epoxide Hydrolase Inhibitor, AUDA, Recuses Neurodegeneration Induced by Amyloid Î² and Tau 10:15 -10:30 am Arunima Debnath. Neuromodulation and the asynchrony between Ca2+ transients and neuronal states 10:30 – 10:45 am Paschalis Kratsios. A noncanonical role for Hox in the C. elegans ventral nerve cord	Moderator: Jennifer Schisa
10:45 am-11:15 am	Breakout sessions for discussion	
11:15 am - 12:15pm	Parallel short talk session 1	
12:15pm-12:45pm	Lunch break (on your own)	
12:45pm-1:45pm	Keynote Speaker - Pamela Padilla Physiological Responses to Dietary Stress and Oxygen Deprivation in *C. elegans*	Moderator: Jennifer Schisa
1:45pm-2:15pm	Breakout sessions, including business meeting Business meeting agenda: 1. Future Meetings 2. Information sharing for future meetings 3. DEI initiatives	
2:15pm-3:15pm	Session 2 (Long talks) 2:15 -2:30 pm Alexandra Socovich. The kinase pig-1/MELK is a conserved cytoskeletal regulator in C. elegans tubulogenesis and in human endothelial cells 2:30 -2:45 pm Xinxing Zhang. A cilia-independent function of BBSome mediated by DLK MAPK signaling in C. elegans 2:45- 3:00 pm Amanda Zacharias. Cell fate plays critical roles in promoting collective cell movements in C. elegans gastrulation and ventral cleft closure during embryogenesis 3:00 -3:15 pm Shijiao Huang. Dietary Restriction mimetic drugs block food perception and induce FMO, a conserved regulator of stress response and metabolism	Moderator: Jamie Alan

Time (EDT)	Event	Coordinator
3:15pm-3:45pm	Breakout sessions for discussion	
3:45pm-4:45pm	Parallel short talk session 2	
4:45pm-5:30 pm	Session 3 (Long talks) 4:45 -5:00 pm Deniz Sifoglu. Neuropeptide modulation of insulin signaling in bacteria-dependent survival 5:00 -5:15 pm Matthew Wirick. daf-16/FOXO blocks adult cell fate in C. elegans dauer larvae via a branched pathway involving lin-41/TRIM71 5:15 -5:30 pm Ambre Sala. Embryo Integrity Regulates Maternal Proteostasis and Stress Resilience	Moderator: Sing Lee
5:30 pm-5:45 pm	Wrap up	Jamie Alan

Breakout Sessions:

3 Breakout sessions:

1) Science Topic Networking
    a. Neurobiology — Moderator: Jamie Alan — Room 1
    b. Stress, Aging, and Pathogenesis — Moderator: Jennifer Schisa — Room 2
    c. Development and Cell Biology — Moderator: Xantha Karp — Room 3

2) Peer Networking:
    a. Business meeting — Moderator: Jamie Alan — Room 1
    b. Graduate students — Moderator: Morteza Sarparast — Room 2
    c. Postdocs — Moderator: TBD — Room 3
    d. Undergrad Students — Moderator: Ben Kessler — Room 4

3) DEI (or catch up on your own):
    a. DEI — Moderator: Sing Lee — Room 1

Keynote Address

Physiological Responses to Dietary Stress and Oxygen Deprivation in *C. elegans*

Pamela A. Padilla
Professor Department of Biological Sciences
Associate Vice President for Research & Innovation
University of North Texas
President SACNAS

Central to many human health issues is a reduction in oxygen levels within tissues (e.g. stroke, cardiac or pulmonary dysfunction, ischemic events and trauma due to blood loss or suffocation). There are physiological circumstances such as hyperglycemia that can negatively impact O_2 deprivation responses. Although substantial efforts have been made to identify O_2 deprivation signaling pathways (e.g. HIF-1), the treatment for tissues exposed to O_2 deprivation and an understanding as to why certain states such as hyperglycemia exacerbates ischemic damage remains a daunting challenge. My lab and others have shown that *C. elegans* are quite tolerant to O_2 deprivation and that preconditioning environments, diet, or genetic changes (e.g. insulin-like signaling) will influence O_2 deprivation responses and survival. Of interest, my lab determined that although animals with a high storage of carbohydrates are better able to survive O_2 deprivation a glucose-supplemented diet negatively impacts O_2 deprivation survival. In addition, a glucose-supplemented diet impacts other biological processes, including developmental progression, gene expression, lipid storage, nervous system function, and the metabolic state of the organism. Furthermore, we have evidence that a parental glucose-supplemented diet will impact the phenotype of offspring fed control diet. Here we will present physiological and transcriptomic changes induced by a glucose-supplemented diet and put forward ideas of how *C. elegans* will help reveal the interplay between diet and the molecular response to environmental stress and O_2 deprivation.

Short talk schedule

11:15am-12:15pm – Parallel short talk session 1

Breakout room 1 – Moderator: Jamie Alan

- Ashley Castelloe. Cryptic Variants in Vulval Development between Nematode Species
- Anuja Dahal . ztf-16 opposes adult cell fate after dauer in Caenorhabditis elegans
- Campbell Brown. Caenorhabditis elegans adult cell fate determination is modulated by ztf-16
- Hyo Sub Choi. Defining a novel mechanism for longevity regulator flavin-containing monooxygenase-2
- Alexandra Wooldredge. Investigating the dietary restriction phenotype caused by disrupted intestinal cell-to-cell communication
- Eduardo Izquierdo. C. elegans Computational Neuroethology: Bridging the gap between connectome, neural dynamics, and behavior using computational models of the complete brain-body-environment system

Breakout room 2 – Moderator: Jennifer Schisa

- Rylee Holek. The regulation of adult cell fate by ztf-16 and lin-29
- Marshall Howington. Investigating the roles and interactions of flavin-containing monooxygenases in C. elegans longevity
- Ashley D'Amour and Neeké Busette. The role of MAP Kinase in modulating condensation of RNA binding proteins in the germ line
- Maria Schiavone and Zhaoyuan Zhang. Multiple event-based and bipedalism-inspired analysis of C. elegans locomotion
- Kin Sing Stephen Lee. The Effect of Cytochrome P450 Metabolites of Dietary Polyunsaturated Fatty Acids on Age-Associated Neurodegeneration
- Derek Vonarx. Development of a Novel Neurodegenerative Model Using C. elegans

Breakout room 3- Moderator: Xantha Karp

- Abimbola Kolawole. UNK-1 interacts with microRNA to promote seam cell multipotency during dauer
- Safa Beydoun. A FuDR alternative for long-term studies in *C. elegans*
- Julianna Escudero. Defining the molecular determinants by which EXC-4/CLICs regulate Rho-family GTPase signaling
- Bahaar Chawla. The role of Condensin IDC's ATPase function in gene regulation
- Chloe Pestrue. The CCT chaperonin selectively regulates phase transitions in the C. elegans germline
- Monica Tamrazi. Genes that slow down degeneration in dystrophic muscles

