Chicago AREA WORM MEETING

3rd ChAWM

Friday December 3rd, 2021. Noon – 5PM

Virtual Meeting

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Opening Remarks and Keynote (12:00 - 1:00PM)

Opening Remarks

Paschalis Kratsios. Dept. of Neurobiology. *University of Chicago.*

Neuron-glia interactions in olfactory neuron diversification

Chiou-Fen Chuang. Dept. of Biological Sciences. *University of Illinois - Chicago.*

Break (1:00 - 1:15PM)

Sponsor Presentation: Alisha John, Research Technology Specialist (*MilliporeSigma*)

Session 1

Full Talks 1 (1:15 - 2:15PM)

Chair: Jayson Smith (Kratsios Lab, *University of Chicago*)

1.- A metastable protein conformational switch reveals cell state transitions in aging and stress.
Laura Bott, Ambre Sala, Renee Brielmann, Richard Morimoto. *Northwestern University*.

2.- A non-canonical role of the *C. elegans* Hox gene *lin-39* as a terminal selector of cholinergic motor neuron identity. Weidong Feng, Honorine Destain, Paschalis Kratsios. *University of Chicago.*

3.- The kinase *pig-1/MELK* is a conserved regulator of tubulogenesis in *C. elegans* and of vertebrate angiogenesis. Alexandra Socovich, Dan Shaye. *University of Illinois - Chicago*.

4.- Dose-response and quantitative genetic analyses reveal complex patterns of natural variation in susceptibility to diverse toxicants. Samuel Widmayer, Timothy Crombie, Janneke Wit, James Collins, Sophia Gibson, and Erik Andersen. *Northwestern University*.

Flash Talks 1 (2:15 - 2:30PM)

Chair: Cindy Voisine (*Northeastern Illinois University*)

5.- FSHR-1 and its candidate ligand FLR-2 regulate neuromuscular signaling balance.
Letitia Bortey, Jennifer Kowalski. *Butler University*.

6.- Lack of canonical beta-tubulin resistance alleles in two benzimidazole-resistant ascarid species.
JB Collins, Lewis Stevens, Robyn Tanny, Abhinaya Venkatesan, John Gilleard, Ray Kaplan, Erik Andersen. *Northwestern University*.

7.- Determining CLIC domains that mediate function in Rho/Rac signaling.
Julianna Escudero, Anthony Arena, Dan Shaye. *University of Illinois - Chicago*.

8.- Understanding the early pathophysiology of Duchenne muscular dystrophy in a nematode model.
Kiley Hughes, Brittany Rivera, Emily Killian, Peyton Carlock, Hannah Ahmed, Nick Leonard, Andres Vidal-Gadea. *Illinois State University*.

Break (2:30 - 2:45PM)

Sponsor Presentation: Jamie Scott, Key Account Manager (*ZEISS Research Microscopy Solutions)*

Session 2

Full Talks 2 (2:45 - 3:45PM)

Chair: Ambre Sala (Morimoto Lab, *Northwestern University*)

9.- Two intrinsic timing mechanisms set start and end times for dendritic arborization of a nociceptive neuron.
Nobuko Suzuki, Yan Zou, Meiyu Shao, Wei Zou, Kang Shen, Chieh Chang. *University of Illinois - Chicago*.

10.- RNA helicase RHA-1 safeguards thermosensitive sperm fertility by promoting small RNA-mediated mRNA clearance. Olivia Gaylord, Jordan Brown, Wei-Sheng Wu, Heng-Chi Lee. *University of Chicago*.

11.- Temperature-stressed *C. elegans* males prioritize food over mating resulting in sterility.
Nicholas Sepulveda, Lisa Petrella. *Marquette University*.

12.- Negative regulation of Raf signaling by the E3/E4 ubiquitin ligase UFD-2.
Robert Townley, Augustin Deniaud, Claudia Rodriguez, Kennedy Stacy, Claire de la Cova. *University of Wisconsin - Milwaukee*.

Flash Talks 2 (3:45 - 4:00PM)

Chair: Erik Andersen (*Northwestern University*)

13.- Germ granules ensure the fidelity of piRNA-mediated transcriptome surveillance in *C. elegans*
Jordan Brown, Wenjun Chen, Wei-Sheng Wu, Tao He, Shikui Tu, Ziping Weng, Donglei Zhang, Heng-Chi Lee. *University of Chicago*.

14.- C48B6.3 - A novel modifier of T-box factor TBX-2 activity.
Akshaya Rajaraman, Mirna Vazquez, Taylor Cairns, Peter Okkema. *University of Illinois - Chicago*.

15.- Physical constraints on cuticle stretch may guide *C. elegans* developmental trajectories.
Joy Nyaanga, Gaotian Zhang, Sasha Shirman, Christina Goss, Niall Mangan, Erik Andersen. *Northwestern University*.

16.- Forward Genetic Screening for Genes Responsible for UNC-2 Regulation in *C. elegans.*
Ame Xiong, Kelly Oh, Hongkyun Kim. *Rosalind Franklin University*.

Break (4:00 - 4:15PM)

Sponsor Presentation: Felicia Roland, Account Manager – Life Science Division (*Leica Microsystems*)

Session 3

Flash Talks 3 (4:15 - 4:30PM)

Chair: Heng-Chi Lee (*University of Chicago*)

17.- Defining the function of EXC-4/CLIC in Gα-Rac signaling using TurboID to identify physical interactors.
Anthony Arena, Dan Shaye. *University of Illinois - Chicago.*

18.- Harnessing phenotypic plasticity to understand the cell biology of neuron aging.
Callista Yee, Sebastian, Sanchez-Luege, Kang, Shen, Claire Richardson. *University of Wisconsin - Madison*

19.- Generation of *in vivo* humanized nematode and *in vitro* human myocyte culture systems for the study of muscular dystrophy. Brittany Rivera, Adams J, Hughes E, Salwilchik E, Aidoo E, Gantert J, Carlock P, Vidal-Gadea AG. *Illinois State University*.

20.- The genetic architectures of gene expression variation in wild *C. elegans*.
Gaotian Zhang, Nicole Roberto, Daehan Lee, Steffen Hahnel, Erik Andersen. *Northwestern University*.

