



**University of Pennsylvania**  
**Department of Physiology**  
**Retreat: 9/16/2022**

## Retreat Outline 9/16/22

<b>8:30 – 9:00</b>	Check-in/Breakfast
<b>9:00 – 9:10</b>	Welcome by J. Kevin Foskett, PhD & DEI Committee
<b>9:10 – 10:10</b>	Morning Session I (Moderator: Robert Cail)
9:10 – 9:25	<b>Olivia Harding</b> - <i>Damaged mitochondria recruit the effector NEMO to activate NF-<math>\kappa</math>B signaling</i>
9:25 – 9:40	<b>Jennifer M. Petrosino</b> – <i>tRNA localization in the heart is a microtubule-dependent process.</i>
9:40 – 9:55	<b>Jose A. Martinez-Sarmiento</b> - <i>Super-resolution imaging reveals temporal changes in chromatin structure and gene activity in single reprogramming cells</i>
9:55 – 10:10	<b>Ekaterina V. Tarasovets</b> – <i>Clustering of the scaffolding protein CENP-T activates recruitment of Ndc80 complexes to assemble a functionally active outer kinetochore.</i>
<b>10:10 – 11:10</b>	Departmental picture + Coffee Break + Poster Session I
<b>11:10 – 12:10</b>	Morning Session II (Moderator: Megan Stefkovich)
11:10 – 11:25	<b>Stephen M. Coscia</b> – <i>An interphase actin wave promotes mitochondrial content mixing to maintain cellular homeostasis.</i>
11:25 – 11:40	<b>Natasha Jaiswal</b> – <i>Role of skeletal muscle AKT signaling in the regulation of glucose homeostasis.</i>
11:40 – 11:55	<b>Emily Fernández García</b> – <i>The mitochondrial Ca<sup>2+</sup> channel MCU is critical for tumor growth by supporting cell cycle progression and proliferation.</i>
11:55 – 12:10	<b>Zoey A. Miller</b> – <i>The bitter taste of cancer: targeting bitter taste receptor 14 to kill head and neck squamous cell carcinoma.</i>
<b>12:10 – 1:15</b>	Lunch
<b>1:15 – 2:00</b>	Afternoon Session I (Moderator: Volodya Demidov)
1:15 – 1:30	<b>Dawei Song</b> – <i>Hyperelastic continuum models for isotropic athermal fibrous networks.</i>
1:30 – 1:45	<b>Rachel H. Ceron</b> – <i>A solution to the long-standing problem of actin expression and purification.</i>
1:45 – 2:00	<b>Pavan Vedula</b> – <i>Actin' Different? Distinct in vivo functions of cytoplasmic actin proteins.</i>
<b>2:00 – 3:00</b>	Coffee Break + Poster Session II
<b>3:00 – 3:45</b>	Keynote: <b>Natali Chanaday, PhD</b> <i>Novel pathways regulating neurotransmitter release.</i>
<b>3:45 – 4:00</b>	Closing + awards
<b>4:00 - 5:00</b>	Reception + softball