3:45pm-4:45pm – Parallel short talk session 2

Breakout room 1 – Moderator: Sing Lee

- Macy Knoblock. Determining the role of daf-16 isoforms in blocking VPC specification during quiescence in Caenorhabditis elegans

- Payton Wolbert. daf-16 blocks precocious expression of an adult cell-fate marker independently of lin-29
- Edgar Correa. The conserved transcription factor UNC-30/PITX1-3 coordinates synaptogenesis with cell identity in C. elegans GABA motor neurons
- Elham Pourmand. Quantitative profiling method for oxylipins in *C. elegans* by liquid chromatography coupled with tandem mass spectrometry
- Benjamin Kessler. Disrupting Polyunsaturated Fatty Acid Biosynthesis Modulates Lifespan and Healthspan
- Ajay Bhat. Metabolic regulation of longevity during high glucose diet by Flavin-containing monooxygenase

Breakout room 2 – Moderator: Jennifer Schisa

- Emily Erdmann. High-throughput COPAS screening for modulators of oocyte development
- Brooklynne Watkins and Katherine Sharp. Characterization of stress-induced phase transitions in the germ line
- Mohamed Elaswad. Characterizing properties of germline RNA-binding proteins that affect condensation
- Himal Roka Pun and Claudia Chabay. An RNAi Screen to Identify Factors that Enhance microRNA Activity After Dauer
- Tope Awe. Iron-handling proteins and Mechanoreceptors are required for magnetic orientation in C. elegans
- Louise Steele. Therapeutic Ultrasound's Effects on the Developing Nervous System of C. elegans

Breakout room 3- Moderator: Xantha Karp

- Laurianne Pene. daf-16 Regulates Transcription of the let-7 MicroRNA in C. elegans Dauer Larvae
- Itzel Rosas Gutierrez. Regulation of the duration of breast cancer dormancy by UNK
- Allison LaMonica and Abrielle Fretz. Aging-related genetic interventions in C. elegans maze learning
- Anthony Osuma. The PBAF chromatin remodeling complex is required for cholinergic motor neuron subtype identity
- Weidong, Feng. The C. elegans Hox gene ceh-13/labial/Hox1 controls motor neuron terminal identity

Guidelines

- Technology
    - If you get kicked out or have to leave the meeting, please rejoin using our zoom link (provided to you by email).
    - Please mute when you are not speaking
- Code of Conduct (adopted from GSA https://genetics-gsa.org/conference-policies/)
    - Interactions between participants must be respectful and harassment-free
    - Do not photograph, screenshot, or record any part of the meeting without permission
    - Live tweeting is allowed unless otherwise specified
- Info for Presenters
    - The moderators will be sharing their screens to show the slides
    - Presenter needs to request control of the computer to advance the slides and use the pointer
    - We have a tight schedule. In order to keep with our schedule, our moderators will be keeping time and we will stop sharing your slides when your time is up
- Parallel short talk sessions
    - Sessions are not organized by theme
    - Go to any session you wish – the goal is to have a good-sized audience at each
    - PIs who are undecided could consider using the following guide to pick a session:

Last name starts with	Breakout room
A-F	1
G-P	2
Q-Z	3

- Questions
    - Trainees are particularly encouraged to ask questions, and questions from trainees will be prioritized to the extent possible
    - Please type your question in the chat, or type “I have a question” in the chat, and the moderator will call on you to ask a question
    - For the parallel short talk sessions:
        - Questions for all speakers will be asked at the end of each session
        - Please specify in the chat who your question is for. The moderator will attempt to direct questions to each speaker
    - Please use the breakout rooms or private chats for additional questions that cannot be addressed due to time constraints
- Breakout rooms
    - Breakout rooms are intended to foster interaction
    - There are planned sessions (Business meeting etc.), and there will also be open breakout rooms where you can talk with anyone you wish
    - Zoom is configured so that you can enter and leave any breakout room

Long Talks

Session 1

Long isoforms of mechanoreceptor pezo-1 control pharyngeal gland cell activity in the nematode Caenorhabditis elegans

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§Contributed equally to this manuscript

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.

Soluble Epoxide Hydrolase Inhibitor, AUDA, Recuses Neurodegeneration Induced by Amyloidβ and Tau

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Abstract:
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 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.

Neuromodulation and the asynchrony between Ca2+ 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 Ca2+ dynamics in ASH, and the quantitative relationship between Ca2+ signals and depolarization amplitudes. We showed that 5-HT potentiates ASH depolarization, but surprisingly, inhibits ASH Ca2+ 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 Ca2+-dependent inhibitory feedback loop: Ca2+ inhibits ASH depolarization through SLO-1 IKCa channels, and 5-HT inhibits EGL-19 L-type Ca2+ 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 Ca2+ transients, representing another paradox: how can OA and 5-HT have opposite effects on ASH-dependent aversive behaviors, but the same effect on ASH Ca2+ transients? Our results show that 5-HT and OA utilize distinct signaling pathways in ASH (GÎ±q for 5-HT and GÎ±o for OA), and that OA does not inhibit EGL-19. We are currently testing the hypothesis that OA hyperpolarizes ASH through GÎ±o-dependent activation of IRK K+ channels. These results further emphasize that neuronal Ca2+ transients, as key reporters of neuronal depolarization, are also critical signaling intermediates in and of themselves, with multiple upstream inputs and downstream consequences.

A noncanonical role for Hox in the C. elegans ventral nerve cord

Weidong Feng, Paschalis Kratsios*

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Hox proteins constitute a phylogenetically conserved family of transcription factors with 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) â€“ 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 â€“ from developmental to adult stages â€“ in MNs of all these eight classes. Using null and auxin-inducible alleles, we observed that in all these neurons 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 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.

Long Talks

Session 2

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

Alexandra M. Socovich* and Daniel Shaye
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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.

Title: A cilia-independent function of BBSome mediated by DLK MAPK signaling in C. elegans

Authors and affiliations: Xinxing Zhang* 1,2, Jinzhi Liu1,2,3, Tong Pan1,2, Jianfeng Liu3, and X.Z. Shawn Xu1,2

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Abstract: 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.

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

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2. Department of Pediatrics, University of Cincinnati Medical School

We examined the role of cell fate in collective cell movements in the C. elegans embryo by using a time-lapse imaging approach with automated lineage reconstruction in embryos mutant for one of five major cell types. We were able to recapitulate previously described disruptions to gastrulation in end-1/end-3 intestinal fate mutants, and we 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 a partially penetrant (~50%) 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 (~550 cells). Taken together, these finding indicate that cell fate plays key roles in regulating gastrulation and ventral cleft closure. Ventral cleft closure, in particular, is a complex process that depends on intestine, muscle, skin 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.