Break (4:30 - 4:40PM)

Flash Talks 4 (4:40 - 4:55PM)

Chair: Dan Shaye (*University of Illinois at Chicago*)

21.- Resolving between two models of magnetic particle-based mechanisms of magnet stimulus transduction in *C. elegans*. Tope Awe, Chance Bainbridge, Ploy Freebairn, Andres Vidal-Gadea. *Illinois State University*

22.- Investigating the neuronal membrane glycoprotein 1 role using *Caenorhabditis elegans*.
Eliana Mailen Fernandez, Yamila Cutraro, Melisa Monteleone, Kiley Hughes, Andres Vidal-Gadea, Marcela Brocco. *National University of San Martin (UNSAM). Argentina*.

23.- Identifying Tissue-specific Susceptibility of *ben-1* to Benzimidazoles.
Sophia Gibson, Erik Andersen. *Northwestern University*.

24.- A quantitative ERK biosensor as a tool to understand FGFR signaling in *C. elegans*.
Claudia Sofia Rodriguez, Melissa Garcia Montes, Te-Wen Lo, Cindy Voisine, Michael Stern, Claire de la Cova. *University of Wisconsin - Milwaukee*.

Closing Remarks (4:55 - 5:00PM)

Dan Shaye. Dept. of Physiology and Biophysics. *University of Illinois at Chicago.*

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ABSTRACTS

1) A metastable protein conformational switch reveals cell state transitions in aging and stress.

Laura Bott, Ambre Sala, Renee Brielmann, Richard Morimoto. *Northwestern University*.

The proteostasis network preserves proteome integrity in all cells and limits the formation of non-native protein species. Decline of this network in aging and chronic stress leads to the accumulation of misfolded and aggregated proteins, a hallmark of many pathological states. To gain insight into the dynamics of the decline, we engineered a protein switch system for probing the cellular folding environment in the model organism *Caenorhabditis elegans*. The protein switch is based on metastable variants of dihydrofolate reductase (DHFR) fused to a conditional proteasome-targeting signal, thereby linking protein levels to folding status inside cells. It visualizes a destabilized conformation of DHFR that is distinct from the folded or terminally misfolded protein. We tracked the spatiotemporal dynamics of the DHFR-based switch during aging and observed a gradual transition from the folded to the destabilized state in adulthood. The rate of conversion correlated with aging rates in individuals. Our results demonstrate that this conformational switch is suitable for in vivo investigations into the cellular folding environment in health and disease states.

2) A non-canonical role of the *C. elegans* Hox gene *lin-39* as a terminal selector of cholinergic motor neuron identity.

Weidong Feng, Honorine Destain, Paschalis Kratsios. *University of Chicago.*

Hox genes encode phylogenetically conserved transcription factors known for their early roles in patterning animal embryos along the anteroposterior axis. Our understanding of Hox gene function during post-embryonic stages, however, remains rudimentary. To bridge this gap, we study the *C. elegans* ventral nerve cord (VNC). In hermaphrodites, the VNC is populated by eight different subtypes of motor neurons (MNs) – two are GABAergic and six are cholinergic. By generating an endogenous reporter allele for the mid-body Hox gene *lin-39* (Scr/Dfd/Hox4-5), we found that it is continuously expressed – from developmental to adult stages – in all GABAergic and cholinergic MN subtypes of the VNC. Interestingly, *lin-39* expression in MNs is maintained via direct transcriptional autoregulation. Using null and auxin-inducible alleles, we observed that *lin-39* is required not only to establish, but also maintain in the adult the expression of effector molecules that define cholinergic MN terminal identity, such as neurotransmitter biosynthesis proteins, ion channels and adhesion molecules. Mechanistically, LIN-39 works together with UNC-3/Ebf, a previously described terminal selector for cholinergic MN identity. Our biochemical analysis supports a model where UNC-3 and LIN-39 bind directly at distinct cis-regulatory motifs upstream of effector genes. Furthermore, we uncovered a cross-regulatory relationship between UNC-3 and LIN-39; LIN-39 activates the expression of unc-3, whereas UNC-3 in turn inhibits the expression of lin-39. The latter inhibitory event helps keep lin-39 levels in check, and not be increased out of control due to the aforementioned *lin-39* positive autoregulation. We conclude that the Hox protein LIN-39 and UNC-3 function as terminal selectors of VNC cholinergic motor neuron identity.

3) The kinase *pig-1/MELK* is a conserved regulator of tubulogenesis in *C. elegans* and of vertebrate angiogenesis.

Alexandra Socovich, Dan Shaye. *University of Illinois - Chicago*.

Biological tube formation, or tubulogenesis, is a complex process that plays an integral role in vascular development and physiology. Kinases are key regulators of most cellular functions and signal transduction pathways, and their well-defined mechanism of action makes them attractive targets for therapeutic development. Therefore, finding kinase regulators of vascular tubulogenesis is a promising translational research approach. The *C. elegans* excretory canal (ExCa), a large unicellular tube, provides a tractable model of tubulogenesis, and vertebrate orthologs of several genes that regulate ExCa tubulogenesis are involved in vascular development and disease. We identified PIG-1 in an RNAi screen for conserved kinases whose loss affected ExCa tubulogenesis, and found that the vertebrate ortholog, MELK, is expressed in human umbilical vein endothelial cells (HUVEC). Furthermore, MELK is required for cell migration and formation of angiogenic sprouts, suggesting that PIG-1/MELK is a conserved regulator of tubulogenesis and angiogenesis. Genetic studies in *C. elegans* led us to identify a novel mode of PIG-1/MELK regulation, and a role for this kinase in regulating formin-family proteins and F-actin accumulation. Finally, we have evidence that loss of Zebrafish Melk causes angiogenic defects. Based on these results, we propose a novel conserved PIG-1/MELK and INF2 pathway that regulates the cytoskeleton in tubulogenesis and angiogenesis. Notably, MELK is upregulated in various aggressive cancers and although inhibitory compounds are undergoing clinical trials, little is known about MELK's physiological function. Thus, our work represents an important step in understanding the function of this clinically relevant kinase.

4) Dose-response and quantitative genetic analyses reveal complex patterns of natural variation in susceptibility to diverse toxicants.

Samuel Widmayer, Timothy Crombie, Janneke Wit, James Collins, Sophia Gibson, and Erik Andersen. *Northwestern University*.

