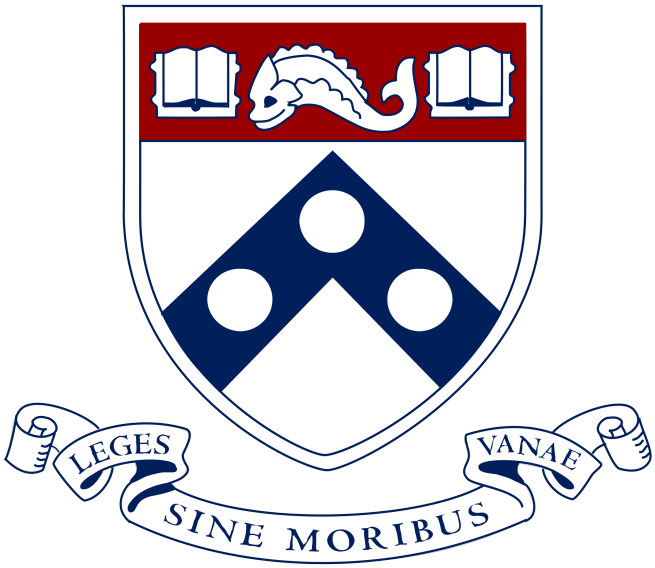
University of Pennsylvania

Department of Physiology Retreat

September 14th, 2023



2023 Physiology Retreat Agenda

8:30 – 9:00	Check In/Breakfast (Enter the Franklin Institute through the Business Entrance)
9:00 – 9:10	Welcome by J. Kevin Foskett, PhD
9:10 – 10:10	Morning Session I (Moderator: Erika Ravitch)
9:10 – 9:25	**Jillian Weissenkampen** - *Epithelial to Mesenchymal Transition of Pancreatic Ductal Adenocarcinoma: Interactions with the Mitochondrial Ca^{2+} Uniporter*
9:25 – 9:40	**Jiangnan (Violet) Xiao** - *Presynaptic store-operated calcium entry supports synaptic vesicle release during prolonged neuron activity*
9:40 – 9:55	**Juliet Goldsmith** - *Proteomic profiling elucidates new aspects of neuronal autophagy in health and disease*
9:55 – 10:10	**Kyle Barrie** - *Structure of the gelsolin-capped actin filament*
10:15 – 11:35	Poster Session + Coffee Break
10:15 – 10:55	Judging for Odd-Numbered Posters
10:55 – 11:35	Judging for Even-Numbered Posters
11:40 – 12:25	Morning Session II (Moderator: Charles Bond)
11:40 – 11:55	**Megan Stefkovich** - *Acute control of hepatic glucose uptake and utilization by insulin*
11:55 – 12:10	**Taisia Shepeliuk** - *Cooperative secretion of platelet dense granules as a regulatory mechanism to promote growth of a thrombus shell*
12:10 – 12:25	**Liz Gallagher** - *Phosphorylation of HSP27 regulates the fluidity of p62 phase condensates in autophagy*
12:30 – 1:30	Lunch
1:30 – 2:30	Afternoon Session I (Moderator: Cameron Thompson)
1:30 – 1:45	**Zoey Miller** - *A Sweet Finding: Targeting Glucose Transporter 1 to Treat Head and Neck Squamous Cell Carcinoma*
1:45 – 2:00	**Bishal Basak** - *Mitochondrial damage triggers degradation of Negative Regulators of Neuronal Autophagy*
2:00 – 2:15	**Jaclyn Welles** - *Investigating the Role of Phosphatidylcholine in NASH-associated fibrosis and inflammation*
2:15 – 2:30	**Jayne Aiken** - *Spastin locally amplifies microtubule dynamics to pattern the axon for presynaptic cargo delivery*
2:30 – 2:40	Departmental Picture (front steps of the Franklin Institute)
2:40 – 3:00	Coffee Break
3:00 – 3:45	Keynote Speaker **Benjamin Prosser** - *Advancing gene-based therapies for neurodevelopmental disorders*
3:45 – 4:00	Closing + Awards
4:30 – 6:30	Reception at City Tap (100 N 18th Street)

Poster Session

10:15 – 11:35 Poster Session + Coffee Break
10:15 – 10:55 Judging for Odd-Numbered Posters (Sub-Session 1)
10:55 – 11:35 Judging for Even-Numbered Posters (Sub-Session 2)

Poster Number	Sub-Session	Presenting Author	Poster Title
1	1	Qing Tang	Detyrosination enrichment on microtubule subsets is established by the interplay between a stochastically-acting enzyme and microtubule stability
2	2	Nicholas Palmer	Structure of formin FH2 domains bound to the actin filament
3	1	Elana Baltrusaitis and Erika Ravitch	Interaction between the mitochondrial adaptor MIRO and the motor adaptor TRAK
4	2	Ekaterina Tarasovetc	Clustering of the scaffolding protein CENP-T activates recruitment of Ndc80 complexes to assemble a functionally active outer kinetochore
5	1	Faviolla Baez-Cruz	*Drosophila* Myo1C and Myo1D are high duty ratio motors with very high ADP affinity
6	2	Luther Pollard	Myosin-1C competes with tropomyosin 3.1 for binding to actin filaments
7	1	Vladimir Demidov and Fedor Balabin	Optical trapping assays to study force-dependent mobility of diffusing microtubule-binding proteins
8	2	Pamela Gallo	Discovering TRPV2 channel molecular function via proximity proteomics reveals novel protein interactions
9	1	Katey Stone	Stress granules as a mechanism of translational control in cardiomyocytes
10	2	Rachel Ceron	Study of ACTG2 mutations reveals different biochemical mechanisms underlying Visceral Myopathy
11	1	Julia Rocereta	Structural differences of mammalian TRPV2 ion channels define ligand sensitivity
12	2	Aleksandr Maiorov	Reconstitution of kinetochore complexes using genetically engineered multimers
13	1	Dominic Santoleri	A novel role for hepatic glucokinase in the regulation of cholesterol gene transcription and synthesis
14	2	Kahealani Uehara	mTORC1 Controls Postprandial Hepatic Glycogen Synthesis Via Ppp1r3b
15	1	Anna Garcia Whitlock	Glucagon Blockade Can Prevent and Treat Hyperglycemia in Stress-Induced Hyperglycemia
16	2	Libby Nunn	Targeting the Activin Type II Receptor Improves Quality of Weight Loss Driven by GLP-1 Receptor Agonism
17	1	Mengqi Xu	Myosin-I facilitates symmetry breaking and promotes the growth of actin comet tails
18	2	Julia Riley	Mitochondrial damage in astrocytes triggers PINK1/Parkin mitophagy and NF-κB-mediated inflammation
19	1	Adam Fenton	FMRP Granules Locally Control Mitochondrial Fission in Neurons
20	2	Cameron Thompson	Possible Regulation of Myosin-19 by the E3-Ligase MARCH5
21	1	Emily Scarborough	Microtubule motors coordinate mRNA export and transport to direct local translation in the cardiomyocyte
22	2	Nick Marotta	Characterization of STXBP1 haploinsufficiency in iPSC derived cortical neurons
23	1	Arpan Bhattacharya	Translational Readthrough Inducing Drug (TRID) Effects On Eukaryotic Termination Investigated At The Single-Molecule Level
24	2	Luke Yochimowitz	Alkaline taste sensation through the alkaliphile chloride channel in *Drosophila*

Abstracts for posters can be found on pages 16-39.

Keynote

Afternoon Keynote Session (3:00 – 3:45)

Advancing gene-based therapies for neurodevelopmental disorders

Benjamin Prosser

Director, Center for Epilepsy and Neuro-Developmental Disorders (ENDD)
Associate Professor, Department of Physiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.
Associate Director, Pennsylvania Muscle Institute, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.

STXBP1 and *SYNGAP1* encode for pre- and post-synaptic proteins that are required for neurotransmission and synaptic plasticity, respectively. Variants in these genes lead to rare, complex and debilitating neurological disorders. Currently there are no treatments capable of altering the course of these diseases, and little is known about the natural progression of these disorders nor their presentation in adults. Our group in the recently formed Center for Epilepsy and Neurodevelopmental Disorders (ENDD) aims to both develop novel gene-based therapies to treat these disorders and to clinically define their presentation and trajectory to enable future clinical trials. Here I will provide both an overview of the challenges and strategies taken to achieve these translational and clinical research goals, as well as a more detailed examination of one of our lead therapeutic strategies – the use of antisense-oligonucleotides (ASOs) to target and manipulate mRNA processing in order to restore expression of STXBP1 and SYNGAP1.

Oral Presentations

Morning Session I – Talk 1 (9:10 – 9:25)

Epithelial to Mesenchymal Transition of Pancreatic Ductal Adenocarcinoma: Interactions with the Mitochondrial Ca^{2+} Uniporter

Jillian S. Weissenrieder, Jason R. Pitarresi, Natalie Weinmann, Rebecca Drager, Usha Paudel, Michael Noji, Zoltan Arany, Anil Rustgi, Ben Z. Stanger, & J. Kevin Foskett

Though novel therapeutics have improved outcomes for patients with many different types of cancers, prognosis for pancreatic ductal adenocarcinoma (PDAC) patients continue to be poor due to late detection, high heterogeneity, early metastasis, and poor responses to current treatment modalities. While Ca^{2+} signaling and mitochondrial function are known to contribute to cancer outcomes in many paradigms, much remains unknown in the context of PDAC, and these may present targets for modulation in cancer therapies. Our previous work suggests that Ca^{2+} flux from the endoplasmic reticulum (ER) into the mitochondria through the mitochondrial Ca^{2+} uniporter (MCU) may drive malignancy. MCU is the main route for Ca^{2+} to enter the mitochondrial matrix, where it drives metabolic activity in the tricarboxylic acid cycle and promotes ATP synthesis by the electron transport chain.