Dietary Restriction mimetic drugs block food perception and induce FMO, a conserved regulator of stress response and metabolism

Shijiao Huang1*, Hillary A Miller2, Marshall B Howington2, Megan L Schaller1, Elizabeth S. Dean1, Angela M. Tuckowski2, Safa Beydoun1, Charles R Evans3, Scott F Leiser1,3

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Flavin-Containing Monooxygenases (FMO) are conserved xenobiotic-detoxifying enzymes. Recent studies have revealed endogenous functions of FMOs in regulating longevity in Caenorhabditis elegans and in regulating aspects of metabolism in mice. Using an C. elegans intestinal reporter for FMO induced by DR but suppressed by attractive smells, we identify three compounds that block food perception, thereby increasing longevity as DR mimetics. These compounds clearly implicate serotonin and dopamine in limiting lifespan in response to food perception by signals through the serotonin receptor 5-HT1A/ser-4 and dopamine receptor DRD2/dop-3. We find aspects of the downstream response to food perception are conserved in mammalian cells as serotonin antagonism produces inductive and pro-longevity phenotypes in mammalian cells. To explore the conserved roles of FMO in mammalian cells, we demonstrate that all five functional mammalian FMOs may play similar endogenous roles to improve resistance to a wide range of toxic stresses in both kidney and liver cells. Furthermore, FMO expression modulates cellular metabolic activity as measured by mitochondrial respiration, glycolysis, and metabolomics analyses. FMO expression augments mitochondrial respiration and significantly changes amino acid and energy metabolism pathways.

Long Talks

Session 3

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 strainsâ€”the B type OP50 versus the K12 type CS180â€”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 activityâ€”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.

daf-16/FOXO blocks adult cell fate in C. elegans dauer larvae via a branched pathway involving lin-41/TRIM71

Matthew J. Wirick(1)*, Allison Cale(1), Isaac T. Smith(1), Amelia F. Alessi(2), Margaret Starostik(2), Himani Galagali(2), Liberta Cuko(1), Kyal Lalk(1), Mikayla N. Schmidt(1), Payton Wolbert(1), Benjamin Olson(1), Kevin Ranke(1), Payton Salomon(1), Alexis Santos(1), Axel Schmitter(1), John K. Kim(2), Xantha Karp(1)

1. Central Michigan University
2. Johns Hopkins University

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.

Embryo Integrity Regulates Maternal Proteostasis and Stress Resilience

Ambre J. Sala, Laura C. Bott, Renee M. Brielmann, Richard I. Morimoto

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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. The reproductive system in particular is a critical tissue for organismal proteostasis regulation, as 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.

Short Talks

Morning Section

Breakout room 1

Cryptic Variants in Vulval Development between Nematode Species

Ashley Castelloe*, Helen Chamberlin, Adriana Dawes

Some species share near-identical phenotypes, despite last sharing a common ancestor millions of years ago. These species are called cryptic species and provide a platform to study the constrains that conserve an advantageous phenotype and prevent evolution. Two species used to explore this idea are Caenorhabditis elegans and Caenorhabditis briggsae. The vulva, or egg-laying organ, of both species passes through a stage of development when cells that can become the vulva are induced to divide. This induction relies on two canonical signaling pathways, EGF and Notch, and results in three induced cells in both species. When inductive signaling is partially inhibited, C. elegans induces no cells, whereas C. briggsae does; the cause of this difference is not known. Our goal is to understand how changes in the signaling pathways of vulval induction allow for different reactions to inductive signal inhibition, despite a common phenotype under unperturbed conditions. We hypothesize that genetic differences in vulval development are due to alternative wiring of the vulval induction networks. Three sources of difference have been proposed: the phosphatase lip-1, Hox gene lin-39 and Wnt signaling (bar-1). We are using insertions of lip-1 and lin-39 to generate worms of one species with an active gene from the alternative species. We will verify the wildtype phenotypes under normal development and look for underinduction under inductive signal inhibition. We expect to find that cross species expression will show underinduction phenotypes matching the inserted geneâ€™s species. We are using the auxin-induced degradation system to knockdown bar-1 during vulval induction. We expect that C. briggsae will show stronger underinduction under this knockdown, while C. elegans will show little to no underinduction. This work opens the door for future studies into how small evolutionary changes that occur in regulatory pathways of development shape organ development.

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.

Caenorhabditis elegans adult cell fate determination is modulated by ztf-16.

Campbell Brown*1, Mark J Hansen1, Ganesh P. Panzade2, Anna Zinovyeva2, Xantha Karp1
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Depending on environmental conditions, C. elegans can either develop continuously from embryo to adult or can undergo an interrupted development through dauer diapause. Regardless of life history, worms express stage-specific cell fates which we visualize through their lateral hypodermal seam cells. Heterochronic genes control this stage-specific cell fate expression. Mutations in heterochronic genes cause stage-specific cell fates to be skipped or reiterated during development. The phenotypes of certain heterochronic mutations are suppressed post-dauerâ€"suggesting differences in the genetic pathways which may be at work in worms with different life histories. Seam cell fate can be visualized using cell fate markers, including the adult cell fate marker col-19p::gfp. Our previous research identified the gene ztf-16 as a regulator of adult cell fate determination in C. elegans. Surprisingly, ztf-16 does not regulate col-19p::gfp expression via lin-29, the most downstream heterochronic gene and a direct activator of col-19 transcription. We used RNA sequencing to identify genes modulated by ztf-16, some of which may regulate col-19p::gfp expression. We identified 24 transcription factors whose expression changes in ztf-16(tm2127) mutants and 32 transcription factors whose expression changes in ztf-16(ma209) mutants (FDR < 0.05). Surprisingly, many genes were regulated differently in these two loss-of-function ztf-16 alleles which show identical phenotypes, suggesting some latent function in these mutant alleles. Neither of these alleles is a full deletion of ztf-16. We therefore used CRISPR to generate new ztf-16 knockout alleles and characterized their phenotype. We also show that the CRISPR generated ztf-16 deletion alleles create phenotypes which are similar to existing alleles. Future work will examine gene expression changes in the full deletion mutants.