# Poster Session I (10:10 – 11:10am)

	Presenting author	Poster Title
1	DEI committee	DEI committee
2	Adam Fenton	FMRP granules are dynamically positioned along mitochondria to regulate fission in neurons
3	Caroline Perry	Testing NAD <sup>+</sup> precursors in a progressive model of Friedreich's Ataxia
4		
5	Elana Baltrusaitis	Mechanism of MIRO-mediated activity and recruitment of cytoskeletal effector TRAK in mitochondrial dynamics
6	P. L. Colosi	Developing quantitative methods to define stoichiometry of large protein complexes using super-resolution microscopy
7	Julia Riley	PINK1/Parkin mitophagy in astrocytes
8	Xuechen Shi	Gigantic polyacrylamide hydrogels for large-batch cell culture
9	Jaclyn E. Welles	Investigating the role of phosphatidylcholine in NAFLD/NASH-associated inflammation and fibrosis
10	Mengqi Xu	Myosin-Is promote the growth of actin 'comet tails'
11	Olivia Harding	Damaged mitochondria recruit the effector NEMO to activate NF-κB signaling
12	Aleksandr Maiorov	Reconstitution of the CENP-C-dependent kinetochore assembly pathway using CENP-C multimers <i>in vitro</i>
13	Jody Dantzig	mCLIFY: monomeric, bright, circularly-permuted yellow fluorescent protein
14	Pamela Gallo	Discovering TRPV2 channel molecular function via proximity proteomics and electron microscopy
15	Anastasia A. Masaltseva	Positive feedback loop in platelet dense granule secretion promotes rapid formation of a thrombus shell
16	Bridget McVeigh	Subcellular localization patterns and protein-protein interaction networks of wild-type human TRPML1 and mild and severe Mucopolipidosis type IV-causing mutations
17	Jillian S. Weissenrieder	The mitochondrial calcium uniporter in pancreatic cancer growth, proliferation, and metastasis
18	Behnaz S. Eftekhari	Electrical stimulation human mesenchymal stem cells on conductive chitosan-polyaniline substrates promotes neural priming
19	Cameron P. Thompson	<i>In vitro</i> study of mitochondrial motility and myosin-19 dynamics
20	Bishal Basak	Striking the balance of mitochondrial quality control between selective and non-selective autophagy
21	Jennine M. Dawicki-McKenna	Mapping PTBP splicing in human brain identifies targets for therapeutic splice switching including <i>SYNGAP1</i>
22	Adriana Naomi Santiago-Ruiz	Super-resolution imaging of Alzheimer's disease hyperphosphorylated tau aggregates
23	Alex J. Felix	Targeting miRNAs using antisense oligonucleotides as a novel therapeutic strategy for STXBP1 encephalopathy
24	Rafaella T. Shima	High-throughput screen to identify NAD <sup>+</sup> boosting compounds

## Poster Session II (2:00 – 3:00pm)

	Presenting author	Poster Title
25	DEI committee	DEI committee
26	Quentin McAfee	Truncated titin in the myocardial sarcomere in human dilated cardiomyopathy
27	Arpan Bhattacharya	Single-molecule studies of a translational readthrough inducing drug (TRID) effect on eukaryotic termination
28	Deborah M. Eaton	ZSF-1 obese rats develop features of HFpEF
29	Him Shweta	Using smFRET to understand structural dynamics of sex-biased RNA helicases DDX3X and DDX3Y
30	Kyle R. Barrie	Mechanism of capping protein regulation by CARMIL
31	David Smith	Membrane lipids as an integral gating component of the large-conductance Ca <sup>2+</sup> - and voltage-gated Slo1 BK channel
32	Li Chen	Arginylation regulates cytoskeleton organization, cell division, and mitochondria function in fission yeast
33	Beishan Chen	NAD precursors as a treatment for acetaminophen-induced liver injury and acute alcohol-induced liver toxicity
34	Adam A. Jabak	Exploring the effects of RNA modifications on eukaryotic translation elongation and termination
35	Kahealani Uehara	mTORC1 controls postprandial hepatic glycogen deposition via <i>Ppp1r3b</i>
36	Megan Stefkovich	Insulin's acute regulation of hepatic glucose uptake and utilization
37	Faviolla A. Baez-Cruz	<i>Drosophila</i> Myo1C and Myo1D are high duty ratio motors with very high ADP affinity
38	Luther W. Pollard	Human Myo19 ensembles bound to calmodulin and regulatory light chain drive multi-micron transport
39	Juliet Goldsmith	Proteomic profiling identifies distinct mechanisms for the autophagic clearance of nucleoid-enriched mitochondria in Parkinson's disease
40	Oindrila Paul	Pulmonary endothelial activation with COVID-19: possible role of reactive oxygen species
41	Anna E. Garcia Whitlock	Glucagon blockade can prevent and treat stress-induced hyperglycemia
42	Fitzroy Byfield	Substrate stiffness, but not the absence of a vimentin cage, modulates the mechanical properties of the nucleus
43	Daria A. Pavlov	Dynamic nuclear-sarcomeric coupling in the beating cardiomyocytes
44	Derek B. McMahon	Savory signaling: T1R umami receptor modulates ER calcium store content and calcium release in airway epithelial cells
45	Rafaella T. Shima	Impact of the mitochondrial NAD transporter SLC25A51 on respiratory complexes of the electron transport chain
46	Dominic Santoleri	Global-run on sequencing in liver identifies <i>Gm11967</i> as an Akt-dependent long noncoding RNA involved in insulin sensitivity
47	Sierra Palumbos	Interplay between secretory autophagy and macroautophagy in neurons
48	Roman R. Kerimov	Development of microfluidic flow chambers to study blood clotting <i>ex vivo</i>