MCU expression is associated with poor outcomes in PDAC patients and disease progression in murine organoid models of cancer development. Deletion of Mcu in murine KPC cells ablates mitochondrial Ca^{2+} uptake, reduces growth and proliferation, and inhibits clonogenicity. Tumor growth and metastatic colonization are also reduced in orthotopic implantation models, with some KPCY-Mcu^{cKO} models completely failing to develop primary lesions. This suggests that MCU, and thus mitochondrial Ca^{2+} , play an important role in tumor growth. Here, we elucidate a heretofore unknown relationship between ER-to-mitochondrial Ca^{2+} flux through Mcu and epithelial to mesenchymal transition (EMT), an important process that contributes to poor outcomes in PDAC. Mcu^{cKO} clones appear more morphologically epithelial than their isogenic, Mcu-expressing counterparts and have reduced basal Snail expression and significantly lower TGFβ secretion. Growth, mobility, and clonogenic deficits in Mcu^{cKO} cell lines are rescued to levels comparable to isogenic, Mcu-expressing cell lines by stable expression of Snail and treatment with TGFβ. Metabolomic studies and mRNA-seq revealed transcriptional and metabolic rewiring which promote cell growth and survival in the context of Snail and TGFβ treatment even in the context of Mcu^{cKO}. Namely, glutamine is more completely metabolized. In addition, levels of a number of metabolites, including pyruvate, malate, and fumarate, in Mcu^{cKO} cells are normalized to levels comparable to Mcu^{WT} isogenic controls by the addition of Snail. These findings suggest that metabolic plasticity in cancer cells enables cell proliferation and motility in the face of Mcu^{cKO}.

Morning Session I – Talk 2 (9:25 – 9:40)

Presynaptic store-operated calcium entry supports synaptic vesicle release during prolonged neuron activity

Jiangnan Xiao & Natali Chanaday

Store-operated calcium entry (SOCE) is activated by depletion of calcium from the endoplasmic reticulum (ER) and mediated by stromal interaction molecule (STIM) proteins. We have previously shown that SOCE activation augments excitatory neurotransmission and potentiates ER-stress-related damage in hippocampal neurons. However, the role of SOCE in normal neuronal physiology remains controversial. Here we investigate the role of STIM isoforms 1 and 2 in modulating synaptic transmission. Using compartment-specific GCaMP sensors, we show that quantal presynaptic and postsynaptic calcium responses to single action potentials are not affected by eliminating STIMs. Presynaptic calcium increase after trains of stimulations are markedly reduced when either STIM1, STIM2 or both proteins are depleted, which is accompanied by a faster depression in synaptic transmission, suggesting that STIMs activate calcium influx during prolonged activity to sustain neurotransmitter release. Furthermore, our results indicate that synaptic vesicle pools are differentially modulated by STIMs in excitatory versus inhibitory synapses. The data presented here uncovers a previously unknown role of STIMs in presynaptic organization and function, suggesting that STIMs are key players in the maintenance of synaptic performance.

Morning Session I – Talk 3 (9:40 – 9:55)

Proteomic profiling elucidates new aspects of neuronal autophagy in health and disease

Juliet Goldsmith & Erika Holzbaur

Autophagy is a cellular recycling mechanism that occurs in all cell types. However, autophagy is especially critical and unique in neurons. Defects in autophagy are a pathological hallmark of neurodegenerative disease, and mutations throughout the autophagy pathway, from cargo selection to degradation efficiency, are causative for Parkinson's disease and Amyotrophic Lateral Sclerosis/Frontotemporal Dementia (ALS/FTD). My postdoctoral research initially asked a simple but long unanswered question: what is autophagy degrading that is critical for neuronal health and homeostasis? Using unbiased proteomics from mouse brain-derived autophagic vesicles and validating in primary and iPSC-derived neurons in culture, I found that mitochondria containing the mitochondrial genome are a major, previously undescribed cargo of autophagosomes in neurons (Goldsmith et al., Neuron 2022). I predict that basal autophagic regulation of mitochondria containing DNA is essential for neuronal function and resilience, specifically with regards to local bioenergetic capacity and neuroinflammation. I propose to investigate the regulation and the function of autophagic clearance of mitochondrial DNA in the context of neurodegenerative disease as an independent researcher. In preliminary data from human iPSC-derived cortical-like and lower motor neurons that harbor ALS/FTD – linked genetic mutations, I have found that a stressed autophagy pathway no longer efficiently clears mitochondria containing DNA. Using this model system, I can now investigate the functional consequence of impaired mtDNA clearance in neurons in culture.

In parallel postdoctoral work, I characterized how autophagy cargos change in two mouse models of Parkinson's disease, finding that despite mutations that impair effective autophagy at different steps in the pathway, distinct compensatory mechanisms are upregulated in these models, namely higher levels of alternate mitophagy adaptors and increased secretion of extracellular vesicles and autophagy cargo such as the pathological molecule alpha-synuclein. Such adaptive mechanisms highlight the critical nature of autophagy in supporting neuron homeostasis. However, the compensatory pathways remain a stopgap fix; upregulation of alternate adaptors does not completely rescue the speed that damaged mitochondria are cleared, nor does secretion effectively degrade cargoes. We predict that the resulting in moderate phenotypes may accumulate or be further exacerbated due to changes in autophagy with age and thus sensitize neurons to degeneration. This manuscript is currently available to read on BioRxiv.

While autophagy has long been an attractive therapeutic target for neurodegenerative disease, attempts to enhance autophagy have broadly failed in clinical trials. I hope that by better understanding why autophagy supports neuron health, we can identify better and more targetable therapeutic angles.

Morning Session I – Talk 4 (9:55 – 10:10)

Structure of the gelsolin-capped actin filament

Kyle Barrie & Roberto Dominguez

Gelsolin is a ubiquitous cytoskeletal regulator that severs and caps actin filaments (F-actin) in blood plasma and in cells. The molecular bases for severing and capping have been revealed by crystal structures of gelsolin's N- or C-terminal halves bound to monomeric actin (G-actin), leaving key mechanistic details unresolved. We report the cryo-EM structure of gelsolin bound to the barbed end of F-actin. The structure reveals that the organization of gelsolin which leads to severing and capping is defined by the linker between its N- and C-terminal halves.

Morning Session II – Talk 1 (11:40 – 11:55)

Acute control of hepatic glucose uptake and utilization by insulin

Megan Stefkovich[1,2]; Talia Coopersmith[1,5]; Dominic Santoleri[1,3]; & Paul Titchenell[1,4]

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Insulin rapidly suppresses glucose production and promotes uptake in the liver postprandially through direct action on hepatocytes, independent of its known effects on transcription. How insulin achieves this is largely unknown, but previous studies show insulin fails to control hepatic glucose balance in insulin resistance. Mechanistically, the serine/threonine kinase AKT mediates insulin's effects on liver transcription. However, much less is known about AKT's transcription-independent ability to control hepatic glucose balance in response to insulin. The goal of this study was to determine 1) the acute effect of AKT on glucose metabolism and 2) the mechanisms involved. To determine how AKT affects glucose flux, we treated hepatocytes with uniformly labeled ^{13}C glucose while inhibiting AKT with a clinically validated small molecule (MK2206). To determine the mechanism, we acutely treated mice with insulin and measured changes in protein levels and post-translational modifications. We found that AKT increases glucose contribution to glycolytic intermediates and lipogenic precursors within 5 minutes, independent of changes to glycogen breakdown. We found that insulin stimulates phosphorylation of an allosteric regulator of glycolysis, PFK2/FBPase2, in hepatocytes at Ser486 in an AKT-dependent manner. Phosphorylation at this residue correlates with increased PFK2 kinase activity and glycolysis. These results indicate that insulin may acutely shift glucose balance in the liver from gluconeogenesis to glycolysis postprandially through AKT-mediated PFK2 phosphorylation. In future studies, we will test the role of PFK2 phosphorylation downstream of insulin signaling in mediating AKT's acute effects on glucose flux in mouse models of normal metabolism and insulin resistance.

Morning Session II – Talk 2 (11:55 – 12:10)

Cooperative secretion of platelet dense granules as a regulatory mechanism to promote growth of a thrombus shell

Taisia O. Shepeliuk[1], Anastasia A. Masaltseva[1,2], Roman R. Kerimov[1,2], Fazoil A. Ataullakhanov[1,2], & Ekaterina L. Grishchuk[1]

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Thrombin generated at the site of vascular injury induces formation of a hemostatic plug, with aggregated platelets forming a stable core and a dynamic outer shell layer. Within the heterogeneous thrombus, activated platelets secrete dense granules (DGs) containing highly concentrated platelet activators. This secretion is crucial for normal hemostasis, but the spatio-temporal regulation of DG secretion remain poorly understood. To circumvent the challenges of studying DG secretion within a growing thrombus, we used fluorescence-based visualization of isolated human platelets in a microfluidics chamber. Real-time imaging revealed that individual thrombin-activated platelets release DGs intermittently in distinct sets rather than continuously. This quantal release is an intrinsic property of activated platelets observed for a wide range of thrombin and ADP concentrations, and it is caused by granule release cooperativity, as evidenced by our finding that supernatant collected from activated platelets significantly amplified thrombin-induced secretion. Moreover, this feedback amplification was reconstituted using a combination of ADP, ATP, and serotonin, which are highly concentrated in DGs. We constructed a quantitative theoretical model of DG secretion and applied it to investigate the impact of cooperative secretion on a growing thrombus. The modeling revealed that the cooperative character of secretion leads to localized bursts of DG release, which significantly elevates ADP concentration, overcoming its immediate dilution by the blood flow. At the injury site, the sustained increase in platelets activators may promote growth of a thrombus shell, thereby insuring robust hemostatic response.