Defining a novel mechanism for longevity regulator flavin-containing monooxygenase-2

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The expression of flavin-containing monooxygenase (fmo)-2 is required for dietary restriction- and hypoxia-mediated longevity and health benefits. Knocking out the fmo-2 gene abrogates the benefits from these interventions, while overexpressing fmo-2 (FMO-2 OE) is sufficient to confer these benefits. These results, combined with the knowledge that FMOs are functionally and structurally well-conserved, make understanding the mechanism(s) of FMO-2 a crucial next step. Using untargeted metabolomics, we find that one-carbon metabolism (OCM) is altered in FMO-2 OE animals. OCM includes the transmethylation and transsulfuration pathways, which utilize nutrient inputs that have been implicated in multiple age-associated diseases and the aging process itself. Based on this finding, we hypothesized that fmo-2 modifies OCM and that these changes are necessary for fmo-2-mediated health and longevity benefits. To test this hypothesis, we analyzed metabolomic profiles and found direct and inverse correlations between OCM metabolite abundance levels and fmo-2 gene expression level. Additionally, using RNAi knockdown of genes in the OCM network, our resulting data suggest that multiple OCM genes genetically interact with fmo-2, consistent with our hypothesis. To determine the biological implication of their interaction, we used a systems analysis, projecting our measured gene expression data on a metabolic flux model to predict the effects of fmo-2 expression on the OCM output fluxes. Our model predicts an inverse correlation between methylation flux and fmo-2 expression level, suggesting that reduction in the flux through methylation processes may be important for fmo-2-mediated lifespan extension. Taken together, our results suggest that fmo-2 plays a role in regulating OCM and that this role is crucial for fmo-2-mediated longevity and health benefits.

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, disrupting a periodic calcium wave. An abnormal inx-16 calcium wave leads to what appears to be a dietary restriction phenotype: an extended lifespan and a smaller brood size laid over a longer time. We hypothesized that inx-16 mutant worms exhibit this 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 is maintained by a sodium proton pump that is regulated by calcium flux. The interrelatedness 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 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 mutantsâ€™ failure to absorb di- and tripeptides results in several phenotypes including a reduced brood size, a shorter body length, and increased thermotolerance. We found a more than 50% reduction in brood size in both inx-16 and pept-1 single mutants, whereas the inx-16;pept-1 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 display increased thermotolerance, yet pept-1â€™s tolerance is more profound. Further, inx-16 mutants differ from wild-type worms in dipeptide uptake but still absorb dipeptide in some intestinal regions, unlike pept-1 mutants. To learn more about the differences between the mutants, we are conducting RT-qPCR analyses. Together, our results suggest that the dietary restriction phenotypes in inx-16 and pept-1 mutants are partially due to disruptions in different pathways.

Title: C. elegans Computational Neuroethology: Bridging the gap between connectome, neural dynamics, and behavior using computational models of the complete brain-body-environment system

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Abstract: One of the grand scientific challenges of this century is to understand how the brain works. In order to address this challenge, we will have to bridge the gap between the information available about the neural circuitry, the neural 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. Furthermore, it is increasingly recognized that the largest challenge we face in attempts to understand the brain is not in collecting the data; instead, what is most needed are new computational models that will allow us to understand these complex systems. So, in addition to experimental tools to map neural connectivity and to image and manipulate neural activity, such a challenge demands the development of computational models of the behaving organisms and tools of analysis to understand them. Caenorhabditis elegans is a uniquely qualified target for such an integrated modeling of a complete animal. In this talk, I will provide the current state of the art on the effort to develop a computational model of the nematode and the challenges. I will also look forward and provide an overview of the medium and long term plans of this massive computational neuroethology effort.

Morning Section

Breakout room 2

The regulation of adult cell fate by ztf-16 and lin-29

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Central Michigan University

Diapause is a period of developmental arrest occurring in response to or in anticipation of adverse environmental conditions. The mechanisms by which developmental pathways are impacted by diapause are unclear. The dauer stage in C. elegans acts as a model for diapause. C. elegans larvae that develop under unfavorable environmental conditions will enter dauer after the second larval molt. By contrast, in favorable environments, C. elegans develops continuously from embryo to adult. Lateral hypodermal seam cells divide at each larval stage and terminally differentiate at adulthood. During dauer, seam cells do not divide and their developmental progression is arrested until recovery. Differentiated adult seam cells express col-19p::gfp, a reporter for an adult-specific collagen. The LIN-29 transcription factor directly activates transcription of col-19p::gfp. Through a prior genetic screen, we found that the ztf-16 mutant exhibited precocious col-19p::gfp expression in both continuous and dauer development. Because LIN-29 directly regulates col-19 expression, we hypothesized that ztf-16 acts upstream of lin-29. Surprisingly, we found that ztf-16 mutants displayed the precocious col-19p::gfp phenotype independently of lin-29. Since ztf-16 does not appear to act upstream of lin-29, we are asking the reverse question: does lin-29 regulate ztf-16? Our approach is to study the impact of the loss of lin-29 on ztf-16 expression, using an endogenously tagged ztf-16::gfp. If the loss of lin-29 shows no change in ztf-16 expression that would indicate that ztf-16 acts in parallel with lin-29. However, a change in ztf-16 expression would suggest that lin-29 acts upstream of ztf-16, thereby regulating col-19p::gfp both directly and indirectly.

Investigating the roles and interactions of flavin-containing monooxygenases in *C. elegans* longevity

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Aging is the leading risk factor for multiple diseases and poses serious health and economic challenges as global life expectancy continues to increase. Thus, our long-term goal in biogerontology is to identify key mechanisms underlying the aging process and to develop approaches that slow aging and improve long-term health. In agreement with this, previous research has defined several genetic and environmental pathways that influence aging in C. elegans. Interestingly, our lab has recently demonstrated that the mechanisms of two well-studied longevity pathways, the dietary restriction (DR) and hypoxic-response pathways, converge on the upregulation of the nematode gene flavin-containing monooxygenase-2 (fmo-2). Tissue-specific upregulation of fmo-2 in the intestine significantly increases healthspan, stress resistance, and longevity in worms, with no obvious side effects, while loss of fmo-2 abrogates these benefits. The downstream pathways of fmo-2 are under investigation, but likely involve changes in flux through one-carbon metabolism (OCM), a nexus of nutrient signaling and metabolic synthesis that is important for other longevity pathways, such as insulin-like signaling.

From the conserved family of flavin-containing monooxygenases, C. elegans express two transmembrane FMOs (FMO-1 and FMO-4) structurally similar to FMO-2. Interestingly, each of these FMOs are also required for health benefits in multiple longevity pathways. My project focuses on these related fmo genes, and how this family of proteins influences healthy aging. My resulting data demonstrate that, similar to fmo-2, fmo-1 is required for DR-, the hypoxic-response-, and OCM-mediated longevity, while fmo-4 is required for some forms of DR-mediated longevity. Intriguingly, both fmo-1 and fmo-4 are required for fmo-2-mediated longevity and stress resistance, and fmo-4 is sufficient to improve stress resistance. These data support a model where fmo-1 and fmo-4 not only have distinct functions from fmo-2 but also function in parallel downstream of fmo-2 as part of an fmo-mediated lifespan pathway. They are also consistent with regulation of longevity and stress resistance being a general property of FMOs in C. elegans.