# Keynote

Afternoon keynote session (3:00 – 3:45)

## **Novel pathways regulating neurotransmitter release**

Natali L. Chanaday

Inside our brains, neurons communicate via electrical signals that cause the release of chemical neurotransmitters. This fast form of neurotransmission allows to control our movement and encode our feelings of joy for science as well as our frustration about those darn experiments and grants that don't turn out. However, in our brain another mysterious form of neurotransmitter release is going on independently of electrical signals at synapses. This pathway of neurotransmitter release was named "spontaneous" in 1952 by Bernard Katz, and 70 years later we are still trying to understand the mechanism regulating this form of neurotransmission and its purpose in the brain. In this seminar we will discuss novel pathways that can modify spontaneous synaptic vesicle trafficking and neurotransmitter release. The first part will focus on store operated calcium entry, a calcium pathway that can increase glutamate release from neurons and exacerbate neurodegeneration during chronic stress. The second section will move the focus to the extracellular space and study secreted vesicles, i.e. extracellular vesicles, that mediate inter-neuronal communication via the exchange of synaptic proteins and can modulate GABA release from the target neurons.

# Oral presentations

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

## **Damaged mitochondria recruit the effector NEMO to activate NF- $\kappa$ B signaling**

Olivia Harding<sup>1,2</sup> & Erika L. F. Holzbaur<sup>1,2</sup>

<sup>1</sup>Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

<sup>2</sup>Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD.

Mitochondrial damage presents an immediate danger to cellular homeostasis. Dysfunctional mitochondria fail to produce the energy required to maintain cellular function and can release reactive oxygen species or initiate other damaged-induced signaling pathways that promote inflammatory responses. Mitophagy is a quality control pathway by which damaged mitochondria are isolated and cleared via the activation of the ubiquitin ligase Parkin and subsequently, mitophagy receptors. Mutations that impair mitophagy or environmental perturbations to the clearance pathway are associated with neurodegenerative diseases including Parkinson's disease and ALS. Here we demonstrate a novel link from mitochondrial dysfunction to the activation of NF- $\kappa$ B, the principal transcription factor that regulates immune activation. Following mitochondrial damage, the NF- $\kappa$ B essential regulator NEMO/IKK $\gamma$  is recruited to mitochondria in a Parkin- and p62/SQSTM1-dependent manner. Although NEMO and the well-studied mitophagy receptor Optineurin (OPTN) are structurally similar, NEMO and OPTN maintain separate domains on the outer mitochondrial membrane. Instead, NEMO colocalizes extensively with p62, suggesting that NEMO and p62 together form phase-condensates that can activate the NF- $\kappa$ B inflammation pathway via the kinase IKK $\beta$ . Indeed, active phospho-IKK $\beta$  colocalizes with NEMO on damaged mitochondria, and cells with mitochondrial damage exhibit upregulation of inflammatory cytokines. These findings demonstrate that damaged mitochondria serve as an intracellular platform for innate immune signaling by promoting the formation of activated IKK complexes in a Parkin-dependent manner. Finally, mitochondria that recruit NEMO are less likely to be engulfed by clearance autophagosomes. Thus, we propose that mitophagy and NF- $\kappa$ B signaling are competing pathways regulating the response to cellular stress.