Genetic differences among individuals can modify the influences of environmental exposures that induce both acute and chronic disease. Toxic exposure is a known risk factor in the onset of many human diseases, but the contributions and abundance of specific alleles to xenobiotic-induced disease risk at the population-level are virtually unknown. Because of the limited power and scale of toxicological assessments across genetically diverse human subjects, a tractable model system is required to characterize hazard levels of xenobiotic compounds and identify genes linked to susceptibility. Toxicological assessments using *C. elegans* have revealed previously unknown and translational features of xenobiotic metabolism, but investigations of natural variation in these responses are extremely limited. To understand population-wide differences in xenobiotic responses, we measured the susceptibility of eight genetically diverse *C. elegans* wild strains to an array of toxicants, including several heavy metals, mitochondrial poisons, organophosphate insecticides, fungicides, herbicides, and one flame retardant using dose-response assessments. We measured phenotypic responses to each compound by adapting a high-throughput fitness assay using the Molecular Devices ImageXpress Nano automated imaging microscope and developed open-source software to extract and analyze animal morphology measurements from images. Wild strains varied significantly in susceptibility to most compounds, motivating us to search for discrete genomic regions associated with differential responses. To accomplish this search, we measured phenotypic responses to a single dose of each compound across a panel of over 100 *C. elegans* strains and performed genome-wide association (GWA) mappings. These analyses revealed dozens of xenobiotic response loci with measurable effects on population-wide toxicant susceptibility and genetically correlated responses to compounds with similar modes of action. We conclude that differential xenobiotic resistance among *C. elegans* strains are highly heritable and driven by discrete genetic factors. Future work will identify these genetic factors and characterize their mode of effect in genetically diverse cohorts.

5) FSHR-1 and its candidate ligand FLR-2 regulate neuromuscular signaling balance.

Letitia Bortey, Jennifer Kowalski. *Butler University*.

Proper neuronal communication is critical to cognitive and motor function. Normal neuronal signaling regulates the balance of chemical messages to the postsynaptic cell. FSHR-1 is a GPCR that functions as the sole *C. elegans* homolog to a family of glycopeptide hormone receptors involved in gonad function and, more recently, implicated in neurological diseases in *C. elegans*. FSHR-1 is involved in multiple functions including germline production, innate immune responses, aversive learning, and is required for normal neuromuscular function. However, a lot still remains unknown about this GPCR and its possible upstream and downstream partners, including its ligand. A possible ligand for FSHR-1 may be the alpha glycopeptide FLR-2, which is the closest homolog to FSH, the human FSHR ligand . Using swimming assays, in which body bends per minute were counted as a readout of neuromuscular function, we found that *flr-2* loss of function mutants showed the same deficits in motility as seen for *fshr-1* loss of function mutants, demonstrating a role for *flr-2* in regulating neuromuscular signaling. Based on these similar phenotypes, I hypothesized that FLR-2 and FSHR-1 work in a ligand-receptor relationship in *C. elegans* within the same neuropeptide signaling pathway to control neuromuscular function. In additional swimming assays, we found that *flshr-1;flr-2* loss of function mutants showed similar decreases in motility as the as *fshr-1* and *flr-2* mutants alone. These non-additive results suggest that *fshr-1* and *flr-2* work within the same pathway and support a model in which *flr-2* is an *fshr-1* ligand. Future studies will further test the compatibility of the FSHR-1 and FLR-2 ligand-receptor relationship by examining if they exhibit similar phenotypes in synaptic vesicle release as well as testing the FLR-2 beta subunit to see if it coincides with the phenotypes we see for alpha subunit mutant. These results contribute to the understanding of GPCRs, neuropeptides, and their potential role in controlling diverse neuronal processes, including neuromuscular function and neuronal signaling balance, which may be important in understanding how to approach neurological diseases in which these functions are disrupted.

6) Lack of canonical beta-tubulin resistance alleles in two benzimidazole-resistant ascarid species.

James Collins, Lewis Stevens, Robyn Tanny, Abhinaya Venkatesan, John Gilleard, Ray Kaplan, Erik Andersen. *Northwestern University*.

In recent decades, management of nematode parasites has become complicated because of the development of anthelmintic resistance. Resistance to benzimidazoles (BZ) is the most well characterized and has been linked to mutations in codons 167, 198, or 200 of the isotype-1 beta-tubulin gene in strongylid nematodes. Reports of BZ-resistance in ascarids are rare, but we recently reported BZ resistance in two ascarid species of poultry (*Ascaridia dissimilis* and *Heterakis gallinarum*) for the first time. We performed whole-genome sequencing of these isolates to determine if any known strongylid BZ-resistance alleles were present in these ascarid species. Illumina short-read and Pac-Bio long-read sequencing were

carried out on DNA extracted from individuals and pools of adult worms. Sequences were initially assembled and queried in BLAST searches using a beta-tubulin sequence from *Ascaridia galli*, a closely related ascarid of poultry. Contigs with beta-tubulin sequences were identified and then aligned for the codons of interest. From these genes, the ortholog of *tbb-2*, the most highly expressed beta-tubulin of ascarids, was identified and analyzed at the codons of interest. To date, in sequences from six samples from these BZ-resistant isolates, no mutations associated with BZ-resistance in strongyles or the model nematode, *Caenorhabditis elegans*, have been found. Three non-synonymous variants were found but none were predicted to be deleterious. These data strongly suggest that the genetic basis of BZ-resistance in ascarids differs from that of strongyles. It is noteworthy that these data are consistent with a previous study of *Ascaris lumbricoides* where, despite strong suspicions of resistance, molecular analyses failed to identify resistance in the *tbb-2* ortholog, suggesting that resistance might have taken a different evolutionary path in ascarids and that investigations must extend beyond these codons and maybe even beta-tubulin genes. Further sequencing and assembly to improve our draft genome and to confirm our initial findings is ongoing. Once a high-quality genome is assembled, we aim to perform controlled crosses of ascarids in poultry and perform a backcross and recurrent selection approach to identify genomic regions underlying the BZ resistance. Once complete, we will not only be able to confirm the lack of traditional resistance mechanisms in ascarids but also discover the genomic regions underlying resistance and ultimately the specific genes.

7) Determining CLIC domains that mediate function in Rho/Rac signaling.