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

Neeké Busette*, Ashley Amour*, Grace Richmond, Mohamed T. Elaswad, Shaughna Langerak, Megan Wood, and Jennifer Schisa

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. 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 31Â°C. We observed reduced levels of anti-diP-MAPK and the assembly of CAR-1 granules in oocytes. Second, we examined the effects of decreased mpk-1 expression on CAR-1 protein condensation, using 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 25Â°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::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.

Multiple event-based and bipedalism-inspired analysis of C. elegans locomotion

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C. elegans are a well-established model organism in numerous fields of experimental biology, such as the biology of aging, neurobiology, and developmental biology. Locomotion is especially of interest for C. elegans, as it constitutes a reliable readout of their physiological and overall status, including aging. Here we focus on C. elegans cultured on NGM (Nematode Growth Medium) plates, where they crawl on 2-dimensional surfaces, engaging in undulatory (â€œsinusoidalâ€) locomotion. The aim of our work is to unveil manifestations of aging in C. elegans
locomotion that go beyond the traditional locomotion analysis. To this end, by employing an open-source tracker, we are developing a pirouette diagnostic tool, based on the sequence of events that mark pirouette performance, i.e., reversal, coiling, and eventually changing motion direction. Preliminary results show that individual pirouette events differ between young and aged animals, with respect to their frequency and magnitude of constituent events. In parallel, we adapt established motor performance indices that are commonly used for locomotion evaluation of bipedal animals, e.g., humans, and we tailor them for use in the analysis of young and aged C. elegans locomotion. The list includes features like gait events that mark individual locomotion cycles, cadence, stride length, stride time, period, Froude number, Froude number range, spatial asymmetry, and temporal asymmetry. Overall, this transdisciplinary approach of analyzing locomotion has the potential to reveal interesting aging-related dynamics that have remained uncharacterized. Our dual goal is to shed light on aspects of locomotive aging that are commonly manifested across species and to further dissect pirouettes, a special C. elegans locomotive feature. By mapping the aging-driven changes to specific genes, we aspire to set the stage for further investigation of the underpinning physiological mechanisms.

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 Alzheimerâ€™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 Parkinsonâ€™s and Alzheimerâ€™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-Î² or tau, which are similar to what has been found in the murine Alzheimerâ€™s disease amyloidosis or tauopathy models. We also demonstrated that a specific Ï‰-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.

Development of a Novel Neurodegenerative Model Using C. elegans

Derek Vonarx*, Morteza Sarparastzardoudi, Benjamin Kessler, Devon Dattmore, Min-Hao Kuo, Jamie K. Alan, Kin Sing Stephen Lee

Alzheimerâ€™s disease (AD) is a neurodegenerative disease that affects over 30 million globally (1). Currently, there is no cure for AD and only limited treatment options. Therefore, a better understanding of the key pathological players will lead to better treatments options and hopefully a cure. One of the major biomarkers of AD are neurofibrillary tangles of fibrillary hyperphosphorylated tau (p-tau). The spatiotemporal distribution of p-tau correlates with cognitive impairment (2). Therefore, p-tau may be a specific target for disease prevention and treatment. To further assess and understand the role of p-tau, a bacterial strain of E. coli has been developed that produces human p-tau (2,3). A modified Fos/Jun complex allows for the GSK-3Î² kinase to phosphorylate tau efficiently in the bacteria, producing human p-tau (3). We hypothesize that feeding delivery can be used to induce neurodegeneration in C. elegans. Three different bacterial strains were used: the vector control, a strain that expresses wild-type tau, and a strain that expresses p-tau. Wild-type N2 C. elegans were fed each bacterial strain independently, and their physical and neuronal fitness were assessed by a thrashing assay. C. elegans fed the tau or p-tau strain thrash significantly less when compared to the vector control at day 5 and older. Further studies will be aimed at addressing the mechanism that underlies this change.

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Morning Section

Breakout Room 3

UNK-1 interacts with microRNA to promote seam cell multipotency during dauer

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Many tissue-specific stem cells maintain multipotency during lengthy periods of quiescence, or nondivision; however, the mechanims by which multipotency is maintained during quiescence are unclear. In favorable conditions, C. elegans larvae undergo continuous development from embryo to adult, while in unfavorable conditions, C. elegans larvae can enter the dauer stage after the second larval molt. During dauer, progenitor cells are quiescent and maintain multipotency. For example, lateral hypodermal seam cells are progenitor cells that divide at each larval stage but remain quiescent during dauer. At adulthood, seam cells terminally differentiate. Hence, there are larval cell fates, and an adult cell fate. Adult fate can be visualized with the col-19p::gfp adult cell fate marker. The transition from larval to adult cell fate is regulated by heterochronic genes. Heterochronic transcription factors and RNA-binding proteins that promote early cell fate are downregulated by microRNAs to allow progression to later cell fates. Interestingly, some heterochronic genes that function during continuous development are dispensable after dauer, indicating that the larval vs. adult cell fate decision is regulated differently in continuous and dauer development. In prior work, we found that unk-1 is required to block adult cell fate during dauer. Notably, unk-1 null mutant dauer larvae express col-19p::gfp. To test whether microRNAs contribute to this phenotype, we examined col-19p::gfp expression in unk-1(0) dauer larvae that lack three microRNAs in the let-7 family, mir-48, mir-84, and mir-241. The loss of these three microRNAs caused a statistically significant but moderate suppression of col-19p::gfp expression. These three microRNAs control the transition from L2 to L3 cell fate, whereas let-7 itself controls a later cell fate transition. Current experiments aim to determine the extent to which let-7 plays a role in the regulation of col-19p::gfp expression. Future investigation will determine the mechanisms by which unk-1 regulates microRNA activity to block adult cell fate during dauer.

A FuDR alternative for long-term studies in *C. elegans.*

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Abstract. *Caenorhabditis elegans* has been used as an instrumental model in aging research due to its large brood size and short lifespan. However, its quick rate of reproduction makes maintaining a synchronous nematode population for longevity studies challenging and time consuming. Multiple methods have been employed in the field ranging from the use of worm strains with temperature sensitive sterility to the use of inhibitors of DNA replication such as 5'-fluorodeoxyuridine (FuDR). In this study, we characterize a small molecule (c22) that impairs eggshell integrity and disrupts early embryogenesis to determine its use as a possible FuDR alternative. We find that c22 prevents egg hatching in a concentration and worm density dependent manner. It extends lifespan of N2 worms fed live but not killed OP50 and it induces FMO-2, a longevity regulating enzyme downstream of two longevity pathways, dietary restriction and hypoxia. Our results suggest that c22 is unlikely to be useful as an alternative to FuDR but may be worth further investigation into its mechanism for lifespan extension.