Morning Session II – Talk 3 (12:10 – 12:25)

Phosphorylation of HSP27 regulates the fluidity of p62 phase condensates in autophagy

Elizabeth R. Gallagher, Peace T. Oloko, & Erika L.F. Holzbaur

Maintenance of intact lysosomes is central to cell viability. Upon injury, lysosomes are rapidly repaired or targeted for degradation. Damaged lysosomes are degraded via selective autophagy, a process by which autophagosomes isolate specific cellular cargo that is marked by binding of selective autophagy adaptor proteins. The adaptor p62/SQSTM1 has a well-established role in several forms of selective autophagy; recently, we identified that p62 is also required for the clearance of injured lysosomes (lysophagy). We found that p62 promotes lysophagy by forming liquid-like condensates at damaged lysosomes, while loss of p62 inhibited lysosomal turnover. Further, we found that p62 condensates are tuned by the heat shock protein HSP27. Depletion of HSP27 decreased the liquidity of p62 condensates following lysosomal damage, observing a decreased rate of fluorescence recovery of GFP-labeled p62 after photo-bleaching and an increased accumulation of insoluble p62. Live-imaging experiments revealed the recruitment of HSP27 to p62-positive damaged lysosomes, which was lost in p62 knockout cells. Next, we investigated the molecular trigger regulating the p62-HSP27 interaction. We found that lysosomal permeabilization induced HSP27 phosphorylation at three serine residues, and mutating these sites blocked HSP27 recruitment to injured lysosomes. HSP27 is a target of the kinase MK2, following its activation by p38 MAPK. Inhibition or depletion of either MK2 or p38 MAPK inhibited HSP27 phosphorylation and thus lysophagy, identifying HSP27 phosphorylation as a critical regulatory step in this pathway. We next asked whether HSP27 maintains p62 condensates in other contexts. To test this hypothesis, we induced proteotoxic stress via proteasome inhibition, and observed activation of both p38 MAPK and MK2, leading in turn to the phosphorylation of HSP27. Mutation of the three regulatory phospho-sites in HSP27 blocked recruitment of the protein to p62-positive protein aggregates. This failure to recruit HSP27 reduced the liquidity of p62 condensates surrounding protein aggregates induced by proteasome inhibition, mirroring the effects induced by inhibition of HSP27 phosphorylation on damaged lysosomes. Thus, our work identifies a conserved mechanism regulating p62-dependent autophagy, in which a p38 MAPK-MK2 axis promotes p62-dependent autophagy via phosphorylation of HSP27.

Afternoon Session I – Talk 1 (1:30 – 1:45)

A Sweet Finding: Targeting Glucose Transporter 1 to Treat Head and Neck Squamous Cell Carcinoma

Zoey Miller & Robert J Lee, PhD

Head and Neck Squamous Cell Carcinomas (HNSCCs) are cancers that arise in the mucosa of the oral/nasal cavities, the larynx, and the pharynx. The five-year patient survival rate is 50%. Patients undergo surgery, radiation, and chemotherapy to treat their cancers. However, these treatments are highly aggressive and invasive, leaving lasting effects. New molecular targets and therapies must be identified to improve outcomes for HNSCC patients. The *SLC2A* family of glucose transporters (GLUTs) are facilitated-transport membrane proteins that import glucose into cells. GLUT1 (*SLC2A1*) is expressed by many cancer cells and facilitates their glucose consumption. It is being investigated as a possible chemotherapeutic target. However, the role of GLUT1 in HNSCCs remains unknown. We examined GLUT1 expression in both immortalized HNSCC cells and patient samples. GLUT1 was the highest expressed GLUT in both HNSCC cell lines and in patient tumor tissues. GLUT1 was upregulated in some patient tumor samples (compared to normal tissues), indicating the possibility of developing targeted therapies. Immunofluorescence staining revealed that GLUT1 is expressed on the plasma membrane and within the cytoplasm (perinuclear) differentially in HNSCC cells. Specific organelle staining showed intracellular co-localization to the Golgi apparatus. Furthermore, we investigated the effects of a characterized small molecule inhibitor of GLUT1, BAY-876. This compound decreased glucose uptake and cell proliferation over the course of 24 hours. In addition, XTT assay revealed that BAY-876 decreased cell metabolism over the course of 6 hours. We found that BAY-876 is more potent than another GLUT1 inhibitor, SFT-31. Inhibition of GLUT1 with BAY-876 does not affect mitochondrial membrane potential, but does induce apoptosis in HNSCC cells. Cells undergo apoptosis regardless of differential GLUT1 localization (plasma membrane vs Golgi apparatus). BAY-876 may have the potential to target GLUT1 to treat HNSCCs, perhaps in a targeted fashion. It is vital to further study GLUT1 and other new targets in HNSCCs to improve treatment options and maintain the quality of life for patients.

Afternoon Session I – Talk 2 (1:45 – 2:00)

Mitochondrial damage triggers degradation of Negative Regulators of Neuronal Autophagy

Bishal Basak & Erika Holzbaur

Mitochondrial dysfunction is one of the leading pathological causes behind the progression of several neurodegenerative disorders such as Parkinson's disease, Amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD). Damaged mitochondria can produce reactive oxygen species, proinflammatory signals and apoptotic factors all of which have detrimental effects on neuronal physiology. In healthy neurons, damaged mitochondria are otherwise degraded by a form of receptor-mediated selective autophagy, also termed as 'mitophagy'. Thus, therapeutic advances to addressing the challenges of clearing out damaged mitochondria from diseased neurons involves detailed molecular understanding of the mitophagy pathway. In this study, we provide a mechanistic insight into how this complex process of mitochondrial quality control is regulated in neurons. We show upon mitochondrial damage in wild type neurons, a stress response pathway, which we coin as the Mitophagic Stress Response (MSR)pathway is activated, which downstream triggers the concerted degradation of proteins that negatively regulate autophagy. These proteins include Myotubularin related protein (MTMR)-5, MTMR2 and Rubicon. We show that the targeted degradation of this class of regulators is specific to neurons, and is mediated by the ubiquitin-proteasomal system (UPS). This degradation is subsequently accompanied by a decrease in the levels of the autophagy receptor - p62/SQSTM1, and a potential increase in autophagosomal acidification, indicating upregulation of autophagic flux. Under basal conditions, we find that the MTMR5-MTMR2 complex repress mitochondrial turnover by trafficking autophagosomes inhibiting early steps of mitochondrial quality control. On the other hand, Rubicon which stably associates with lysosomes, prevents autophagosome-lysosome fusion, thereby inhibiting final steps of the autophagy pathway. Thus, during mitochondrial damage upon stress, the degradation of MTMR5 and MTMR2 promote the uptake of damaged mitochondria by autophagosomes, and the degradation of Rubicon facilitates the fusion of these autophagosomes carrying damaged mitochondria with lysosomes. We elucidate a graded neuronal response in the activation of this stress pathway, that connects the UPS to the autophagy pathway to mediate an increase in clearance of damaged neuronal mitochondria. Our work thus identifies three potential targets whose depletion can trigger clearance of damaged mitochondria in neurons from ALS, PD and AD patients where the process of mitophagy is compromised.

Afternoon Session I – Talk 3 (2:00 – 2:15)

Investigating the Role of Phosphatidylcholine in NASH-associated fibrosis and inflammation

Jaclyn E. Welles[1], Kahealani Uehara[2], Michael V. Gonzalez,[3], James P. Garifallou[4], James D. Weissenkampen[5], David Li[6], Rebecca G. Wells[7], & Paul M. Titchenell[8]

University of Pennsylvania – Perelman School of Medicine[6,7], Dept. of Physiology[1,2,8], Dept. of Genetics[5], Philadelphia, PA
Children's Hospital of Philadelphia – Center for Applied Genomics, Abramson Research Center, Philadelphia, PA[3,4]

Phosphatidylcholine (PC) is a lipid macromolecule demonstrated to play an integral role in multiple intracellular processes critical in lipid homeostasis (i.e., VLDL-TAG secretion; lipid metabolism; lipid droplet dynamics). Although the reduction of dietary PC intake has been demonstrated in several human and animal studies, to contribute to the onset of nonalcoholic fatty liver disease (NAFLD), PC production is also significantly decreased in patients with nonalcoholic steatohepatitis (NASH), an irreversible, advanced metabolic disorder associated with significant increase in fibrosis and inflammation. It remains unknown however, how the reductions in hepatic PC biosynthesis, promotes NASH-associated fibrosis and inflammation. Thus, in this study, we used a diet-focused model, the low methionine, choline-deficient, 42% kcal/fat high-fat diet (LMCD-HFD), and a genetic model using a hepatocyte-specific *PCYT1A* KO to address our hypothesis. *PCYT1A* is the gene that encodes for CTP: phosphocholine cytidyltransferase, alpha (CCT-α), the rate-limiting enzyme required for ~70% of hepatic PC production via the Kennedy pathway. Given the complexity of NASH pathogenesis, and the heterogeneity of cells within the liver, we used 10X-based single-nuclei RNA-Seq (sNUC RNA-Seq) to perform this study.