Julianna Escudero, Anthony Arena, Dan Shaye. *University of Illinois - Chicago*.

The *C. elegans* excretory canal (ExCa) is a unicellular tube that elongates throughout the length of the entire animal. This makes the ExCa a good model to study the genetic regulation and cell biological processes that underlie biological tube formation (tubulogenesis) in *C. elegans* and in angiogenesis (a process of blood vessel formation). Some genes that regulate ExCa tubulogenesis are also involved in angiogenesis, such as the *exc-4* gene. The first mutants of this gene were retrieved in a screen looking for animals with a cystic ExCa. *exc-4* was also cloned and found to encode a chloride intracellular channel (CLIC). Work from our collaborators in Dr. Jan Kitajewski's lab implicated two vertebrate orthologs, CLIC1 and CLIC4, in angiogenesis in vitro (using cultured human umbilical vascular endothelial cells, or HUVEC) and *in vivo* (in mice). In collaboration with the Kitajewski Lab we have now shown that EXC-4 in the worm, and CLIC1 and CLIC4 in endothelial cells, modulate signaling pathways that act via heterotrimeric G proteins (Gα/ß/γ) and the small GTPases Rho and Rac. To further understand the role of CLICs in Rho/Rac signaling it is important to investigate the extent of functional conservation between human (CLIC1 and CLIC4) and *C. elegans* (EXC-4) CLICs. On the N-terminus of invertebrate CLICs, the putative transmembrane domain (PTMD) has been found to be an important determinant for membrane localization. Human CLICs have SH2 and SH3-binding motifs in the C-terminus and other functionally conserved residues. We hypothesize that CLIC function in Rho/Rac signaling is achieved via features of the C-terminus while localization is achieved through the N-terminus. We will be examining the functional domains of human CLIC1 and CLIC4 in *C. elegans* by making chimeric constructs to analyze the interspecies conservation through *exc-4(0)* rescue.

8) Understanding the early pathophysiology of Duchenne muscular dystrophy in a nematode model.

Kiley Hughes, Brittany Rivera, Emily Killian, Peyton Carlock, Hannah Ahmed, Nick Leonard, Andres Vidal-Gadea. *Illinois State University*.

Duchenne muscular dystrophy (DMD) is an x-linked degenerative disease that affects one out of every 3,500 males. This disease is produced by loss of function mutations in the dystrophin gene that results in the absence of the dystrophin protein from muscles and other tissues. Loss of dystrophin leads to progressive muscle weakness, loss of ambulation, and premature death. At the cellular level, patients present with increased sarcoplasmic calcium, loss of sarcomeric integrity, and mitochondrial damage. There is no cure for DMD. Understanding the progression of the disease, and developing effective treatments has been hampered by lack of animal models able to recapitulate the disease at both the genotypic and phenotypic levels. Thus, it remains elusive if increased sarcoplasmic calcium observed in dystrophic muscles follows or leads to the mechanical insults caused by the muscle's disrupted contractile machinery. This knowledge has important implications for patients, as it may lead to identification of other molecular targets susceptible to intervention for the treatment of DMD. Our lab has recently shown that dystrophic *C. elegans* nematodes (*dys-1*) recapitulate key phenotypes associated with dystrophic patients. Here, we use the external development of *C. elegans* embryos to understand the earliest steps in the progression of Duchenne muscular dystrophy.

9) Two intrinsic timing mechanisms set start and end times for dendritic arborization of a nociceptive neuron.
Nobuko Suzuki, Yan Zou, Meiyu Shao, Wei Zou, Kang Shen, Chieh Chang. *University of Illinois - Chicago*

Choreographic dendritic arborization takes place within a defined time frame, but the timing mechanism is not known. Here, we report that a precisely timed *lin-4-lin-14* regulatory circuit triggers an initial dendritic growth activity whereas a precisely timed *let-7-lin-41* regulatory circuit signals a subsequent developmental decline in dendritic growth ability, hence restricting dendritic arborization within a set time frame. *lin-4 and let-7* circuits are expressed in the right place at the right time to set start and end times for PVD dendritic arborization. Replacing the endogenous *lin-4* promoter at the *lin-4* locus with a late-onset *let-7* promoter delays PVD dendrite arborization whereas replacing the endogenous *let-7* promoter at the *let-7* locus with an early-onset *lin-4* promoter causes precocious decline in PVD dendritic growth ability. Our results indicate that the *lin-4-lin-14* and the *lin-28-let-7-lin-41* regulatory circuits control the timing of PVD dendrite arborization through antagonistic regulation of DMA-1 receptors on PVD dendrites.

10) RNA helicase RHA-1 safeguards thermosensitive sperm fertility by promoting small RNA-mediated mRNA clearance.
Olivia Gaylord, Jordan Brown, Wei-Sheng Wu, Heng-Chi Lee**.**

Male fertility and sperm development are thermosensitive processes whereby germline exposure to high temperature reduces viable sperm. Small RNAs and Argonaute proteins play critical roles in male fertility, particularly at high temperature conditions. In *C. elegans*, several small RNA pathways function in spermatogenic germlines and target germline-expressed mRNAs. Argonaute CSR-1 targets many spermatogenesis genes and recent evidence highlights a dual role for CSR-1 in mRNA protection and mRNA clearance, the second requiring CSR-1 catalytic activity to slice mRNA targets. Whether and how these distinct functions of CSR-1 promote male fertility remains largely unknown. Intriguingly, a germline enriched helicase RNA helicase A (RHA-1) is also required for male fertility at high temperature. Here we show in the rha-1 mutant, CSR-1 small RNAs exhibit a slight decrease in overall abundance. Strikingly, the CSR-1 small RNAs are preferentially depleted from the coding sequence rather than the 3' UTR of target mRNAs. This depletion in mRNA coding sequences resembles the reported CSR-1 small RNA defects found in the CSR-1 slicer mutant. When analyzing the mRNA levels of CSR-1 target genes, the rha-1 mutant shows increased CSR-1 mRNA targets, including spermatogenesis genes. Taken together, our results suggest RHA-1 facilitates CSR-1 slicing of mRNA transcripts to promote mRNA clearance. Our study further highlights the critical role of small RNA-mediated mRNA clearance in sperm fertility.