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.

The role of Condensin IDC's ATPase function in gene regulation

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Condensin IDC, a five-subunit protein complex, is one of the key players in C. elegans dosage compensation, the process that equalizes gene expression between biological sexes. Like the canonical mitotic condensins, it consists of two SMC (structural maintenance of chromosome) ATPase proteins and a set of non-SMC regulatory subunits. Work on the canonical condensins has shown that ATP hydrolysis is required for condensins to move along the chromosomes. However, the exact contribution of the ATPase activity within condensin IDC's role in dosage compensation is not known.

Our study aims to understand the role of condensin IDC's ATPase function by generating dpy-27 mutants that will disrupt ATPase function and evaluating the effect on X chromosome structure, gene regulation, and other functions of the dosage compensation complex (DCC) in those mutants. These studies will help us understand if condensin IDC is a true motor like the other condensins or if it only serves as a scaffold for the members of the DCC.

We generated one mutation in condensin IDC subunit dpy-27 through CRISPR which disrupts ATP hydrolysis by mutating the essential glutamate residue in the Walker B motif to a glutamine. Using this mutant, we have found that the subunits of condensin IDC localize with each other via immunofluorescence (IF), but they are present throughout the whole nucleus, rather than localized to the X chromosome. To understand how mutating DPY-27 affects other members of the DCC, we assayed for H4K20me1 which is enriched on the X chromosome by DPY-21, another member of the DCC. In dpy-27 mutant nuclei, there is no enrichment of H4K20me1 on the X chromosome as shown by IF.

Our preliminary results suggest that losing the ATPase function of condensin IDC causes loss of the DCC from the X chromosome and therefore lack of gene regulation, resulting in a maternal effect lethal phenotype.

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

Authors:
Chloe Pestrue, Elizabeth Breton, Brooklynne Watkins, Mohamed Elaswad, Katherine Sharp, and Jennifer Schisa

When phase transitions of proteins are dysregulated, proteins may 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 phase transitions is incompletely 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 examine the role of CCT in regulating phase transitions of RNA-binding proteins in different developmental contexts. We first used RNA interference (RNAi) to knockdown the expression of cct subunits in fog-2 females. In meiotically-arrested oocytes, 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. 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 leads to aggregation of a subset of RNA-binding proteins. Possible models include CAR-1 and CGH-1 as substrates directly folded by CCT, or an indirect regulator of RNA-binding protein condensation may be a CCT substrate.

Genes that slow down degeneration in dystrophic muscles

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Duchenne muscular dystrophy (DMD) is a degenerative muscular disorder that affects 1 in 3,500 males and is characterized by progressive muscle weakness, loss of ambulation, and premature death. DMD is caused by an absence of the dystrophin protein. Dystrophin connects the actin cytoskeleton to the extracellular matrix, which stabilizes the sarcolemma during muscle contraction. Other hallmarks of this disease include elevated calcium levels, oxidative stress, and mitochondrial damage. It is not precisely understood how the loss of dystrophin affects the molecular mechanisms that lead to degeneration. We are investigating gene expression in dystrophic C. elegans that genetically model Duchenne muscular dystrophy through mutations in the worm dystrophin homolog (dys-1). Under high exertion exercise, the dys-1(eg33) strain of dystrophic worms recapitulates the most severe features of DMD. However, a dystrophic strain with a similarly missense mutation near the eg33 loci (dys-1(cx18)), displays a significantly less severe phenotype. RNA seq data from muscle specific tissue identified several genes that have differential expression between these two dystrophic strains. We have identified differentially expressed genes with known roles in calcium handling, muscle contractile ability, and mitochondrial function. We are using RNA interference as a screen to identify genes that decrease calcium levels in the muscle of dys-1(eg33) animals. Genes that show decreased calcium levels when silenced will then be further studied to see how this manipulation impacts locomotor ability and longevity. This approach can help identify therapeutic targets, which can be used to improve the quality of life for Duchenne patients.

Afternoon Section

Breakout room 1

Determining the role of daf-16 isoforms in blocking VPC specification during quiescence in Caenorhabditis elegans

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Central Michigan University

In order to function properly, adult stem cells must maintain multipotency, the ability to give rise to multiple cell types, during periods of quiescence. In favorable conditions, Caenorhabditis elegans develops continuously through four larval stages to adulthood. In response to adverse environmental conditions, C. elegans can enter a temporarily quiescent stage as dauer larvae. During this stage, progenitor cells must maintain quiescence and multipotency. The evolutionarily conserved FOXO transcription factor is a candidate for maintaining multipotency during quiescence. daf-16, the sole FOXO ortholog in C. elegans, promotes dauer formation, longevity, and stress resistance. We can use vulval precursor cells (VPCs) in C. elegans as a stem cell model to determine the role of FOXO in maintaining multipotency during quiescence. Six VPCs are born in the L1 stage and remain multipotent until L3, when signal transduction pathways specify the VPCs to adopt one of three cell fates: 1Â°, 2Â°, or 3Â°. If larvae enter dauer, this specification is delayed until they have recovered. 1Â° VPC specification is mediated by EGFR/Ras signaling and can be visualized using the 1Â° cell fate marker lag-2p::yfp. daf-16 is required to block VPC specification during dauer, but the mechanism by which daf-16 regulates 1Ëš cell fate specification is unknown. The daf-16 gene encodes three major isoforms, daf-16a, daf-16b, and daf-16f, each with its own promoter. Isoforms a and f are thought to be the primary regulators of dauer formation, longevity, and stress-resistance. In contrast, work in our lab has shown that all three isoforms act to oppose adult cell fate in seam cells during dauer. To determine which isoforms of daf-16 are important in blocking VPC specification during dauer, we can use isoform-specific mutants then score for lag-2p::yfp expression. We found that loss of either daf-16a or daf-16f caused a minor increase in lag-2p::yfp expression compared to control dauers. However, the effect of simultaneous loss of daf-16a and daf-16f is still unclear. Ongoing experiments are attempting to resolve this question. We expect that the results of these experiments will allow us to determine the relative role of the different daf-16 isoforms in maintaining VPC multipotency during quiescence.

daf-16 blocks precocious expression of an adult cell-fate marker independently of lin-29

