May 29, 2024
Avogadro's Number
Fort Collins, CO

Schedule

Time	Speaker	University / Lab	Topic
9:30 – 9:45 AM	***Coffee and breakfast***		
9:45 – 9:50	Fred Hoerndli & Ann Wehman		Welcome
9:50 – 10:15	Kelsie Eichel	CU Boulder	Cell-extrinsic and intrinsic mechanisms of axon initial segment development
10:20 – 10:45	Thiago Knittel	CSU / Montgomery Lab	The RNA binding protein RDE-4 is required for loading siRNAs into Argonautes
10:45 – 10:55	***Coffee break***		
11:00 – 11:25	Allison Hall	Regis University	Afadin is required for epidermal morphogenesis and functionally interfaces with the cadherin-catenin complex and the RhoGAP PAC-1/ARHGAP21
11:30 – 11:55	Gabrielle Reimann	U Wyoming / Fay Lab	Class II PI3Ks in Membrane Trafficking
12:00 – 1:20 PM	**Lunch and posters**		
1:20 – 2:20	Coni Hoerndli Fred Hoerndli, Christian Frøkjær-Jensen & Ann Wehman	Workshop OR Panel	Workshop: Designing Graphical Abstracts with Illustrator Panel: Pros and Cons of a PhD/Postdoc/Group Leader Abroad
2:20 – 2:30	***Coffee break***		
2:30 – 2:40	Ambika Basu	CSU / Nishimura Lab	Guiding mRNA to nuclear periphery: Lessons from *imb-2*
2:45 – 2:55	Shae Milne	U Wyoming/ Fay Lab	Phosphatidylserine translocase recruits RME-1 and regulates endocytic recycling
3:00 – 3:10	Kaz Knight	CSU / Hoerndli Lab	Investigating the role of post-synaptic mitochondria dynamics in activity-dependent plasticity
3:15 – 3:30	**Coffee and Cookies**		
3:30 – 4:30	**Christian Frøkjær-Jensen**	**KAUST**	**Large-scale gene perturbation and engineering**
4:30 – 5:00	Fred Hoerndli & Ann Wehman		Concluding remarks and brainstorming for next year

Talks:

- **Cell-extrinsic and intrinsic mechanisms of axon initial segment development**
    - *Kelsie Eichel, Eichel lab, CU Boulder*

  The axon initial segment (AIS) is a critical neuronal domain that is essential for maintaining neuronal polarity and generating action potentials. The AIS is characterized by the enrichment of the large protein ankyrinG, which assembles many AIS proteins through its scaffolding functions. The development of the AIS is known to require cell-intrinsic mechanisms, including kinesin-based transport of AIS proteins and the coupling of the cytoskeleton and membrane proteins to stabilize the AIS structure. We previously found that *C. elegans* neurons have hallmark features of an AIS (Eichel et al. Nature 2022), thus establishing a novel intact animal model to investigate AIS biology. Here, we leverage this in vivo system to investigate neurodevelopmental mechanisms across diverse neurons. In doing so, we identify an unexpected cell-extrinsic mechanism that instructs AIS development and converges with known cell-intrinsic mechanisms. We find that the AIS, as defined by an enrichment of an ortholog of ankyrin, develops in a stereotyped region and similar tissue environment in distinct neurons. This suggests that cell-extrinsic cues in the surrounding tissue environment may instruct AIS development. In fact, the conserved heparin sulfate proteoglycan Perlecan/UNC-52 comprises the extracellular matrix and overlaps with the location of the AIS in multiple neurons. Loss of function mutations in perlecan/*unc-52* disrupts AIS development, causing a decrease in ankyrin localization at the AIS and the AIS to take on morphological features of the dendrite. These results reveal that cell-extrinsic mechanisms also shape the development and organization of the AIS in the intact nervous system. Using combinatorial mutational analysis, we find that cell-intrinsic and cell-extrinsic mechanisms converge in vivo to develop the critical neuronal AIS domain. Future work will identify the molecular underpinnings of these converging mechanisms and investigate the functional consequences of such a stereotyped patterning of the AIS across the nervous system.