11) Temperature-stressed *C. elegans* males prioritize food over mating resulting in sterility.
Nicholas Sepulveda, Lisa Petrella. *Marquette University*.

Sexual reproduction is temperature sensitive: as temperature goes up, fertility goes down. As the consequences of manmade climate change continue to manifest, it is imperative to understand how both ecologically and economically vital species are negatively impacted by temperature stress. Because male infertility tends to define reproductive failure across taxa, we endeavored to understand precisely how temperature stress induces male sterility using wild isolates of *Caenorhabditis elegans* as a model. While in some other organisms it has be shown that male infertility is primarily due to changes in sperm production and quality, we have shown that underappreciated changes in behavior are the principal component contributing to sterility under temperature stress in *C. elegans*. Succinctly: temperature stressed *C. elegans* males are not interested in mating with hermaphrodites and thus stand virtually no chance at reproducing even if they have functional sperm. We hypothesized that diminished male interest in mating could be attributed to: (1) reduced pheromone response, (2) impaired touch response, (3) increased food drive, (4) lowered movement velocity and physical endurance, and/or (5) decreased mating drive. Surprisingly, we found that temperature stress increases pheromone response, a potentially maladaptive change that does not result in mating success. We also found that temperature stress perturbs the balance in male food and reproductive drives: temperature stressed males appeared to prioritize food over mating and in the presence of food never start to search for mates. In addition, we uncovered evidence that temperature-stressed males have diminished movement velocity and physical endurance, which may also contribute to their sterility. Collectively our results paint a portrait where a temperature stressed male's fertility is reduced by a preoccupation with pheromones and food, lowered mating drive and weakened physical capacity. Changes in behavior caused by temperature stress may prove to be conserved components of fertility that have been previously under-studied, and thus merit additional scrutiny as climate change-associated decreases in fertility become more common in taxa as diverse as worms and humans.

12) Negative regulation of Raf signaling by the E3/E4 ubiquitin ligase UFD-2.

Robert Townley, Augustin Deniaud, Claudia Rodriguez, Kennedy Stacy, Claire de la Cova. *University of Wisconsin - Milwaukee*.

Signaling by the small GTPase Ras activates a conserved kinase cascade involving Raf, MEK, and ERK. In malignant melanomas, the human Raf family member BRAF is frequently altered by the missense mutation BRAF(V600E), resulting in unregulated kinase activation. In C. elegans, the Raf ortholog LIN-45 acts in vulval precursor cells (VPCs) to promote the primary (1º) vulval fate. We previously showed that expression of LIN-45(V627E), which is equivalent to human BRAF(V600E), causes increased ERK activation, ectopic 1º vulval fate in VPCs, and a Multivulva (Muv) phenotype in adults. To discover novel regulators of LIN-45, we performed a genetic screen for mutations that enhance the Muv phenotype resulting from LIN-45(V627E). Here we report the isolation of UFD-2, a conserved E3/E4 ubiquitin ligase and ortholog of human UBE4B, as a negative regulator of LIN-45. During fate specification of VPCs, LIN-45 protein degradation is initiated by phosphorylation at a conserved Cdc4-phosphodegron motif that is subsequently recognized by the E3 ubiquitin ligase SEL-10. As UFD-2 has a well-known role in poly-ubiquitination of proteins targeted to the proteasome, we investigated whether it is also affects LIN-45 degradation. We find that loss of *ufd-2* results in increased levels of both wild-type LIN-45 and mutant LIN-45(V627E) protein. Using a quantitative biosensor of ERK kinase activity, we show that loss of *ufd-2* causes increased ERK activation, confirming that UFD-2 acts to negatively regulate LIN-45 signaling. We present evidence that UFD-2 acts in the same pathway as SEL-10 to promote LIN-45 degradation; however, we also discovered unexpected differences. Unlike SEL-10, regulation by UFD-2 requires sequences at both the N and C-termini of LIN-45. To define the specific protein functions required, we generated LIN-45 mutant forms that abrogate protein interactions found in structural studies of human Raf complexes. Our data show that LIN-45 mutants lacking either the cysteine-rich domain or unable to bind 14-3-3 proteins are degraded independently of UFD-2. We propose a new model whereby Raf proteins present in complexes with Ras or 14-3-3 proteins require UFD-2 for their efficient poly-ubiquitination and degradation, constituting a previously unrecognized mechanism of Raf regulation.

13) Germ granules ensure the fidelity of piRNA-mediated transcriptome surveillance in *C. elegans*

Jordan Brown, Wenjun Chen, Wei-Sheng Wu, Tao He, Shikui Tu, Ziping Weng, Donglei Zhang, Heng-Chi Lee. *University of Chicago*.

Germ granules are perinuclear, membraneless organelles that are essential for fertility across the animal kingdom. In *C. elegans*, germ granules contain the machinery required by the piRNA pathway – the genetic immune system that preserves worm fertility and genome integrity by targeting and destroying non-self mRNA sequences, including transposons. Although this striking localization pattern suggests a functional relationship between the piRNA pathway and germ granules, whether germ granules are required for piRNA biogenesis or function is not known. By analyzing mutants shown previously to be deficient in germ granule assembly, we found that germ granules are largely dispensable for piRNA biogenesis, but are required to trigger silencing against wild type piRNA targets. Strikingly, although many piRNA targets become activated in the absence of germ granules, we found that hundreds of endogenous genes are aberrantly targeted and silenced by piRNAs. Taken together, our results argue that perinuclear germ granules function critically to promote the fidelity of piRNA-based transcriptome surveillance in *C. elegans*.

14) C48B6.3 - A novel modifier of T-box factor TBX-2 activity.

Akshaya Rajaraman, Mirna Vazquez, Taylor Cairns, Peter Okkema. *University of Illinois - Chicago*.