Cluster analyses using SEURAT, identified eight (8) clusters consisting of ~35,000 nuclei, with distinct gene expression signatures in the livers from our experimental groups: Control, L-CCT-α KO, and LMCD-HFD. Notably, in both NASH models, we observed the expansion of an "unknown" cell cluster, which demonstrated a cluster-specific upregulation of gene diaphanous related formin 3 (*Diaph3*). Diaph3 is an actin nucleator, critical in cytokinesis, which has been identified a poor-prognostic marker in colorectal and liver cancer. No studies investigating the role of Diaph3 in metabolic disorders have been performed to date. KEGG analysis of this Diaph3+ cluster, showed enrichment of genes associated with microtubule organization, microtubule motor activity, and cell cycle. Cell identity analysis using *ENRICHR*, identified enrichment of genes associated with hepatoblast, pluripotent stem cells, and cell cycling. Cell cycle scoring and regression analysis identified significant distribution of nuclei within the S and G2/M phases exclusively in this cluster suggesting that these Diaph3+ cells may be a disease-specific proliferative cell cluster. Slingshot analysis of this Diaph3+ cluster, shows trajectory patterns, similar to hepatocytes and endothelial cells in identity, yet distinct from either suggesting a novel identity. *In vitro* assays using HUH-7 cells overexpressing a Diaph3-GFP plasmid, showed significant upregulation in cell proliferation, which correlated in significant increases in the expression of hepatoblast/stem cell markers *FANCI*, *BUB1B*, and *NDC80*. Huh-7 cells overexpressing Diaph3-GFP, also exhibited changes in cell morphology and size when compared to cells transfected with the Empty Vector (control). Preliminary elastography studies performed in collaborative studies on NASH livers from both models, demonstrated increased stiffness in both the LMCD-HFD and L-CCT-α KO livers when compared to controls. Thus, given the fact that the expansion of Diaph3+ cells is more significant in the livers of L-CCT-α KO mice, which exhibited less stiffness when compared to LMCD-HFD livers, and that Diaph3 plays an integral role in regulating microtubule and actin cytoskeleton dynamics, we *hypothesize* that Diaph3+ may be acting as a novel mechanosensor capable of sensing, and integrating ***early*** changes during the transition from a steatotic liver (NAFLD) to an inflammatory, fibrotic liver (NASH).

Afternoon Session I – Talk 4 (2:15 – 2:30)

Spastin locally amplifies microtubule dynamics to pattern the axon for presynaptic cargo delivery

Jayne Aiken & Erika L. F. Holzbaur

Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Neurons rely on long-range trafficking of synaptic components to form and maintain the complex neural networks that encode the human experience. With a single neuron capable of forming thousands of distinct en passant synapses along its axon, spatially precise delivery of the necessary synaptic components is paramount. How these synapses are patterned, and how efficient delivery of synaptic components is regulated, remains largely unknown. Here, we reveal a novel role for the microtubule severing enzyme spastin in locally enhancing microtubule polymerization to influence presynaptic cargo pausing and retention along the axon. In human neurons derived from induced pluripotent stem cells (iPSCs), we identify sites stably enriched for presynaptic components, termed 'protosynapses', which are distributed along the axon prior to the robust assembly of mature presynapses apposed by postsynaptic contacts. These sites are capable of cycling synaptic vesicles, are enriched with spastin, and are hotspots for new microtubule growth and synaptic vesicle precursor (SVP) pausing/retention. Disruption of neuronal spastin, either by CRISPRi-mediated depletion or transient overexpression, interrupts the localized enrichment of dynamic microtubule plus ends and diminishes SVP accumulation. Using an innovative human heterologous synapse model, where microfluidically isolated human axons recognize and form presynaptic connections with neuroligin-expressing non-neuronal cells, we reveal that neurons deficient for spastin do not achieve the same level of presynaptic component accumulation as control neurons. We propose a model where spastin acts locally as an amplifier of microtubule polymerization to pattern specific regions of the axon for synaptogenesis and guide synaptic cargo delivery.

Poster Presentations

Poster 1

Detyrosination enrichment on microtubule subsets is established by the interplay between a stochastically-acting enzyme and microtubule stability

Qing Tang[1], Sebastian Sensale[2,3, †, *], Charles Bond[1, †], Victor Xing[1], Andy Qiao[2], Siewert Hugelier[1], Arian Arab[1], Gaurav Arya[2], & Melike Lakadamyali[1,*]

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Microtubules in cells consist of functionally diverse subpopulations carrying distinct post-translational modifications (PTMs). Akin to the histone code, the tubulin code regulates a myriad of microtubule functions ranging from intracellular transport to chromosome segregation. Yet, how individual PTMs only occur on subsets of microtubules to contribute to microtubule specialization is not well understood. In particular, microtubule detyrosination, the removal of the C-terminal tyrosine on α-tubulin subunits, marks the stable population of microtubules and modifies how microtubules interact with other microtubule-associated proteins to regulate a wide range of cellular processes. Previously, we found that, in certain cell types, only ~30% of microtubules are highly enriched with the detyrosination mark and that detyrosination spans most of the length of a microtubule, often adjacent to a completely tyrosinated microtubule. How the activity of a cytosolic detyrosinase, Vasohibin (VASH) leads to only a small subpopulation of highly detyrosinated microtubules is unclear. Here, using quantitative super-resolution microscopy, we visualized nascent microtubule detyrosination events in cells consisting of 1-3 detyrosinated α-tubulin subunits after Nocodazole washout. Microtubule detyrosination accumulates slowly and in a disperse pattern across the microtubule length. By visualizing single molecules of VASH in live cells, we found that VASH engages with microtubules stochastically on a short time scale suggesting limited removal of tyrosine per interaction, consistent with the super-resolution results. Combining these quantitative imaging results with simulations incorporating parameters from our experiments, we provide evidence for a stochastic model for cells to establish a subset of detyrosinated microtubules via a detyrosination-stabilization feedback mechanism.

Poster 2

Structure of formin FH2 domains bound to the actin filament

Nicholas Palmer & Roberto Dominguez

The actin cytoskeleton is a tightly controlled and orchestrated process in cells which plays a role in many functions within the cell. Formins are a diverse class of actin binding proteins that control the nucleation and elongation of actin filaments. Key to formins function is their ability to remain processivity bound to the fast growing barbed. The mechanism behind formin processivity is unknown. Inverted formin 2 (INF2) is a formin which in addition to barbed end elongation has the unique functions of actin severing and depolymerization. Here we use cryo-electron microscopy to determine the structure of INF2 in its native states bound to actin filaments. We find multiple conformations of INF2's FH2 domains that give key insights into the mechanisms of barbed end elongation and clues into the mechanism behind INF2's unique ability to sever actin filaments. Further biochemical and cell studies based on these structures will strengthen our understanding of formin regulation of the actin cytoskeleton.

Poster 3

Interaction between the mitochondrial adaptor MIRO and the motor adaptor TRAK

Elana Baltrusaitis, Erika Ravitch, & Roberto Dominguez

MIRO (mitochondrial Rho GTPase) consists of two GTPase domains flanking two Ca^{2+}-binding EF-hand domains. A C-terminal transmembrane helix anchors MIRO to the outer mitochondrial membrane, where it functions as a general adaptor for the recruitment of cytoskeletal proteins that control mitochondria dynamics. One protein recruited by MIRO is TRAK (trafficking kinesin-binding protein), which in turn recruits the microtubule-based motors kinesin-1 and dynein-dynactin. The mechanism by which MIRO recruits TRAK to mitochondria is not well understood. Here, we map and quantitatively characterize the interaction of human MIRO1 and TRAK1 and test its potential regulation by Ca^{2+} and/or GTP binding to MIRO1. TRAK1 binds MIRO1 with low micromolar affinity. The interaction was mapped to a fragment comprising MIRO1's EF-hands and C-terminal GTPase domain and to a conserved stretch of amino acids within TRAK1 residues 394-434, immediately C-terminal to the Spindly motif. MIRO1's EF-hands bind Ca^{2+} with dissociation constants (K_D) of 3.3 mM and 130 nM. This suggests that under normal cellular conditions, where the Ca^{2+} concentration ranges from 0.1-0.2 mM, one EF-hand may be constitutively bound to Ca^{2+}, whereas the other EF-hand binds Ca^{2+} in a regulated manner, depending on its local concentration. Yet, the MIRO1-TRAK1 interaction is independent of Ca^{2+} or GTP binding to MIRO1. The interaction is also independent of TRAK1 dimerization, such that a TRAK1 dimer can be expected to bind two MIRO1 molecules on the mitochondrial surface.

Poster 4

Clustering of the scaffolding protein CENP-T activates recruitment of Ndc80 complexes to assemble a functionally active outer kinetochore

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Proper kinetochore assembly requires binding of multiple copies of different proteins in an ordered and localized manner. In particular, the human kinetochore scaffolding protein CENP-T recruits two Ndc80 molecules, which are required for stable interaction with spindle microtubules. However, when the CENP-T-kinetochore localization domain is removed, individual CENP-T molecules do not associate robustly with Ndc80 when present in the cytoplasm, highlighting the importance of the centromere localization of CENP-T. We hypothesized that clustering of CENP-T molecules at centromeres plays a role in Ndc80 recruitment. To test this, we created genetically-encoded 60-subunit clusters of CENP-T's outer kinetochore recruitment domain and expressed them in human cells. Clustered CENP-T, but not monomeric CENP-T 1-242 , robustly recruited Ndc80 and other outer kinetochore proteins. Moreover, complexes formed by CENP-T clusters and outer kinetochore components isolated from cells bind to microtubules and move processively with dynamic microtubule tips. To investigate the mechanism that leads to assembly of a functionally active outer kinetochore on clustered CENP-T, we tested whether this phenomenon can be recapitulated using recombinant components. We used a real time TIRF fluorescence assay to monitor interactions between GFP-tagged Ndc80 and CENP-T 1-242 present in either clustered or monomeric forms. Both forms of CENP-T bind to two Ndc80 complexes, but we found that Ndc80 molecules dissociate significantly faster from monomeric compared to clustered CENP-T. In addition, the stability of CENP-T-Ndc80 binding increases over time, indicating a "maturation" process of Ndc80 binding sites on CENP-T. Such maturation was concurrent with the presence of multiple weakly-bound Ndc80 molecules around CENP-T clusters. The formation of such molecular clouds was not observed with monomeric CENP-T molecules, which experienced much slower maturation compared to clustered CENP-T. Thus, the enhancement of Ndc80 recruitment and stabilization of its binding to CENP-T is an intrinsic feature of CENP-T clusters and is associated with different kinetics of Ndc80 binding and maturation. We propose that, in cells, these molecular mechanisms promote efficient and stable binding of Ndc80 and other outer kinetochore components specifically at the centromere loci, while avoiding interactions between the soluble components in the cytoplasm.