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

### **tRNA localization in the heart is a microtubule-dependent process.**

Jennifer M. Petrosino<sup>1,2</sup>, Keita Uchida<sup>1</sup> & Benjamin L. Prosser<sup>2</sup>

<sup>1</sup>Department of Physiology, Pennsylvania Muscle Institute, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.

<sup>2</sup>Department of Physiology, Pennsylvania Muscle Institute, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.

In response to pathological and physiological stress, the heart can undergo remodeling and cardiomyocyte hypertrophy. For hypertrophy to occur, cardiomyocytes must increase levels of protein synthesis and subsequently correctly localize those proteins. However, before the synthesis of new proteins can even occur, the cardiomyocyte first relies on the active transport of messenger and ribosomal RNAs along the microtubule network. Transfer RNAs (tRNA), the most abundant non-coding RNA, are historically believed to solely rely on passive diffusion for transport within cells, and function as nexus molecules linking transcription and translation. Here, we give the first report detailing that tRNAs in cardiomyocytes require the microtubule network for proper localization. Super-resolution microscopy suggests that tRNAs are distributed along the microtubule tracks throughout the cardiomyocyte. Imaging of endogenous tRNA<sup>Gly-GCC</sup>, tRNA<sup>Gly-CCC</sup>, and tRNA<sup>Pro-AGG</sup>, by fluorescent *in situ* hybridization following 500nM nocodazole treatment, demonstrated that in the absence of a microtubule network tRNAs accumulate in and around the nucleus, and in patterns that are distinct to each tRNA species. Additionally, treatment with the pro-hypertrophic stimulus phenylephrine resulted in distinct changes in tRNA<sup>Gly-GCC</sup> distribution and biogenesis. To further examine the ability for tRNAs to undergo active transport in cardiomyocytes, Kinesin 1, the predominate motor protein in the heart was knocked down using an adenovirus. Indeed, the loss of Kinesin 1 resulted in the mislocalization of tRNAs and their peri-nuclear accumulation, ultimately suggesting a role for Kinesin 1 in the transport of tRNA. Together our findings support the novel notion that tRNAs undergo active transport, and require the microtubule network for proper localization, in the mammalian heart.

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

### **Super-resolution imaging reveals temporal changes in chromatin structure and gene activity in single reprogramming cells**

Jose A. Martinez-Sarmiento<sup>1,3,4</sup>, Maria P. Cosma<sup>3,4,5,6</sup> & Melike Lakadamyali<sup>1,2</sup>

<sup>1</sup>Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

<sup>2</sup>Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

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<sup>5</sup>Guangzhou Regenerative Medicine and Health Guangdong Laboratory (GRMH-GDL), Guangzhou, China

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During reprogramming, the predominantly compact chromatin of somatic cells extensively reorganizes to acquire a pluripotent-like configuration; however, understanding this process and their associated gene expression changes is precluded by the limitations of iPSC systems to investigate the correctly reprogramming cells. To address these questions, we employed the highly efficient heterokaryon system (somatic/pluripotent cell fusion) to study, at the single-cell level, the spatial and epigenetic changes to the chromatin nano-structure and gene reactivation at the onset of pluripotency conversion using advanced microscopy techniques. Using super-resolution microscopy, we revealed that within 6h and 48h following fusion of human fibroblasts with mouse ESCs, there is a dramatic decrease of the repressive histone modification marks H3K9me3 and H3K27me3 at the somatic nucleus; and surprisingly, no overall changes in the active marks H3K9ac and H3K4me3. These changes preceded a global chromatin de-condensation occurring 48h after fusion. Notably, we detected by RNA-FISH nascent OCT4 transcripts at 6h and 12h after fusion, followed by mature RNA production at 24h and 48h in >70% of heterokaryons. Conversely, NANOG showed a delayed reactivation kinetics with nascent expression detected until 48h after fusion, in ~20% of heterokaryons. Finally, using OligoSTORM we showed that OCT4 and NANOG genomic regions undergo differential nano structural changes with NANOG exhibiting increased opening. Altogether, our findings suggest that during reprogramming cells undergo a global chromatin de-compaction preceded by repressive histone mark decommission; whereas OCT4 and NANOG exhibit differential gene reactivation and chromatin decondensation dynamics with OCT4 being uncoupled from the global chromatin decondensation stage.