T-box transcription factors are critical developmental regulators in all animals and altered levels of T-box factor activity are associated with a variety of human diseases. To understand the mechanisms controlling T-box factor activity, we are focusing on the *C. elegans* T-box factor TBX-2, which functions with the Groucho-family co-repressor UNC-37 to specify pharyngeal muscles derived from the ABa blastomere. Double mutants containing hypomorphic *tbx-2(bx59)* and *unc-37(e262)* alleles exhibit a temperature-sensitive synthetic lethal phenotype, and we identified 8 new mutants that suppress this lethality. Linkage analysis indicates that 3 of these suppressor mutations are very tightly linked to *unc-37(e262)*. Using whole genome sequencing we found one of these suppressor strains contains a second site mutation in *unc-37*, suggesting that the screen is effective in identifying biologically relevant mutations, while the other two strains contain independent missense mutations in the uncharacterized gene, C48B6.3. The candidate C48B6.3 suppressor mutations result in non-conservative amino acid substitutions in the conserved DUF4588 region of this protein. C48B6.3(RNAi) phenocopies the suppression of *unc-37(e262); tbx-2(bx59)* lethality and increases TBX-2 protein expression assayed using an endogenously tagged *tbx-2::gfp* allele. We hypothesize that C48B6.3 negatively regulates TBX-2 protein levels, and the suppressed phenotype in C48B6.3 mutants results from increased TBX-2 activity. We are currently further characterizing our C48B6.3 mutants and a null mutant obtained from the Vancouver KO group (thanks Don Moerman!). C48B6.3 orthologs are found in

all eukaryotes, but their function is not well characterized in any organism. We hope our studies will provide insight into the function of these highly conserved genes and help us understand how T-box factor activity is regulated.

15) Physical constraints on cuticle stretch may guide *C. elegans* developmental trajectories.

Joy Nyaanga, Gaotian Zhang, Sasha Shirman, Christina Goss, Niall Mangan, Erik Andersen. *Northwestern University*.

Organismal growth is regulated on a genetic level, as changes in gene expression patterns and signaling dictate much of development. However, environmental conditions (e.g. nutrients and temperature) also have strong impacts on growth. For a comprehensive understanding of organismal growth, the links among genetics, environment, and metabolic regulation must be considered. Studies of single cells have revealed that growth regulation can be achieved using time or size sensing control methods. In multicellular organisms, regulatory mechanisms must not only control single cell growth but also integrate it across organs and tissues during development. The nematode *Caenorhabditis elegans* enables the investigation of growth control in metazoans because it has conserved metazoan pathways and processes and can be cultured by the thousands in controlled laboratory conditions. We developed a high-throughput phenotyping platform that facilitates a quantitative assessment of *C. elegans* growth at high precision. Using this platform, we collected growth measurements of thousands of individuals throughout the 72 hours of larval development, measured feeding behavior to pinpoint developmental transitions associated with decreased feeding, and quantified highly accurate changes in animal size and shape during development. We observed simultaneous increases in animal length, decreases in width, and maintenance of volume at each larval transition, suggesting that body shape in addition to size plays a role in the control of *C. elegans* growth. We propose a model of growth control whereby *C. elegans* senses body size through physical constraints on cuticle stretch and undergoes larval-stage transitions when the cuticle reaches its maximum capacity for stretch. This work lays the foundation for a mechanistic dissection of how both genetics and environmental cues control organismal growth.

16) Forward Genetic Screening for Genes Responsible for UNC-2 Regulation in *C. elegans.*

Ame Xiong, Kelly Oh, Hongkyun Kim. *Rosalind Franklin University*.

Neuronal communication is dependent on the conversion of electrical impulses into chemical signaling in order to release neurotransmitters at synaptic sites. Facilitating this release are pre-synaptic voltage-gated Ca2+ channels (VGCCs). VGCCs provide the necessary influx of calcium to trigger the fusion of readily releasable pool (RRP) of synaptic vesicles with neurotransmitters to the presynaptic membrane. Mutations in genes encoding VGCCs cause synaptic dysfunction and increase the risk of neurological and psychiatric disorders including autism, schizophrenia, and bipolar disorder. In C. elegans, the *unc-2* gene, which encodes a pore-forming α1 subunit of P/Q-type CaV2 VGCC, controls calcium influx and is specifically expressed in the nervous system. As in mammals, UNC-2 requires UNC-36, an auxiliary subunit $\alpha 2\delta 3$, for its normal function. UNC-36 form a complex with UNC-2 and influence UNC-2 channel function and localization at pre-synaptic sites. In a genetic study, we perform a forward-genetic screen in *C. elegans* with endogenously-tagged *unc-2* in the *unc-36* mutant background to identify novel genes regulating UNC-2 channel function and trafficking. Identification and characterization of genes involved in UNC-2 channel regulation can further help elucidate the genetic components of pathological mechanisms seen in psychiatric disorders.

17) Defining the function of EXC-4/CLIC in Gα-Rac signaling using TurboID to identify physical interactors.

Anthony Arena, Dan Shaye. *University of Illinois - Chicago.*

We use the *C. elegans* excretory canal (ExCa) cell, a unicellular tube, as a model to identify and study conserved tubulogenesis regulators. EXC-4, a *C. elegans* Chloride Intracellular Channel (CLIC) protein, was first defined for its role in ExCa tubulogenesis and subsequent work using mammalian models showed that the *exc-4* orthologs CLIC1 and CLIC4 regulate angiogenesis. Our recent collaborative studies (Arena and Shaye, submittted, Mao et al., 2021) show that EXC-4/CLICs are conserved regulators of Gα and Rac signaling, but how CLICs function in this pathway remains unknown. Previous work has shown that EXC-4 localization to the apical plasma membrane is crucial for its function and that membrane targeted CLIC1 can rescue *exc-4* null (*0*) mutants—indicating conserved function. CLIC1 and CLIC4 are cytoplasmic at steady state, but are rapidly recruited, to the plasma membrane upon activation of Gα and Rho/Rac signaling. Therefore, in both *C. elegans* and human cells, EXC-4/CLIC membrane localization is critical for their role in signaling. To understand how CLICs perform this conserved function we want to identify direct EXC-4/CLIC interactors. We hypothesize that functional EXC-4/CLIC-containing complexes will be readily identified in the ExCa because EXC-4 is constitutively localized to the membrane in this cell, where Gα-Rho/Rac signaling is initiated. To identify interactors, we are using TurboID, which rapidly and efficiently biotinylates proteins within ~10nm *in vivo*. Using an ExCa-specific promoter we

are expressing various EXC-4::TurboID fusions as “baits” to identify interactors, and we expect to present our initial proteomic results from this approach at the meeting.

18) Harnessing phenotypic plasticity to understand the cell biology of neuron aging.