Poster 5

Drosophila Myo1C and Myo1D are high duty ratio motors with very high ADP affinity

Faviolla A. Baez-Cruz & E. Michael Ostap

Unconventional class-I myosins (myosin-Is) are single-headed, actin-based motors that link membranes to the cytoskeleton and participate in a range of cellular functions. Drosophila has two myosin-I isoforms, myosin-1C (Myo1C) and myosin-1D (Myo1D), which have roles in establishing organismal chirality during development, likely through the interaction with E-cadherin and β-catenin at sites of cell-cell adhesion. Remarkably, ectopic expression of either Myo1C or Myo1D in non-chiral organs (e.g., epithelium and trachea) result in generation of organs with distinct chirality, depending on the myosin-I isoform expressed. The activity of the myosin-I motor domains was shown to be crucial for this induced chirality, with the handedness of chirality depending on which motor domain was expressed. To better understand the motor properties of these myosins, we performed a detailed biochemical investigation of recombinant, full length Myo1C and Myo1D obtained by expression in Sf9 cells using baculovirus. The steady-state ATPase activities of the motors are actin-activated, with Myo1D having a V_{max} 12.5-fold larger than Myo1C, consistent with slower actin gliding speeds of Myo1C found via *in vitro* motility assay. ATP binding is fast and non-rate-limiting in both myosins. The maximum rate of actin-activated phosphate release from Myo1C (0.4 s^{-1}) is similar in magnitude to its ADP release rate (1 s^{-1}). However, the rate of ADP release from Myo1D (8 s-1) is substantially slower than the rate of phosphate release (28 s^{-1}). Notably, the ADP affinities for both Myo1C (89 nM) and Myo1D (44 nM) are among the tightest measured for any myosins, and will likely result in substantial steady-state population of the force-bearing AM.ADP states under physiological nucleotide concentrations.

Poster 6

Myosin-1C competes with tropomyosin 3.1 for binding to actin filaments

Luther W. Pollard, Malgorzata Boczkowska, Roberto Dominguez, & E. Michael Ostap

The expression of approximately 40 different tropomyosin (Tpm) isoforms confers different identities upon actin filaments, resulting in subcellular compartments with different filament dynamics and morphologies. Strikingly, these compartments have been shown to have differential selectivity for members of the myosin superfamily. For example, several Tpm isoforms are known to inhibit class-I myosins while activating class-II myosin motility. The mechanism of Tpm-myosin selectivity is unknown. It is possible that the exclusion of myosin isoforms results from steric blocking due to structural differences at the Tpm-actin binding site. Alternatively, differential regulation could be due to the intrinsic kinetic properties of myosins, and their ability to switch on the dynamic tropomyosin-actin filament. Here, we investigate the mechanism of Myo1C inhibition by Tpm3.1, a short Tpm isoform abundantly expressed in non-muscle cells. We show that filament gliding in the presence of Tpm3.1 requires higher Myo1C concentrations attached to coverslips than necessary for undecorated actin gliding. Tpm3.1 also reduces the number of filaments bound by Myo1C as a function of increasing ionic strength in the motility assay. Actin gliding speeds are substantially inhibited in the presence of saturating Tpm3.1; however, motility does occur. In contrast, we found that Tpm3.1 completely inhibits the actin-activated steady-state ATPase activity of Myo1C. To determine if the inhibitory effect is due to steric or kinetic differences, we performed cosedimentation assays of Tpm3.1-bound actin and Myo1C in the absence of ATP and found that high Myo1C concentrations displace Tpm3.1 from the actin pellet, showing that Myo1C and Tpm3.1 actin-binding are mutually exclusive. Together, these data suggest that the mechanism of reduced gliding velocity is due to a reduction in the number of heads bound to filaments as a result of steric blocking by Tpm3.1. We propose that high local enrichment of Myo1C, such as on the coverslip surface in motility assays, is enough to outcompete some of the Tpm3.1, thus enabling sub-optimal motility. One possibility is that Myo1C enriched at membrane surfaces alongside the dynamic assembly of branched actin networks can exclude Tpm3.1 from these networks. This work was supported by NIH grant 5R37GM057247 and NSF grant CMMI 15-48571 to EMO.

Poster 7

Optical trapping assays to study force-dependent mobility of diffusing microtubule-binding proteins

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A number of microtubule associated proteins (MAPs) have a remarkable ability to diffuse along the microtubule wall. During cell division, kinetochore-localized diffusing MAPs, such as Ndc80 protein complex, maintain diffusive bonds between the chromosome and microtubule. These mobile interactions mediate chromosome motions even under significant forces, but the underlying molecular mechanisms are poorly understood. Quantitative analysis of these mechanisms requires specialized equipment and approaches which permit application of controllable forces to individual gliding proteins or their small ensembles. We employ advanced laser trapping techniques based on three-bead configuration, in which a microtubule is attached to two trapped beads, forming a dumbbell. The suspended microtubule is brought in contact with MAP molecules conjugated to the coverslip-immobilized bead, called pedestal. The ultrafast force-clamp (UFFC) technique allows application of forces from 2 to 15 pN and the ability to resolve sub-millisecond binding events with nanometer accuracy. Using this technique, we have previously shown that the Ndc80 protein exhibits microtubule polarity-dependent mobility, acting as a mobile catch-bond when pulled toward the plus end of the microtubule, and as a mobile slip-bond in the opposite direction. This feature likely allows Ndc80 to prevent strong forces from detaching kinetochores from the plus ends of kinetochore-bound microtubules. This poster will describe our efforts to improve the UFFC approach by introducing microprocessor-driven feedback loops, which will record the dumbbell beads positions at up to 1 MHz. Such improvement is anticipated to obtain the sub-nanometer accuracy and sub-millisecond resolution to examine the character of Ndc80 translocation along microtubule. Ndc80 is recruited to the kinetochore by CENP-T, but the role of mechanical force in Ndc80 recruitment is unknown. To address this gap of knowledge, we are developing a laser trapping approach to observe uninterrupted gliding of Ndc80 for several seconds, much longer than is afforded by the UFFC. In this “dragging assay”, the CENP-T-coated pedestal is moved at a constant velocity for 5-7 μm along microtubule dumbbell in the presence of soluble Ndc80. This poster will describe our progress in developing protocols for dragging assay, as well as the procedures to functionalize pedestals and assemble microtubule dumbbells.

Poster 8

Discovering TRPV2 channel molecular function via proximity proteomics reveals novel protein interactions

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Transient receptor potential vanilloid 2 (TRPV2) is a calcium-permeable non-selective cation channel, which belongs to the vanilloid subfamily of the transient receptor potential (TRP) channels. TRPV2 is broadly expressed in the human body and has been implicated in many physiological and pathophysiological processes from neuronal development and cardiac function to progression and metastasis of different forms of cancer. Yet, the exact molecular function of TRPV2 in the cellular environment together with its accurate subcellular localization, its endogenous modulators, and its protein interaction network are largely unknown. To further our knowledge on TRPV2 endogenous function, we adopted a peroxidase (APEX2)-based proximity-tagging method to comprehensively identify TRPV2 interactome using mass spectrometry and subcellular localization using electron microscopy. Our results revealed unexpected TRPV2 subcellular localization and identified protein-protein interaction network within ~20 nm distance of the TRPV2 channel. We were able to identify proteins that are already known to affect TRPV2 function and discovered novel proteins that provided an insight into TRPV2 molecular function. Together, this information will allow us to further elucidate the endogenous role of TRPV2 in many physiological processes.

Poster 9

Stress granules as a mechanism of translational control in cardiomyocytes

Katey R. Stone, Emily A. Scarborough, & Benjamin L. Prosser

The heart is largely made up of post-mitotic, non-regenerative cardiomyocytes (CM) that require tight coupling of translational control and proper proteostasis to constantly respond to translational needs over an organism's lifetime. However, the mechanisms by which CMs spatially and temporally control translation are largely unknown. Stress granules (SG) are non-membranous phase-separated granules containing mRNA and mRNA binding proteins. SGs have been shown to function in translational downregulation in other cell types. Although some studies suggest that SGs may assemble in neonatal CMs, the occurrence and function of SGs in mature myocardium have not been well-studied. We hypothesize that CMs use the assembly and disassembly of SGs to tune translational activity for general proteostasis and upon stress to accommodate hypertrophy. To test this, we use immunofluorescence (IF) and RNA fluorescence in situ hybridization (FISH) to visualize mRNA and RNA binding proteins (RBP) in neonatal and adult CMs. Additionally, methionine depletion and methionine analogs in combination with click-chemistry are used to measure translation. We use overexpression of SG-associated RBP G3BP1 and heat shock as methods of inducing stress and causing SG assembly. We find that neonatal CMs form distinct puncta that contain G3BP1 and polyA mRNA in some cells under control conditions and in all cells after heat shock. We find that neonatal CMs overexpressing GFP-G3BP1 at a high level form distinct puncta that colocalize with SG-associated RBP PABPC-1 and show a drastic reduction in translation, while those expressing at a low level do not form puncta and show no change in translation compared to those not expressing GFP-G3BP1. We find that mature adult CMs form distinct puncta in G3BP1 and polyA mRNA as well as show reduced translation following heat shock. Together, these data indicate that CMs form SGs that incorporate canonical SG proteins, and when SGs are present, rates of translation are decreased. In the future, we will further identify the composition of SGs in CMs and the stressors that induce their assembly, and determine their role in modulation of translation and subsequent cardiac hypertrophy.