- **The RNA binding protein RDE-4 is required for loading siRNAs into Argonautes**
    - *Thiago Knittel, Montgomery lab, CSU Biology*

  Small RNAs (sRNAs) play crucial roles in regulating gene expression and genomic stability by associating with a class of RNA binding proteins called Argonautes that mediate mRNA silencing of targets through base pairing interactions. Some sRNAs are produced by cleavage of longer double-stranded RNA (dsRNA) precursors by the endoribonuclease Dicer. However, the mechanism underlying loading of Dicer-dependent sRNAs into Argonautes remain elusive in *C. elegans*. Here we show that RDE-4, a dsRNA binding protein thought to promote Dicer cleavage, plays a critical role in facilitating the loading of Dicer-dependent sRNAs into Argonaute proteins. Our findings reveal that RDE-4 serves dual functions during RNA interference: firstly, it promotes dsRNA cleavage and secondly it promotes small RNA-Argonaute interactions.

- ***C. elegans* Afadin is required for epidermal morphogenesis and functionally interfaces with the cadherin-catenin complex and RhoGAP PAC-1/ARHGAP21**
    - *Allison Hall, Hall lab, Regis University*

  During epithelial morphogenesis, the apical junctions connecting cells must remodel as cells change shape and make new connections with their neighbors. In the *C. elegans* embryo, new apical junctions form when epidermal cells migrate and seal with one another to encase the embryo in skin (ventral enclosure), and junctions remodel when epidermal cells change shape to squeeze the embryo into a worm shape (elongation). The junctional cadherin-catenin complex (CCC), which links epithelial cells to each other and to cortical actomyosin, is essential for *C. elegans* epidermal morphogenesis. RNAi genetic enhancement screens have identified several genes encoding proteins that interact with the CCC to promote epidermal morphogenesis, including the scaffolding protein Afadin (AFD-1), whose depletion alone results in only minor morphogenesis defects. Here, by creating a null mutation in afd-1, we show that afd-1 provides a significant contribution to ventral enclosure and elongation on its own. Unexpectedly, we find that afd-1 mutant phenotypes are strongly modified by diet, revealing a previously unappreciated parental nutritional input to morphogenesis. We identify functional interactions between AFD-1 and the CCC by demonstrating that E-cadherin is required for the polarized distribution of AFD-1 to cell contact sites in early embryos. Finally, we show that afd-1 promotes the enrichment of polarity regulator, and CCC-interacting protein, PAC-1/ARHGAP21 to cell contact sites, and we identify genetic interactions suggesting that afd-1 and pac-1 regulate epidermal morphogenesis at least in part through parallel mechanisms. Our findings reveal that *C. elegans* AFD-1 makes a significant contribution to epidermal morphogenesis and functionally interfaces with core and associated CCC proteins.

- **Class II PI3Ks in *C. elegans* Membrane Trafficking**
  - *Gabrielle Reimann, Fay lab, U Wyoming*

  Molting is an evolutionarily conserved process that relies on fundamental cellular functions, such as membrane trafficking. In *C. elegans*, two highly conserved members of the NIMA-kinase family, NEKL-2 and NEKL-3, regulate clathrin mediated endocytosis and membrane trafficking. From a genetic screen conducted to identify other genes involved in the NEKL kinase pathway, we identified a conserved lipid kinase, piki-1. PIKI-1 is a class II phosphatidylinositol 3-kinase (PI3K) that phosphorylates the 3'-OH position on the inositol ring of phosphatidylinositol to produce phosphatidylinositol 3-phosphate and phosphatidylinositol 3,4-bisphosphate. The transient nature of phosphoinositide signaling, low abundance of phosphoinositides, and additional complexities have posed challenges in elucidating the exact function of class II PI3Ks in membrane trafficking. Using a genetic approach, we describe the role of PIKI-1 in membrane trafficking. PIKI-1 is needed for proper recruitment of early endosomal effectors such as RAB-5 but is not needed for the internalization of clathrin-coated pits in *C. elegans*. Furthermore, loss of function mutations in piki-1 suppress NEKL associated defects in the early endosome and correct actin organization. Here we have characterized the role of PIKI-1 in membrane trafficking in *C. elegans* and expanded on the role of class II PI3Ks in regulation of early endosomes and actin polymerization.