Callista Yee, Sebastian, Sanchez-Luege, Kang, Shen, Claire Richardson. *University of Wisconsin - Madison*

Neurons live as long as the animal in which they reside. Just as animals show characteristic signs of aging, neuron aging is likewise accompanied by characteristic cellular features, including morphological alterations, synapse loss, and metabolic dysfunction. How these features come about remains unclear. Understanding the mechanisms underlying neuron aging is important because aging is the primary risk factor for neurodegenerative disease: loss of cellular homeostasis in neurons likely contributes to this increased risk. We investigated how aging affects neuron cell biology by exploiting the ability of the nematode *Caenorhabditis elegans* to de-couple the symptoms of aging from chronological age. Under standard laboratory growth conditions, *C. elegans* live in a proliferative state, in which they develop, age and die in two weeks. In the alternate organismal state called dauer, however, animals survive for months. Importantly, their neurons continue to function. We asked, how is neuron aging stalled in the dauer state? This initial work focused on aspects of neuron morphological aging. We first determined that morphological aging is regulated cell-intrinsically. Through a neuron-specific genetic manipulation, we induced a dauer-like suspension of neuron morphological aging within aging (non-dauer) animals. We term this manipulation “dauerization.” Next, we used dauerization to ask, what are the cell-intrinsic drivers of neuron morphological aging? Surprisingly, we found that dauerization intensely suppresses the constitutive endocytic pathway. Synaptic vesicle cycling was uniquely preserved. We propose that this physiological suppression of the constitutive endocytic pathway simultaneously turns down multiple pro-aging processes supported by the endosomal system. A flip-side to our results is the well-established connection between abnormal endocytic pathway function and neurodegenerative diseases, suggestive that there is a conserved interplay between the endocytic pathway and aging. Funding: HHMI, NIH R01-NS103037 and R01-NS091144 to KS, Human Frontier Science Program LT000127/2016-L to CY.

19) Generation of *in vivo* humanized nematode and *in vitro* human myocyte culture systems for the study of muscular dystrophy.

Brittany Rivera, J Adams, E Hughes, E Salwilchik, E Aidoo, J Gantert, P Carlock, A Vidal-Gadea. *Illinois State University*.

Duchenne muscular dystrophy (Dmd) is an x-linked, recessive genetic disorder that affects 1 in 3500 male births. It is characterized by progressive deterioration of muscle tissue. The disease is caused by loss of function mutation dystrophin (hDMD). *C. elegans* dystrophic (dys-1) mutants have been used extensively to model Dmd and show key features of the disease such as calcium dysfunction, mitochondrial degeneration, loss of mobility, and shortened life span. While dystrophin is highly conserved between humans and nematodes, and while dystrophic *C. elegans* recapitulate many salient phenotypes of Dmd, it remains unclear to what extent these similar phenotypes are truly representative of the human disorder. To quantify the extent to which worm-derived insights are likely to be transferable to human patients we generated a humanized Dmd nematode using human hDMD cDNA that rescues loss of *dys-1* in the musculature of dystrophic worms. To further link our findings to the target human muscle cells we incorporated the in vitro use of human myocyte cultures that complement our in vivo nematode approach to the study of Dmd. We believe that our approach will help drive the generation and validation of new insights into this disorder.

20) The genetic architectures of gene expression variation in wild *C. elegans*.

Gaotian Zhang, Nicole Roberto, Daehan Lee, Steffen Hahnel, Erik Andersen. *Northwestern University*.

Regulation of gene expression provides an efficient and flexible way for organisms to respond and adapt to variable environments. For this reason, it underlies phenotypic diversity within and between species. Genome-wide gene expression variation in the nematode *Caenorhabditis elegans* has been observed for thousands of expression quantitative trait loci (eQTL) over the past two decades. However, most studies used two-parent recombinant inbred lines derived from crosses of the laboratory-adapted reference strain, N2, and the genetically diverse Hawaiian strain, CB4856. Consequently, the observed variation in gene expression and their identified eQTL were limited to the differences among a small number of *C. elegans* strains and only revealed a small fraction of the natural diversity of gene expression and regulation in *C. elegans* species. Here, we investigated the natural variation in gene expression of 208 genetically distinct *C. elegans* wild strains by performing bulk mRNA sequencing on synchronized young adult hermaphrodites. We used genome-wide association mappings to identify 6,545 eQTL associated with variation in expression of 5,291 transcripts of 4,520 genes. We found that local eQTL (located close to the genes that they influence) explained most of the narrow-sense heritability in expression variation and showed larger regulatory effects than distant eQTL (located further away from the genes that they influence).

We identified 67 hotspots that comprise 1,828 distant eQTL across the C. elegans genome. We further found a diverse collection of potential regulatory mechanisms that underlie these distant eQTL hotspots. Additionally, we applied mediation analysis to gene expression variation data and other quantitative variation to elucidate putative mechanisms that can play a role in organism-level trait variation. Our results provide an unprecedentedly large resource of transcriptome profiles and genome-wide regulatory regions that will facilitate future studies and efficient methods to locate causal genes to identify the underlying mechanisms for *C. elegans* complex traits.

21.- Resolving between two models of magnetic particle-based mechanisms of magnet stimulus transduction in *C. elegans*.

Tope Awe, Chance Bainbridge, Ploy Freebairn, Andres Vidal-Gadea. *Illinois State University*

Many animals can detect the geomagnetic field (GMF), but little is understood about the mechansims used in transducing magnetic stimuli. Proposed mechanisms of magnetic transduction include the light-dependent chemical-based and the light-independent magnetic particles-based hypotheses. There are two models of the magnetic particles-based mechanism. One that relies on particles stretch receptors (magnetomechanical) and the other, as recently proposed by Bell et al., (2019), involves thermoreceptors (rotating magnetocaloric effect). We have previously shown that *C. elegans* orient to GMF in a light-independent manner, mediated by the polymodal AFD neurons. We are performing experiments to test the potential use of magnetocaloric and magnetomechanic mechanism in *C. elegans*. To this end, we exposed worms to either strong magnetic pulses, or to oscillating magnetic fields, and testes their ability to orient to magnetic fields. Our data favor a magnetomechanic mechanism for magnetic orientation in worms. *C. elegans* is an excellent system for studying the molecular, cellular, and behavioral basis of magnetic field detection.