Poster 10

Study of ACTG2 mutations reveals different biochemical mechanisms underlying Visceral Myopathy

Rachel H. Ceron, Faviolla A. Báez-Cruz, Nicholas Palmer, Peter J. Carman, Malgorzata Boczkowska, E. Michael Ostap, Robert O. Heuckeroth, & Roberto Dominguez*

Visceral myopathies are rare and debilitating diseases involving dysfunction of the smooth muscle that contracts the bowel, bladder and uterus. Weakness of visceral smooth muscle hinders intestinal motility and bladder emptying leading to abdominal distension, constipation, and vomiting. Patients with visceral myopathy often require intravenous nutrition, bladder catheterization, and surgical procedures to release gas or flush the bowel. While the genetic etiology for most cases of visceral myopathy remains a mystery, the most common known cause is mutation of the gamma smooth muscle actin gene (*ACTG2*). Other reported mutations affect proteins that interact with ACTG2, including the smooth muscle myosin complex (MYH11, MYL9 and MLCK) and actin binding proteins (LMOD1). Over 30 unique mutations in *ACTG2* have been associated with visceral myopathy, but how these mutations cause disease is poorly understood. To determine how disease-causing *ACTG2* mutations affect actin biochemistry, we developed a method to express and purify human actin variants in the lab. With this method, we produced four disease-causing ACTG2 variants and studied polymerization kinetics, filament length and strength, and interaction with relevant actin binding proteins (LMDO1, Tpm1.4, and smooth muscle myosin) for comparison with WT ACTG2. We discovered that different ACTG2 variants exhibited unique deficits in actin polymerization kinetics, filament strength, ability to interact with actin-binding proteins, and protein stability in solution. Additionally, mixing ACTG2 variants with WT ACTG2 and/or decorating filaments with smooth muscle tropomyosin reduced the biochemical deficits. Our work establishes biochemical mechanisms underlying visceral myopathy which we hope will help guide future *in vivo* studies.

Poster 11

Structural differences of mammalian TRPV2 ion channels define ligand sensitivity

Julia Rocereta & Vera Moiseenkova-Bell

Transient receptor potential vanilloid 2 (TRPV2) is a non-specific cation channel with broad distribution across tissues and organs and is believed to play a role in many physiological processes, such as neuronal development, immunity, and cancer progression. Tissue distribution, functionality, and ligand sensitivity vary across mammalian TRPV2 isoforms. We have previously characterized the structures and functions of rat TRPV2 (rTRPV2) in the presence of known TRPV2 ligands. Here, we have resolved the cryo-EM structure of the wildtype human TRPV2 (hTRPV2) isoform and compared to known TRPV2 structures. This model highlights components within hTRPV2 that account for differences in ligand sensitivity and is necessary to design improved drugs that bind and effectively modulate hTRPV2.

Poster 12

Reconstitution of kinetochore complexes using genetically engineered multimers

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Prior studies in cells and in vitro revealed general features of human kinetochore architecture and assembly pathways. In particular, the scaffolding CENP-C protein, which is present in multiple copies at centromeric location, plays a crucial role in recruiting outer kinetochore components. Mis12 complex binds N-terminal end of CENP-C, then the Ndc80 complex binds Mis12 through its globular domains Spc24/Spc25. These studies also unveiled the role of Aurora B kinase phosphorylation of the Mis12 complex, which enhances the binding affinity of the Mis12 complex to CENP-C. Past reconstructions in vitro used Mis12 and CENP-C proteins at concentrations exceeding their physiological levels. Moreover, CENP-C was used in a soluble form, while in cells the CENP-C scaffold is clustered at the centromere. Our recent work using other scaffolding protein CENP-T showed that binding interactions may differ significantly between the proteins in the soluble vs. clustered form, so some important features of the underlying binding mechanisms may remain unresolved. To overcome these limitations, we developed a novel approach in which the N-terminal ends of CENP-C are clustered using genetically engineered multimers (GEMs). This technique enables precise control of system components, as well as accurate measurement of the Mis12 complex binding to clustered CENP-C. We used real-time TIRF microscopy to track interactions between GFP-labeled CENP-C clusters containing 32 copies and the recombinant Alexa647-labeled Mis12 complex bound to the KNL1 fragment. At 100 nM of Mis12/KNL1, its binding saturation to CENP-C clusters was observed within few minutes, with a 1:1 ratio of Mis12/KNL1 to CENP-C molecules, as expected based on prior work. Subsequent addition of soluble Ndc80 complex used at 200 nM, which is similar to the estimated cellular concentration, led to recruitment of ~ 0.5 Ndc80 complexes per CENP-C, however, only about a third of these complexes were stable, as evidenced by the Ndc80-GFP brightness of the CENP-C/Mis12/KNL1 clusters. Surprisingly, when complex formation was assayed by incubating CENP-C clsuters with a mixture of 100 nM Mis12/KNL1 and 200 nM Ndc80, the binding of Ndc80 molecules increased two-fold, whereas the Mis12 recruitment to CENP-C clusters was not affected. This result may indicate that Mis12/KNL1 and Ndc80 first form a complex in solution and then their complex binds to CENP-C particles. Thus, Ndc80 binding recruitment is sensitive to the specific binding pathway and is stronger when Mis12 complex is present in a soluble form. Interestingly, the proportion of stably bound Ndc80 complexes remained unchanged regardless of the recruitment pathway, and the mechanisms limiting formation of stable Ndc80 assembly with CENP-C/Mis12/KNL1 clusters are unknown. The poster will describe our current efforts to test the role of Aurora B kinase in regulating both initial binding of Ndc80 to CENP-C/Mis12/KNL1 and formation of their stable assemblies.

Poster 13

A novel role for hepatic glucokinase in the regulation of cholesterol gene transcription and synthesis

Dominic Santoleri, Matthew Gavin, & Paul Titchenell

Gck is the primary glucose-sensing enzyme in the liver. It is responsible for converting glucose into G6P so it can be utilized by the liver either for energy or stored for later use as glycogen. Gck drives hepatic glucose uptake when stimulated by insulin and glucose, helping to regulate blood glucose levels. When fasting, hepatic Gck is inactivated by Glucokinase Regulatory Protein (GKRP), permitting the liver to release its glucose stores to feed the rest of the body. In order to test how Gck activity regulates liver metabolism, we performed RNA-seq on mice with acute deletion of Gck. Through RNA-seq, we found that Gck strongly suppresses genes involved in cholesterol synthesis, which we verified using isotope labeling and found increased triglyceride and cholesterol levels in L-GckKO mice.These results are contrary to strong evidence that Gck activation, either by Gck-activating drugs or by mutations in GKRP, results in elevated cholesterol levels. In particular, Genome-wide association studies (GWAS) have identified several GKRP single-nucleotide polymorphisms (SNPs) to be some of the highest correlators to increased triglyceride and cholesterol levels, which is attributed to increased glucose flux into triglyceride and cholesterol synthesis due to activated Gck. However, our evidence that Gck deletion has the same effect on cholesterol synthesis suggests that Gck may control cholesterol synthesis via a mechanism independent of its catalytic activity and glucose flux.

Poster 14

mTORC1 Controls Postprandial Hepatic Glycogen Synthesis Via Ppp1r3b

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In response to a meal, insulin drives hepatic glycogen synthesis to help coordinate systemic glucose homeostasis. The mechanistic target of rapamycin complex 1 (mTORC1) is a well-established insulin target and contributes to the postprandial control of liver lipid metabolism, autophagy, and protein synthesis. However, its role in hepatic glucose metabolism is less well understood. Here, we used metabolomics, isotope tracing, and mouse genetics to define a role for liver mTORC1 signaling in the control of postprandial glycolytic intermediates and glycogen deposition. We show that mTORC1 activity is required for glycogen synthase activity and glycogenesis. Mechanistically, hepatic mTORC1 promotes the feeding-dependent induction of Ppp1r3b, a phosphatase important for glycogen synthase activity whose polymorphisms are linked to human diabetes. Re-expression of Ppp1r3b in livers lacking mTORC1 signaling enhances glycogen synthase activity and restores postprandial glycogen content. Collectively, we identify a role for mTORC1 signaling in the regulation of Ppp1r3b expression and the subsequent control of postprandial hepatic glycogen synthesis.

Poster 15

Glucagon Blockade Can Prevent and Treat Hyperglycemia in Stress-Induced Hyperglycemia

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INTRODUCTION: Trauma patients are at risk of delayed death due to multiple organ failure and a strategy that may improve survival is controlling stress-induced hyperglycemia (SIH). SIH is a state of elevated blood glucose after injury or illness that is associated with organ failure and mortality. SIH is often attributed to insulin resistance as lowering glucose with insulin is associated with improved outcomes, however, mechanisms are unclear including how glucose contributes to pathophysiology or if other pathways drive SIH. Namely, trauma patients exhibit increased glucagon. Glucagon is a gluconeogenic hormone that promotes glucose production from the liver by activating protein kinase A and we found that inhibiting this enzyme prevents SIH. Excess glucagon has been implicated in the pathogenesis of Type II Diabetes and blocking glucagon has been explored as a treatment for chronic hyperglycemia. We hypothesized that blocking glucagon will prevent SIH.