- **Guiding mRNA to nuclear periphery: Lessons from *imb-2* in *C. elegans***
  - *Ambika Basu, Osborne-Nishimura lab, CSU BMB*

  The maternal mRNA transcript of Importin beta-2 (*imb-2*) in *Caenorhabditis elegans* accumulates at the nuclear periphery along with its encoded protein. Through assays inhibiting translation, we discovered that *imb-2*'s localization at the nuclear periphery relies on active translation. To further understand signals that guide the *imb-2* transcript to the nuclear periphery, we investigated whether its mRNA or peptide sequence plays a role. To test the sufficiency of the 3'UTR of *imb-2* in driving localization, the 3'UTR was appended to a reporter construct. We observed it failed to localize, indicating insufficiency. Experiments with translation-inhibitors cycloheximide and puromycin revealed an intact ribosome nascent chain complex is required for *imb-2* localization. This suggests that there is information embedded in either the peptide or RNA sequences that is directing nuclear localization of the nascent chain, the ribosome, and the associated *imb-2* mRNA. Hence, we went on to ask if the mRNA coding sequence or amino acid sequence is necessary for mRNA localization. By recoding the mRNA coding sequence while maintaining the IMB-2 protein sequence using redundant codon usage, we observed loss of mRNA and protein localization, as well as mRNA instability. This led us to conclude that the peptide sequence alone cannot replicate the localization of the wild-type mRNA. Also, the mRNA instability is consistent with our previous findings of mRNA degradation when a naïve reporter was appended to *imb-2* 3'UTR. Taken together, *imb-2* nuclear periphery localization depends on a combination of mRNA and amino acid encoded information to ensure mRNA stability, translational complex formation, and transport.

- **Phosphatidylserine translocase recruits RME-1 and regulates endocytic recycling**
    - *Shae Milne, Fay Lab, U Wyoming*

  C. elegans TAT-1 is a P4-ATPase which has previously been shown to regulate endocytic trafficking by controlling the ratio of phospholipids in internal versus external leaflets of cellular membranes. TAT-1 homologues have been shown to flip phosphatidylserine (PtdSer), and to a lesser degree phosphatidylethanolamine to the cytosolic leaflet. This gene was isolated in a forward genetic screen for suppressors of a lethal phenotype caused by loss of NIMA kinases, *nekl-2* and *nekl-3*. Interestingly, we have shown that NEKL-3 may positively regulate the localization and activity of EH domain containing protein RME-1, directly or indirectly. Additionally, we have confirmed that not only does TAT-1 flippase activity recruit RME-1 to endosomal membranes by acting as a positive regulator, but that loss of *tat-1* rescues deficient trafficking of RME-1 caused by loss of NEKL-3. Further work aims to link the regulation of endocytic recycling to the NEKL kinases via the regulation of PtdSer and recruitment of RME-1 by taking advantage of phospholipid biosensors.