Payton A. Wolbert*, Matthew J. Wirick, Xantha Karp

Central Michigan University

Stem cells have the unique ability to differentiate and self-renew. Multipotency of our human stem cells is maintained during quiescence, a period of cell cycle arrest. We study an analogous cell type to human stem cells, C. elegans seam cells, to understand how multipotency is maintained during quiescence. Larval seam cells are multipotent whereas adult seam cells are differentiated and express the adult cell fate marker, col-19p::gfp. In unfavorable conditions, L2 larvae molt into the quiescent and stress-resistant dauer stage. Forkheadbox-O (FOXO) proteins are conserved transcription factors that promote stem cell maintenance, stress resistance, and quiescence. The FOXO ortholog in C. elegans, daf-16, promotes dauer formation. We find that daf-16(0) mutants express precocious col-19p::gfp during dauer. The LIN-29 transcription factor is the most downstream regulator of adult cell fate and binds to the col-19 promoter to directly activate transcription. Therefore, we hypothesized that daf-16 would act upstream of lin-29 to regulate col-19p::gfp. To test this hypothesis, previous Karp lab members examined the effect of loss of lin-29 on the col-19p::gfp phenotype of daf-16(0) dauer larvae. These experiments utilized lin-29(n546), which is a nonsense mutation. Surprisingly, the loss of lin-29 did not appear to affect precious col-19p::gfp expression. This suggests that the typical activator of col-19 is not working strongly during dauer. Although lin-29(n546) is the canonical null allele for lin-29, it is possible that there may still have been some protein expressed. To rule out this possibility, we obtained a deletion allele from the GroÃŸhans lab that completely knocks out lin-29, lin-29(xe37). Preliminary data show that col-19p::gfp is still expressed in the daf-16(0); lin-29(xe37) dauers, consistent with our prior data. Ongoing analysis will determine whether there is any difference in levels of GFP expression between lin-29(xe37) and lin-29(+) dauer larvae. Nonetheless, these data demonstrate that daf-16 regulates col-19p::gfp expression at least partially independently of lin-29, despite lin-29 being a direct activator of adult-specific cell fate.

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.

Quantitative profiling method for oxylipins in *C. elelgans* by liquid chromatography coupled with tandem mass spectrometry

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The endogenous levels of specific cytochrome P450 (CYP) polyunsaturated fatty acids (PUFAs) metabolites correlate with the disease progression in many animal models, including diabetes, neuropathy, hypertension, cancer, and neurodegenerative diseases. (ref) The risk of the diseases mentioned above increases with aging. Therefore, in this study, we will specifically determine the effect of aging on the CYP metabolism of PUFAs and investigate the relationship between CYP PUFA metabolites' levels and age-associated neuronal functional decline. We hypothesize that aging affects CYP PUFA metabolism.

In this regard, we will use *C. elegans* as a model organism in our study owing to its long history of being used to investigate aging. The corresponding findings have been translated to mammals and humans, like the inference of insulin signaling and calorie restriction in aging. Furthermore, the short half-life, the ease of handling, and vast genetic tools availability greatly facilitate the aging study in *C. elegans*. Also, the aging pathways and neuronal structure in *C. elegans* are conserved in humans. (ref)

In this study, we will develop analytical tools to measure the endogenous levels of CYP PUFA metabolites over the lifespan of *C. elegans* using state-of-the-art ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). These metabolites are very potent but present in low abundance. The dramatic increase in sensitivity in UPLC-MS/MS allows us to monitor these metabolites over the lifespan of C. elegans with minimum samples. In this presentation, we will show that *C. elegans* also produce very similar classes of CYP PUFA metabolites as mammals and humans using our preliminary SPE-UPLC-MS/MS method. We will also report that some CYP PUFA metabolites correlate with the lifespan and/or neuronal health of *C. elegans*. The results from this project will significantly improve our understanding of the role of dietary PUFAs and associated metabolism on aging and neurodegeneration and will uncover new mechanisms of how aging affects neurodegeneration through modulation of PUFA metabolic pathways.

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Quantitative profiling method for oxylipin metabolome by liquid chromatography electrospray ionization tandem mass spectrometry

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.

Metabolic regulation of longevity during high glucose diet by Flavin-containing monooxygenase

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Metabolic re-wiring due to changes in the composition or quantity of diet can influence health and lifespan. Dietary restriction (DR), the reduction of nutrient intake without malnutrition, improves health and longevity across multiple species. Conversely, intake of diet rich in sugar and fat is known to exacerbate age-related diseases and shorten lifespan. DR mediated longevity has been vastly studied across different model systems, however, there is limited knowledge of how excess nutrients regulate aging. We previously found that DR in C. elegans leads to induction of a family of xenobiotic metabolizing enzymes, flavin-containing monooxygenases, that are both necessary and sufficient to improve health and longevity. Interestingly, mammalian FMOs can influence carbohydrate and lipid metabolism and their expression is altered in human diabetic patients and rodent models of diabetes. We now show that while addition of high glucose (HG) to media shortens wild type worm lifespan, overexpression of FMO-2 rescues this negative effect of HG on longevity. Metabolic profiling shows that overexpression of FMO-2 significantly alters one-carbon metabolism (OCM), leading to decreased methylation flux. Further genetic studies reveal that enzymes in different metabolic nodes of OCM interact with FMO-2 in regulating longevity during regular and high glucose diet. Based on our preliminary data we propose that the FMO-2-mediated protective effect against HG acts via re-wiring OCM metabolism which leads to decreased methylation flux and subsequently alters downstream metabolism.

Afternoon Section

Breakout room 2

Title: High-throughput COPAS screening for modulators of oocyte development

Authors: Emily A. Erdmann*, Olivia Abraham, Melanie Forbes, Margaret Holdeman, Heather A. Hundley

Abstract: ADARs (Adenosine DeAminases that act on RNA) are a class of RNA binding protein that can catalyze the deamination of adenosine to inosine, known as A-to-I RNA editing. ADARs can alter gene expression either by competing for binding sites with other RNA binding proteins or by editing. ADARs are present in all animals and have been shown to play important roles in development, innate immunity, and oncogenesis. C. elegans is a useful model system for studying ADARs, as knockout animals are viable. While ADAR knockout worms show mild defects such as changes in chemotaxis behavior and lifespan, the lack of obvious and easily measurable ADAR phenotypes has made it difficult to identify factors that genetically interact with ADARs. Here, we describe a phenotype resulting from the expression of a vitellogenin::green fluorescent protein (GFP) construct that is easily quantifiable using a COPAS Biosort instrument that determines both developmental stage and GFP expression of intact worms. We show that this phenotype is rescued when ADR-2, the functional deaminase in C. elegans, is lost. We have used this phenotype in a high-throughput, RNAi-based screen to identify RNA binding proteins that genetically interact with ADARs.