METHODS: We used a mouse model known as fixed pressure trauma and hemorrhage (TH) to recapitulate SIH. This includes laparotomy, bilateral placement of femoral artery catheters, and 90 minutes of hemorrhage-induced hypotension. Glucose, insulin, and glucagon were measured at before and after 90 minutes hemorrhage (TH0 versus TH90, respectively). We performed the TH protocol in two models of glucagon blockade. A genetic model used the Cre-loxP inducible knockout system to make mice lacking the glucagon receptor in liver (L-GcgrKO). A second model used a glucagon receptor antagonist (GRA) drug to block glucagon interaction with its receptor. The first GRA cohort was pretreated with GRA via oral gavage an hour before TH. The second was given GRA via intraperitoneal injection in mice that exhibited SIH at 30 minutes into TH (TH30, defined by blood glucose >200 mg/dL).

RESULTS: Glucose, insulin, and glucagon increased at TH90 compared to TH0 in wild-type mice ($p<0.001$, $p<0.05$, and $p<0.05$ respectively). L-GcgrKO mice were protected from SIH including decreased glucose levels at TH90 compared to controls (Figure 1A, $p<0.0001$) and lower insulin ($p<0.05$). Radioactive tracer infusion experiments suggest this is due to a reduction in hepatic glucose production. Mice pretreated with the GRA for an hour before the TH protocol were also protected from SIH, namely having lower glucose compared to controls ($p<0.01$, Figure1B). Finally, mice treated with the GRA at TH30 exhibited lower glucose levels at TH90 ($p<0.01$, Figure 1C) and a trend toward lower insulin ($p=0.07$).

CONCLUSION: Glucagon is a significant driver of SIH and blocking glucagon is sufficient to prevent and treat the elevated glucose levels seen in SIH. Future work will determine if treating SIH with a GRA can improve survival and prevent multiple organ failure.

Poster 16

Targeting the Activin Type II Receptor Improves Quality of Weight Loss Driven by GLP-1 Receptor Agonism

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Obesity is a chronic, multifactorial disease that continues to rise in prevalence worldwide, and common lifestyle interventions such as alterations to diet and levels of physical activity often fail to help individuals lose and, importantly, sustain significant amounts of weight loss. The recent FDA approval of glucagon-like polypeptide 1 (GLP-1) receptor agonist semaglutide represents a significant breakthrough in effective pharmacological interventions for weight loss. In clinical trials, high-dose weekly injections led to mean total body weight loss of 14.9% over 68 weeks in adults, however, roughly 40% of this weight loss is due to loss of lean mass. Proposed treatment strategies that seek to improve the quality of weight loss, or composition lean vs. fat mass lost, may help to improve long-term sustainability of weight loss. Targeting signaling pathways regulating the size of skeletal muscle is a promising approach to modulating body composition during weight loss; signaling through the activin type II receptor (ActRII) or through the PI3K/Akt/mTOR axis works to promote or oppose skeletal muscle atrophy, respectively. In this study, we utilized a combined treatment strategy of anti-ActRII antibody bimagrumab and GLP-1 receptor agonist semaglutide to evaluate changes in body composition and systemic physiology in a mouse model of obesity, as well as muscle-specific deletion of Akt to elucidate the signaling pathways required for skeletal muscle hypertrophy resulting from blockade of ActRII signaling. We show that bimagrumab induces a nearly 10% increase in lean mass weight in both lean and obese mice over a two-week period, and in obese mice, treatment with bimagrumab plus semaglutide successfully prevents loss of lean mass while leading to fat mass loss greater than with semaglutide alone. These results indicate that targeting ActRII improves body composition during weight loss driven by GLP-1 receptor agonism. In future studies, we will evaluate the effects of ActRII blockade on energy expenditure and its potential to more effectively maintain weight loss long-term.

Poster 17

Myosin-I facilitates symmetry breaking and promotes the growth of actin comet tails

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Actin assembly stimulated by the Arp2/3 complex provides pushing forces for a variety of cellular processes. Type-I myosins (myosin-Is) are a class of actin binding motor proteins that are commonly found alongside Arp2/3-mediated branched actin networks at membrane interfaces and participate in actin-mediated force generation. Yet, the molecular effects of how myosin-Is interact with the actin network remain elusive. To further investigate the roles of myosin-I in actin-mediated force generation, we reconstituted an *in vitro* actin-based motility system, where branched actin networks were nucleated by Arp2/3 complex from a micron-sized bead surface coated with Arp2/3 activating factors. Actin filaments first formed a symmetric shell around the bead, which transitioned into a polarized comet tail after symmetry breaking and propelled the bead forward. We site-specifically coupled a range of densities of myosin-Is to the bead surface and assessed their effects on actin polymerization, network architecture, and symmetry breaking. We found that myosin reduced the density of actin network while maintaining comparable or even faster comet growth rates. Under conditions where actin shells were too dense to be fractured by polymerization forces alone, myosin promoted symmetry-breaking. Furthermore, when actin assembly was arrested around the bead surface before symmetry breaking, we found that myosin motor activity alone can break the sparse actin shell. By employing rigor myosin, we confirmed that these emergent effects primarily stem from the force-generation power-stroke of myosin-I, rather than its dynamic actin tethering properties. Our results suggest that myosin-I is likely involved in the branched actin assembly by facilitating more efficient force-production through its power-stroke.

Poster 18

Mitochondrial damage in astrocytes triggers PINK1/Parkin mitophagy and NF-κB-mediated inflammation

Julia F. Riley & Erika L.F. Holzbaur

Astrocytes are the most abundant cell type in the brain, and their ability to promote neuroinflammation is a key aspect of Parkinson's disease (PD) pathology. Mitochondrial dysfunction, another hallmark of PD, triggers inflammation in many cell types. In fact, mutations in the enzymes PINK1 and Parkin, which facilitate clearance of damaged mitochondria via mitophagy, are sufficient to cause PD. However, the mechanism of clearance for damaged mitochondria and the precise inflammatory consequences of prolonged mitochondrial damage have not been investigated in astrocytes. Here, we use OXPHOS inhibitors to induce mitochondrial damage in a murine astrocyte model. We show that damaged mitochondria accumulate both phospho-ubiquitin and Parkin in a dose-dependent manner, thus targeting them for clearance via PINK1/Parkin mitophagy. To determine whether this mechanism is conserved in the presence of neurons, we developed a novel model for co-culturing iPSC-derived human neurons and murine astrocytes. Using this model, we corroborate our findings in monoculture that mitophagy occurs in astrocytes. Our lab recently discovered that mitochondria targeted for mitophagy can also recruit the NF-κB effector molecule (NEMO), allowing them to act as platforms directly promoting NF-κB inflammation. Using the same damage paradigm that induces mitophagy in astrocytes, we observe robust NEMO recruitment to damaged mitochondria. Through qPCR, we confirm that levels of the NF-κB-associated cytokines TNFα and IL6 are elevated in astrocytes following mitochondrial damage, and this response is ameliorated in PINK1$^{-/-}$ astrocytes. Together, these results suggest that the pathway responsible for turnover of damaged mitochondria in astrocytes also stimulates NF-κB activation, implicating this as a novel mechanism by which mitochondrial damage in astrocytes can promote neuroinflammation.

Poster 19

FMRP Granules Locally Control Mitochondrial Fission in Neurons

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Mitochondrial health and function are maintained in neurons by network remodeling and local translation, which allow for rapid responses to changing cellular demands. Yet, it is not understood how neurons orchestrate the timing and positioning of translation or how local translation is coupled with mitochondrial dynamics to maintain network integrity. The Fragile X Messenger Ribonucleoprotein Protein (FMRP) is a critical regulator of translation in neurons whose absence causes Fragile X Syndrome, a severe neurodevelopmental disorder. Loss of FMRP disrupts mitochondrial health in neurons, resulting in a fragmented network with impaired metabolic function. However, the mechanism by which FMRP supports mitochondrial homeostasis in neurons is not known. Here, we use DNA-PAINT super-resolution microscopy and live-cell confocal microscopy to demonstrate close contacts between FMRP granules and mitochondria in mammalian neurons. FMRP preferentially clusters at sub-organellar regions of mitochondria: the ends and the midzone. End contacts are dynamic and allow for long-distance co-transport of FMRP with mitochondria. Midzone-associated FMRP marks sites of mitochondrial fission, where the FMRP granule stays associated with the mitochondrial end following fission. Endosomes are known to associate with sites of mitochondrial fission and to deliver RNA granules to mitochondria in neurons. We demonstrate that endosomes contact FMRP granules and contribute to their positioning at mitochondrial ends and fission sites. Using a combination of live-imaging, RNA *in situ* hybridization, immunocytochemistry, and cryo-electron tomography, we demonstrate that mitochondria-associated FMRP granules are ribosome-rich sites of protein synthesis, which contain mRNAs for nuclear-encoded proteins that drive mitochondrial function and dynamics. Further, mitochondrial fission at FMRP granules is dependent on protein synthesis and facilitated by local translation of Mitochondrial Fission Factor within these granules. These findings reveal a role for FMRP in the control of mitochondrial fission and suggest that FMRP granules serve as platforms to selectively regulate the dynamics of individual mitochondria in distal parts of neurons.