- **Investigating the role of post-synaptic mitochondria dynamics in activity-dependent plasticity**
    - *Kaz Knight, Hoerndli lab, CSU BMS*

  The AMPA sub-type of glutamate receptors (AMPARs) are necessary for excitatory synaptic function. Specifically, dynamic AMPAR transport and localization throughout dendrites is critical for synaptic strengthening as well as learning and memory. We are beginning to unravel the effects of neuronal activity as well as subsequent calcium influx and its downstream signaling on synaptic AMPAR localization. However, how subcellular compartments, such as mitochondria, contribute to localized calcium signaling, and how they affect AMPAR transport and delivery dynamics are not well understood. Recent work from our lab using in vivo imaging of calcium and reactive oxygen species (ROS) in single mitochondria in neurons of intact *C. elegans* animals has shown that neuronal activity drives mitochondrial ROS formation and is dependent on mitochondrial calcium influx. During these experiments, we observed diversity in calcium influx and ROS production in mitochondria, suggesting potential differences in feedback signaling based on mitochondrial proximity to synaptic inputs. To characterize these differences, we express the calcium indicator GCaMP6f inside the mitochondrial matrix and tag the outer membrane with tdTomato to quantify mitochondrial shape and calcium influx after varying optogenetic stimulations of excitatory presynaptic inputs. Recent experiments have aimed to link specific mitochondrial location in the AVA dendrite and function to distinct synaptic changes in AMPAR content relating to behavioral changes elicited by short and long-term optogenetic paradigms. Overall, our in vivo analysis suggests that mitochondria morphology and functions respond to neuronal activity to regulate subsequent local synaptic changes.

Posters:

- Single cell analysis of smFISH in *C. elegans* embryo
  - *Naly Torres, Osborne-Nishimura lab, CSU BMB*

- SPN-4 Promotes Maternally Inherited mRNA Clearance in Caenorhabditis elegans
  - *Karissa Coleman, Osborne-Nishimura lab, CSU BMB*

- Investigating the non-conducting role of Kv2 at ER-PM junctions, a combined *in vivo* and *in vitro* approach
  - *Arielle Michaelis, Hoerndli lab, CSU BMS*

- Chemotaxis and locomotive behavior of *C. elegans* N2, *alh-2* and CGC1
  - *Tiffini Lovell, Hoerndli lab, CSU BMS*

- Investigating the role of OSM-6 in *Caenorhabditis elegans* behavior
  - *Jacob Lewey, Stone-Roy lab, CSU BMS*

- **Modeling the Effects of an Emerging Toxicant, Wildfire Smoke, on Reproductive Toxicity using *Caenorhabditis elegans***
  - *Jacob Smoot, Montrose Lab, CSU EHS*

- **Cellular stress following wildfire smoke exposure in *C. elegans***
  - *Abdullatif Alsulami, Moreno Lab, CSU EHS*

- **G-quadruplexes modulate TDP-43 aggregation in ALS**
  - *Eman Elshalia, Horowitz lab, DU*

- **Phagolysosomes use LC3 lipidation to break down the membrane of a non-apoptotic corpse independent of macroautophagy**
    - *Cassidy Kline, Wehman lab, DU*

List of participants:

Participant Name	Institution	Lab	Title	Email	Role
Fred Hoerndli	CSU/BMS	Hoerndli	Assistant Professor	Frederic.Hoerndli@colostate.edu	Chair and Panelist
Ann Wehman	DU	Wehman	Assistant Professor	ann.wehman@du.edu	Co-chair and Panelist
Kelsie Eichel	CU Boulder	Eichel	Assistant Professor	kelsie.eichel@colorado.edu	Talk
Allison Hall	Regis	Hall	Assistant Professor	ahall013@regis.edu	Talk
Coni Hoerndli	CH Science Design		Science Illustrator	coni.hoerndli@gmail.com	Workshop
Thiago Knittel	CSU/Biology	Montgomery	Postdoc	knittel.tl@outlook.com	Talk
Ambika Basu	CSU/BMB	Nishimura	Graduate Student	ambika.basu@colostate.edu	Talk
Kaz Knight	CSU/BMS	Hoerndli	Graduate Student	kaz.knight@colostate.edu	Talk
Shae Milne	U Wyoming	Fay	Technician	smilne1@uwyo.edu	Talk
Gabrielle Reimann	U Wyoming	Fay	Technician	greimann@uwyo.edu	Talk
Christian Frøkjær-Jensen	KAUST	Frøkjær-Jensen	Associate Professor	cfjensen@kaust.edu.sa	Keynote and Panelist
Karissa Coleman	CSU/BMB	Nishimura	Undergraduate Student	karissac@colostate.edu	Poster
Naly Torres	CSU/BMB	Nishimura	Graduate Student	naly.torres@colostate.edu	Poster
Jacob Lewey	CSU/BMS	Stone-Roy	Graduate Student	jacob.lewey@colostate.edu	Poster
Tiffini Lovell	CSU/BMS	Hoerndli	Research Assistant	tiffini.lovell@colostate.edu	Poster
Arielle Michaelis	CSU/BMS	Hoerndli	Graduate Student	arielle.michaelis@colostate.edu	Poster
Jacob Smoot	CSU/EHS	Montrose	Graduate Student	Jacob.smoot@colostate.edu	Poster
Abdullatif Alsulami	CSU/EHS	Moreno	Graduate Student	abdullatif.alsulami@colostate.edu	Poster
Eman Elshalia	DU	Horowitz	Graduate Student	Eman.Elshalia@du.edu	Poster
Cassidy Kline	DU	Wehman	Graduate Student	Cassidy.Kline@du.edu	Poster

Participant Name	Institution	Lab	Title	Email	Role
Levi Johnson	CSU/Ag	Kelly	Graduate Student	levisoilguy@gmail.com	
Tai Montgomery	CSU/Biology	Montgomery	Associate Professor	Tai.Montgomery@colostate.edu	
Brooke Montgomery	CSU/Biology	Montgomery	Technician	Brooke.montgomery@colostate.edu	
Erin Nishimura	CSU/BMB	Nishimura	Associate Professor	Erin.Nishimura@colostate.edu	
David King	CSU/BMB	Nishimura	Senior Scientist	david.king@colostate.edu	
Jessica Hill	CSU/BMB	Nishimura	Postdoc	Jessica.Lynn.Hill@colostate.edu	
Steven Graham	CSU/BMB	Nishimura	Graduate Student	Steven.Graham@colostate.edu	
Nora Tayefeh	CSU/BMB	Nishimura	Undergraduate Student	nora.tayefeh@colostate.edu	
Ennis Deihl	CSU/BMS	Hoerndli	Lab Manager	e.deihl@colostate.edu	
Zephyr Lenninger	CSU/BMS	Hoerndli	Graduate Student	zephyrl@colostate.edu	
Tom LaRocca	CSU/EHS	LaRocca	Assistant Professor	Tom.LaRocca@colostate.edu	
Rachel Doser	CSU/EHS	LaRocca	Postdoc	Rachel.Doser@colostate.edu	
Aly Cavalier	CSU/EHS	LaRocca	Graduate Student	Alyssa.Cavalier@colostate.edu	
Devin Wahl	CSU/EHS	LaRocca	Postdoc	Devin.Wahl@colostate.edu	
Luke Montrose	CSU/EHS	Montrose	Assistant Professor	luke.montrose@colostate.edu	
Chris Link	CU Boulder	Link	Associate Professor	linkc@colorado.edu	
Jasper Rowe	DU	Sher	Graduate Student	jasper.rowe@du.edu	
Bethany Lucas	Regis	Lucas	Assistant Professor	blucas001@regis.edu	
Mercedes Lopez	Regis	Hall	Undergraduate Student	milopez023@regis.edu	
David Fay	U Wyoming	Fay	Professor	davidfay@uwyo.edu	
Phil Edeen	U Wyoming	Fay	Technician	pedeen@uwyo.edu	
Owen Funk	U Wyoming	Fay	Postdoc	ofunk@uwyo.edu	