Characterization of stress-induced phase transitions in the germ line

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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, induces 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. To begin to characterize the imaging stress, we performed a time course study that revealed that DAF-16 trans-locates to nuclei within 20 minutes of a worm being placed on an agarose pad. We are currently testing additional stress reporters to further characterize the type of stress that is occurring during extended imaging. We are also investigating whether the stress affects transcriptional activity. Taken together, we conclude that this inadvertent imaging stress differentially affects the condensation of RNA-binding proteins in the germline. Interestingly, the opposing effects on MEX-3/CGH-1 condensation and PGL-1/GLH-1 condensation are also observed in response to heat stress. Our results suggest that researchers studying RBP condensates need to be cautious and rigorous in their imaging methods to avoid confounding factors that can impact investigations of condensation.

Characterizing properties of germline RNA-binding proteins that affect condensation

Authors who contributed to work (not in a particular order):
Mohamed T. Elaswad*, Brooklynne Watkins, Katherine Sharp, and Jennifer A. Schisa

In the C. elegans germline, stresses such as extended meiotic arrest can induce the condensation of several RNA-binding proteins (RBPs) into large ribonucleoprotein (RNP) granules in oocytes. The RNP granules include P-granule proteins, P-body proteins, and stress granule proteins and are hypothesized to maintain oocyte quality by regulating RNA metabolism. In this study, we are characterizing the properties of RBPs in meiotically-arrested oocytes. First, to determine if hydrophobic interactions are required for condensation of CGH-1, MEX-3, or PGL-1 into large RNPs, we performed hexanediol assays in fog-2 females. Hexanediol (HD) disrupts hydrophobic interactions and has been used to categorize liquid-like and gel-like RBPs. Our preliminary results indicate that CGH-1 and MEX-3 do not require hydrophobic interactions to condense as the granules appear insensitive to HD; however, the granules are sensitive to SDS, suggesting that they are not insoluble aggregates. We are currently investigating the effects of HD on PGL-1. In non-arrested oocytes, PGL-1 granules disperse in response to HD; therefore, it will be intriguing to see if PGL-1 sensitivity to HD is altered when complexed in large RNP granules. Second, we investigated the effects of heat stress on RBP condensation. We find that while heat promotes MEX-3 condensation, heat promotes dispersal of PGL-1, suggesting that PGL-1 is more liquid-like than MEX-3. We are currently testing CGH-1 sensitivity to heat. Lastly, we are conducting FRAP experiments to assess the mobility of RBPs within large RNP granules. Taken together, we hope our studies of RBP properties will provide insight into the basis for their differential responses to stress. We also hope to determine if the large RNP granules in arrested oocytes contain multiple phases, as has been observed for P granules in embryos.

An RNAi Screen to Identify Factors that Enhance microRNA Activity After Dauer.

Himal Roka Pun*, Claudia Chabay*, Xantha Karp

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.

Iron-handling proteins and Mechanoreceptors are required for magnetic orientation in C. elegans

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Many species of organisms can sense and orient to the earthâ€™s magnetic field. Little is known about the molecular and cellular mechanisms responsible for magnetoreception. One hypothesized mechanism involves biogenic magnetic particles exerting force on adjacent mechanoreceptors when pulled by the force of the earthâ€™s magnetic field. There is evidence for biogenic magnetic particles in the tissues of magnetotaxing animals, including C. elegans. The AFD neuron play an important role in the magnetotactic behavior of C. elegans. However, it is unknow if the magnetosensitivity of the AFD neurons depends on a magnetite-mechanoreceptor based mechanism. We used RNA interference to silence the expression of known mechanoreceptors and iron-handling genes and identified mechanoreceptors and iron-handling proteins are required for magnetic orientation by C. elegans. To determine if and how these proteins are involved in magnetic transduction I will determine their cellular and subcellular expression patterns. These results will help us understand how AFD neurons sense magnetic stimuli and could potentially help better understand the mechanism for magnetoreception in other animals.

Therapeutic Ultrasound 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 â€œbioeffectsâ€ 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.

Afternoon Section

Breakout room 3

daf-16 Regulates Transcription of the let-7 MicroRNA in C. elegans Dauer Larvae

Laurianne Pene*, Matthew J. Wirick, Xantha Karp

Central Michigan University

Tissue-specific stem cells self-renew and differentiate to replenish tissues as needed. Stem cells maintain multipotency during periods of nondivision, or quiescence. Caenorhabditis elegans can serve as a model organism to study this phenomenon. Under unfavorable conditions, C. elegans larvae can enter dauer after the second larval molt. Dauer can last for months during which time progenitor cells maintain multipotency. The DAF-16 transcription factor promotes dauer formation and also blocks cell fate specification to maintain multipotent fate in vulval precursor cells during dauer. Recent work in our lab has shown that daf-16 also blocks adult cell fate in lateral hypodermal seam cells. Adult cell fate and differentiation are promoted by the let-7 microRNA which therefore opposes multipotency. We found that expression of the mature let-7 microRNA is increased in daf-16(0) mutants. Levels of mature microRNA may be affected by regulation at any step of biogenesis or by regulation of microRNA stability. However, the simplest hypothesis is that this regulation is transcriptional given that DAF-16 is a transcription factor. Expression of a let-7p::gfp transcriptional reporter was used to test this hypothesis. We found that let-7p::gfp expression was significantly higher in daf-16(RNAi) dauer larvae compared to lacZ(RNAi) controls. Therefore, let-7 transcription is regulated by daf-16, either directly or indirectly. Given that daf-16 and let-7 are conserved in mammals, it will be interesting to see if this regulation occurs in mammalian stem cells.

Regulation of the duration of breast cancer dormancy by UNK

Itzel Rosas Gutierrez*, D. Stave Kohtz, Xantha Karp

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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.

Aging-related genetic interventions in C. elegans maze learning

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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 recently demonstrated 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 showed that C. elegans young adults locate food in T-shaped mazes and, following that experience, learn to reach a specific maze arm, in a newly characterized food-triggered multisensory behavior. Here we build on our findings that reveal an aging-related decline in C. elegans maze learning ability, which can still be reversed at mid-age by 24hr food deprivation. We explore the effect of genetic interventions that extend lifespan either by inducing dietary restriction (eat-2) or by interfering with the insulin signaling pathway (daf-2), and we find that both have a significant impact on C. elegans performance in the maze environment. In addition, we investigate the impact of pan-neuronal expression of APL-1, the C. elegans ortholog of human APP (amyloid precursor protein), from the cleavage of which the Î²-amyloid peptide is produced, a major component of the senile plaques which are formed in the brains of patients with Alzheimerâ€™s disease. Combined, our findings indicate that maze learning ability is strongly affected by aging-related physiological changes, some of which are reversible by environmental and genetic interventions.

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.

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.