Poster 20

Possible Regulation of Myosin-19 by the E3-Ligase MARCH5

Cameron P. Thompson, Erika L.F. Holzbaur, & E. Michael Ostap

Myosin-19 (Myo19) is a mitochondrially-localized, actin-based motor that has been shown to play a role in regulating mitochondrial morphology and health. Previous work has shown that Myo19 localization to the mitochondria is tightly controlled, and motors not bound to the organelle are largely degraded. The mechanisms for control of this system have not been fully discerned. Initial experiments have shown that Myo19 is ubiquitylated in the cell, in agreement with previously reported whole proteome ubiquitylation analysis. Previous work from our lab found that the E3-ligase MARCH5 co-purifies with Myo19, inspiring our further investigation into the potential role of MARCH5 in Myo19 regulation. Through knockdown experiments, we found that both total cellular and mitochondrially localized protein levels of endogenous Myo19 are not changed by loss of MARCH5. Interestingly, MARCH5 expression levels affect the presence of a proteolyzed subpopulation of Myo19 that is ~20kDa smaller than the full-length motor. Furthermore, it appears that the proteolysis is occurring at the N-terminus of the motor which would render it unable to bind actin filaments. We hypothesize that MARCH5 is involved in the partial proteolytic regulation of Myo19 that produces a polypeptide incapable of actin binding. We speculate that this regulatory pathway could explain how the cell tightly controls the levels of functional Myo19 on the mitochondria, therefore ensuring proper organelle morphology and health.

Poster 21

Microtubule motors coordinate mRNA export and transport to direct local translation in the cardiomyocyte

Emily A. Scarborough, Rani M. Randell, & Benjamin L. Prosser

Introduction
The post-mitotic cardiomyocyte (CM) must maintain proteostasis while also preserving the ability to rapidly remodel, particularly during times of cardiac growth. How the CM couples transcription and translation in space and time for normal maintenance of the cell is understudied, and thus it is also unclear how these programs are co-opted to enable hypertrophy. Our lab's previous work has indicated the necessity of microtubules (MTs) and kinesin-1 in proper localization of transcripts, translation and growth of the CM, yet the molecular mechanisms that direct specific mRNAs to subcellular regions for local translation remain opaque.

Research Questions
We hypothesize that changes to MT PTMs and/or the stability of the MT network are sufficient to change the subcellular localization of motors, mRNA and translation. We additionally postulate that changes to the MT network during initial cardiac growth facilitates mRNA transport and translation.

Methods/Approach
To visualize mRNA and protein localization in CMs, we couple immunofluorescence (IF) and smFISH. To measure translation of specific transcripts or bulk translation, we methionine-deplete and use methionine-analogues and click chemistry. To measure cell size, we use live cell imaging of a membrane dye.

Results
We find that the CM contains at least three MT-dependent subdomains for mRNA localization and local translation, and that some transcripts require proper localization for translation. Partial stabilization through sub-stoichiometric taxol treatment results in remaining dynamic MTs (tyrosinated tubulin) at the nuclear short axis in the absence of stable MTs (detyrosinated tubulin), leading to nuclear wrinkling. We observe mRNA, kinesin-1, dynactin1 and NUP153 relocalization to these wrinkles. This relocalization of mRNA and mRNA export machinery is sufficient to relocalize active translation in the adult cardiomyocyte. Additionally, taxol treatment in neonatal rat CMs results in a similarly asymmetric mRNA and nucleoporin localization, concomitant with an increase in width along this axis.

Conclusions
A precise balance of dynein/dynactin1 and kinesin-1 in the CM is necessary for proper mRNA localization, which is at least partly established by MT PTMs. Partial stabilization of the MT network reveals a role for motors and nucleoporins in coupling mRNA export and transport. We propose that perturbed mRNA export/localization may alter downstream translation to direct the growth of the CM.

Poster 22

Characterization of STXBP1 haploinsufficiency in iPSC derived cortical neurons

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Objective:
Characterize phenotypes of *STXBP1* mutations within iPSC derived glutamatergic neurons for potential avenues of therapeutic readouts.

Background:
Syntaxin binding protein 1 (STXBP1), is a pre-synaptic protein that plays an essential role in SNARE complex formation and function critical for neurotransmitter release from synaptic vesicles, vesicle docking and fusion to the membrane. Often, patients diagnosed with mutations in *STXBP1* suffer from STXBP1 encephalopathy, a neurodevelopmental disorder, that is hallmarked by epilepsy and intellectual disabilities.

Methods:
STXBP1 Patient derived iPSC lines and isogenic controls were differentiated into glutamatergic and GABAergic neurons. Immunofluorescence (IF), western blotting (WB), and quantitative PCR (qPCR) were utilized to confirm *STXBP1* haploinsufficiency in iPSC neurons. Multielectrode arrays (MEA) were used to investigate electrophysiological defects due to *STXBP1* mutations. Ca^{2+} live cell imaging was used to characterize altered Ca^{2+} kinetics.

Results:
WB and qPCR analysis revealed that our iPSC derived neurons harboring *STXBP1* mutations had significantly reduced mRNA and protein levels allowing confirmation of *STXBP1* haploinsufficiency compared to isogenic controls. Further, IF stains revealed altered STXBP1 and Syntaxin1 signal intensities as well as altered cellular localization in mutant *STXBP1* neurons compared to corrected neurons. Ca^{2+} live cell imaging demonstrated shortened decay kinetics in spontaneous bursting, as well as altered fatigue kinetics under evoked conditions. Finally, MEA analysis at the spike, burst, and network burst revealed that *STXBP1* mutations in neurons alter mean firing rates, network burst and burst durations, and burst frequency.

Conclusion:
In sum, *STXBP1* mutations in iPSC derived cortical neurons recapitulate haploinsufficiency confirmed by WB and qPCR and reveal altered cellular localization of STXBP1 and SNARE complex proteins. In addition, *STXBP1* mutant neurons display altered Ca^{2+} bursting kinetics and electrophysiological signatures detected by MEA. These findings indicate that this model is appropriate for investigating pathological mechanisms of STXBP1 haploinsufficiency. Importantly, this model can further be utilized in examining therapeutic efficacy aimed at upregulating wild type STXBP1 levels.

Poster 23

Translational Readthrough Inducing Drug (TRID) Effects On Eukaryotic Termination Investigated At The Single-Molecule Level

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Many genetic disorders are caused by premature stop codon (PSC) mutations, but only one TRID (Translational readthrough-inducing drug), ataluren, has been approved for clinical use. Recently, we used single-molecule TIRF with a cell-free *in vitro* assay and found that ataluren competitively inhibits productive release factor complex (RFC, eRF1.eRF3.GTP) binding to the pre-termination complex. We found that such inhibition occurs before or at the peptidyl-tRNA hydrolysis step (Huang *et al., Nat. Comm.,* 2022, 13: 2413). Here we report new results using an sm-FRET assay with a Cy3-labeled peptidyl-tRNA bound in the ribosomal P-site adjacent to a UGA stop codon in the A-site and Cy5-labeled human eRF1. Upon RFC binding, we observe transient FRET efficiency, E = ~0.25, between Cy5-eRF1 and Cy3-tRNA, consistent with the successful accommodation of eRF1 within the A-site. Following peptidyl-tRNA hydrolysis, we find a strong correlation between eRF1 and tRNA dissociation times, unlike the weak correlation between peptide release and tRNA dissociation times that we observed previously (Huang *et al., ibid*.). Additionally, we found that ataluren and added near-cognate suppressor tRNA each had a significant inhibitory effect on the arrival time of RFCs at the ribosome, and that ataluren in combination with the aminoglycoside G418 had an even stronger inhibitory effect compared with ataluren or G418 alone. These results support our earlier suggestion (Ng *et al., PNAS*, 2021, 118: e2020599118) that ataluren or ataluren-like TRIDs could potentiate the therapeutic effects of aminoglycosides on PSC diseases. We expect that our ongoing studies will aid in elucidating the readthrough mechanisms of other TRIDs leading to enhanced and safer treatments of PSC diseases.

Poster 24

Alkaline taste sensation through the alkaliphile chloride channel in *Drosophila*

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Animals, ranging from insects to mammals, rely on their sense of taste to detect various types of food. Food pH is crucial in assessing its palatability and nutritional value, influencing food sampling and preference. Since maintaining an appropriate pH is vital for almost all physiological processes, excessive consumption of alkaline food can lead to alkalosis, a condition associated with severe health problems such as coma. While acid-sensing mechanisms have been extensively studied, the mechanisms underlying the perception of alkaline foods remained unclear.

In this study, we employed the fruit fly, *Drosophila melanogaster*, as a model organism to investigate the molecular and cellular mechanisms involved in the detection of alkaline food. Through a genetic screen, we identified alkaliphile (Alka), a member of the ligand-gated chloride channel (LGCC) family in flies, as an alkaline taste receptor. Alka was expressed in a distinct group of gustatory receptor neurons (GRNs) in the fly's peripheral taste organ. While wild-type flies naturally displayed an aversion to alkaline food, *alka* mutant flies exhibited significant impairments in their ability to avoid such food. Consistently, we observed a profound reduction in the number of action potentials fired by the alkaline GRNs of *alka* mutant flies compared to the wild type when exposed to high-pH stimuli. Furthermore, our patch-clamp recordings showed that Alka formed a chloride (Cl^-) channel, which was selectively activated by hydroxide ions (OH^-). Moreover, through optogenetic activation of *alka*-expressing GRNs, we found that the flies rejected sweet food. Conversely, inhibiting these alkaline GRNs rendered the flies insensitive to alkaline food.

In summary, our work establishes Alka as a novel class of taste receptors dedicated to sensing alkaline pH in flies. This discovery expands our understanding of taste receptors and their role in pH detection, opening up new avenues for exploring the mechanisms underlying alkaline taste perception across different species.