22.- Investigating the neuronal membrane glycoprotein 1 role using *Caenorhabditis elegans*.

Eliana Mailen Fernandez, Yamila Cutraro, Melisa Monteleone, Kiley Hughes, Andres Vidal-Gadea, Marcela Brocco. *National University of San Martin (UNSAM). Argentina*.

Chronic stress is one of the main risk factors for depression and other neuropsychiatric diseases. Long exposure to chronic stress results in changes in brain gene expression that are deleterious for organisms. Due its relevance to human health, our goal is to investigate the molecular pathways disrupted by chronic stress to understand how this leads to such diseases. Using rodent models of chronic stress, we found that stress alters neuronal protein GPM6A. GPM6A participates in neuronal differentiation and morphology establishment and human GPM6A has been linked to schizophrenia, bipolar disorder, claustrophobia and suicide patients. This links GPM6A to the stress phenomenon and depression. Nevertheless, there is a gap between the cellular GPM6A functions and its role in systemic stress response. To fill this gap, we use the nematode *Caenorhabditis elegans* as model due to shared features between nematode and mammal nervous system and because of the genetic tools available. *C. elegans* exhibits a GPM6A ortholog, the neuronal membrane glycoprotein 1 (NMGP-1), thus, here we used *C. elegans* as a simpler model to study NMGP-1 participation in stress response. First, worms expressing GFP under the *nmgp-1* promoter indicated us that *nmgp-1* expresses in sensory amphid and phasmid neurons and in the egg-laying apparatus. Second, we have characterized NMGP-1 functions using RNAi knockdown and two non-null, hypomorphic mutant alleles. Analysis of dsRNA (*nmgp-1*)-treated or mutant alleles showed an increased recovering time from the stress-resistant dauer stage and a reduced egg-laying rate with respect to control worms. In addition, defects in egg-laying induced egg retention (bag of worms) in *nmgp-1*-deficient worms. Also, worms lacking NMGP-1 showed a normal response to the attractant diacetyl, but an altered repulsive response to SDS. Moreover, morphologically, *nmgp-1*(RNAi) worms showed alterations on ASJ chemosensory neurons located at the nerve ring, responsible of dauer exit. Altogether these results suggest that NMGP-1 is involved in the stress response in *C. elegans*. To move forward, we will present and discuss a battery of experiments to score stress response: Temperature acute and chronic exposure, oxidative and osmotic stress. The characterization of stress response in worms lacking *nmgp-1* will allow us to deepen on the stress molecular bases and mental diseases.

23.- Identifying Tissue-specific Susceptibility of *ben-1* to Benzimidazoles.

Sophia Gibson, Erik Andersen. *Northwestern University*.

Infections with parasitic nematodes cause an enormous global burden in both human and livestock populations. Resistance to the limited arsenal of anthelmintic drugs to combat these infections benzimidazoles (BZ) is widespread with resistance to BZ compounds in livestock particularly common. Previous studies using the free-living nematode Caenorhabditis elegans to model parasitic nematode resistance have shown that mutations in the *C. elegans* beta-tubulin

gene *ben-1*, as well as knocking out the gene, confer resistance to BZ drugs. However, the mechanism of resistance and the tissue-specific susceptibility are not well known. To identify in which tissue(s) *ben-1* function underlies BZ susceptibility, we have generated transgenic strains expressing *ben-1* fused to different tissue-specific promoters including hypodermis, muscle, neurons, intestine, and ubiquitous expression. Performing high-throughput assays to measure and compare the quantitative responses to BZ compounds between different transgenic lines allows us to identify in which tissue(s) *ben-1* function underlies BZ susceptibility. We observed significant deficiencies in development in worms expressing *ben-1* in neurons, comparable to expression under the *ben-1* promoter, suggesting that *ben-1* function in neurons underlies susceptibility to BZ. We are continuing to narrow down in which neurons out of the 118 neuron classes *ben-1* function causes susceptibility by subsetting based on neurotransmitter and performing the same high-throughput assays.

24.- A quantitative ERK biosensor as a tool to understand FGFR signaling in *C. elegans*.

Claudia Sofia Rodriguez, Melissa Garcia Montes, Te-Wen Lo, Cindy Voisine, Michael Stern, Claire de la Cova. *University of Wisconsin - Milwaukee*.

The hypodermis of the roundworm Caenorhabditis elegans is critical for fluid homeostasis. EGL-15 is the sole Fibroblast Growth Factor Receptor (FGFR) of *C. elegans* and is expressed in the hypodermis. Upon ligand binding, EGL-15 activates signal transduction by the RAS-RAF-MEK-ERK pathway that has been implicated in many human diseases. EGL-15 is negatively regulated by CLR-1, a receptor tyrosine phosphatase. In the hypodermis, loss of *clr-1* activity causes a fluid balance defect termed the “clear” (Clr) phenotype. EGL-15 dysregulation also results in defects that include the hyperactive Clr phenotype, as well as a “suppressor of clear” (Soc) phenotype, caused by mutations that compromise EGL-15 activity. Furthermore, different levels of ERK activity have been shown to result in distinct cellular and tissue phenotypes and questions remain on how FGFR regulation can result in dynamic ERK signaling. We hypothesize that the activation state of the downstream kinase ERK is increased in Clr mutants and decreased in Soc mutants. To quantify ERK activity in *egl-15* and *clr-1* mutants, we utilized a fluorescent biosensor termed ERK-Kinase Translocation Reporter (ERK-KTR). The ERK-KTR protein is an ERK substrate, and its phosphorylation state is monitored through nuclear/cytoplasmic localization within the cell. In the presence of ERK activity, the ERK-KTR is phosphorylated, and its localization becomes cytoplasmic. In the absence of ERK activity, the ERK-KTR remains nuclear-enriched. We quantified ERK-KTR localization in the hypodermis as a ratio of cytoplasm/nucleus signal, where a higher Cyto/Nuc ratio indicates higher ERK activity. We demonstrate that the ERK-KTR is a faithful reporter of ERK activation in the hypodermis. In addition, we show that *clr-1* mutants have significantly elevated ERK activity compared to wild type, indicative of increased EGL-15 activity. We aim to use the ERK-KTR to better understand FGFR regulation and the quantitative impact of *egl-15* mutations.