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

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

Ekaterina V. Tarasovets<sup>1</sup>, Gunter B. Sissoko<sup>2</sup>, Aleksandr Maiorov<sup>1</sup>, Iain M. Cheeseman<sup>2</sup> & Ekaterina L. Grishchuk<sup>1</sup>

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<sup>2</sup>MIT Department of Biology, Cambridge, MA; Whitehead Institute for Biomedical Research, Cambridge MA.

Proper kinetochore assembly requires coordinated recruitment of multiple copies of different proteins. We recently discovered that clusters of the kinetochore scaffolding protein CENP-T, but not monomeric CENP-T molecules, trigger assembly of kinetochore-like particles in the cytoplasm of HeLa cells. The assembled “T-particles” host microtubule-binder Ndc80 and other outer kinetochore proteins; moreover, isolated from cells they bind to microtubules and move processively with dynamic microtubule tips. To investigate the mechanism that leads to assembly of an 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 present in either clustered or monomeric forms. Both forms of CENP-T bind two Ndc80 complexes, but Ndc80 molecules dissociate significantly faster from monomeric compared to clustered CENP-T. 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 weakly-bound Ndc80 molecules around CENP-T clusters. The formation of such molecular clouds was not observed with monomeric CENP-T, which experienced much slower maturation. 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.

## Morning Session II – Talk 5 (11:10 – 11:25)

### **An interphase actin wave promotes mitochondrial content mixing to maintain cellular homeostasis**

Stephen M. Coscia<sup>1</sup>, Andrew S. Moore<sup>2</sup> & Erika L. F. Holzbaur<sup>1</sup>

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<sup>2</sup>Howard Hughes Medical Institute, Janelia Research Campus, Ashburn, VA, USA.

Cellular health is dependent on mitochondrial homeostasis and the proper regulation of mitochondrial dynamics. We discovered a mechanism regulating mitochondrial dynamics via a cycling wave of actin polymerization/depolymerization. In metaphase the force of actin polymerization propels mitochondria in a comet-tail mechanism, resulting in equivalent partitioning of mother cell mitochondria between daughters. Actin cycling persists in interphase, however during this cell cycle stage F-actin assembly leads to mitochondrial fission, as we observed fragmentation dependent on the essential fission mediator DRP1. We hypothesized that cycling produces force which is resisted by tethering of mitochondria to microtubules, leading to mitochondrial tubulation and thus fission. In support, upon chemical microtubule depolymerization, actin wave-associated mitochondria ceased to fragment and instead displayed enhanced motility as revealed by tracking and displacement index analysis. Next, we focused on the machinery driving cycling, which is blocked by inhibitors of the kinase CDK1. We probed for the involvement of putative CDK1-regulated F-actin nucleators, including the formin FMNL1. Depletion of FMNL1 blocked actin cycling and expression of a non-phosphorylatable mutant at the CDK1 site had a dominant negative effect. We next probed the interphase function of this wave. We noted a loss of mitochondrial membrane potential, as measured by TMRE uptake, in cells depleted of FMNL1. Moreover, Seahorse analysis indicated that FMNL1-depleted cells consumed oxygen at a slower rate, leading to lower ATP levels. While these data indicate that interphase cycling is required to maintain mitochondrial health, we found that the actin wave continues to propagate following CCCP-induced mitochondrial depolarization, indicative that altered mitochondrial health does not feed-back to inhibit cycling. Finally, we asked if the interphase wave promotes mitochondrial content mixing, required to maintain mitochondrial function. In support, inhibition of cycling blunted the spread of mitochondrially-targeted photoactivatable GFP and mitochondrial tracking revealed that mitochondria fragmented by the wave often re-fused with distinct neighbors. Thus, we propose that interphase actin cycling maintains mitochondrial health by enhancing content mixing to promote inter-organelle complementation.

## Morning Session II – Talk 6 (11:25 – 11:40)

### **Role of skeletal muscle AKT signaling in the regulation of glucose homeostasis**

Natasha Jaiswal<sup>1</sup>, Matthew Gavin<sup>1</sup>, Louise Lantier<sup>2</sup>, David H. Wasserman<sup>2</sup> & Paul M. Titchenell<sup>1,3</sup>

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Insulin resistance is considered to be the principal factor underlying several metabolic diseases including type II diabetes. Since, skeletal muscle is the predominant site of insulin-mediated glucose uptake in the postprandial state, a reduction in the insulin signaling pathway of diabetic skeletal muscle is widely considered to be the primary cause of postprandial hyperglycemia. The serine/threonine kinase AKT is a central regulator of insulin action and a decrease in AKT activity is observed in muscle from insulin-resistant mice and humans. This has understandably led to the dogma that impaired AKT activity in skeletal muscle causes insulin resistance and defects in glucose homeostasis. To test the direct requirement of skeletal muscle AKT signaling on systemic glucose metabolism, we generated several mouse models of skeletal muscle AKT deficiency. Unexpectedly, mice lacking AKT2 alone or both muscle AKT isoforms (M-AKTDKO) were insulin sensitive and displayed normal rates of glucose uptake in response to insulin. Mechanistically, our phosphoproteomics study reveals activation of PDK-1, a PI3K substrate, in M-AKTDKO muscles in response to insulin. This was associated with the significant inhibition in phosphorylation of IRS2 at Ser303 and Ser577 (recently identified AKT-dependent phosphosites on IRS2 that limit PI3K signaling) and activation of the AMPK pathway in M-AKTDKO muscles in response to insulin. Intriguingly, mice with combined inhibition and both AKT and AMPK pathway were insulin resistant and glucose intolerant. Collectively, these data suggest the new role of AKT in regulating insulin-mediated AMPK pathway to control glucose uptake via negative feedback inhibition of PI3K/IRS2 activity.

## Morning Session II – Talk 7 (11:40 – 11:55)

### **The mitochondrial Ca<sup>2+</sup> channel MCU is critical for tumor growth by supporting cell cycle progression and proliferation**

Emily Fernández García<sup>1</sup>, Jillian Weissenrieder<sup>1</sup> & J. Kevin Foskett<sup>1,2</sup>

<sup>1</sup>Departments of Physiology, Perelman School of Medicine, University of Pennsylvania Philadelphia, PA 19104.

<sup>2</sup>Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania Philadelphia, PA 19104.

The mitochondrial uniporter (MCU) Ca<sup>2+</sup> ion channel, the primary mechanism for Ca<sup>2+</sup> uptake into mitochondria, has been associated with malignancy and progression of cancer. Here we employed *in vitro* transformation models with MCU genetically eliminated to examine roles of MCU in tumor formation and progression *in vivo*. In addition, we examined malignant capabilities *in vitro*, as well as mitochondrial bioenergetics and cytoplasmic Ca<sup>2+</sup> signaling in the presence and absence of MCU expression. Transformation of primary fibroblasts *in vitro* was associated with increased MCU expression and enhanced mitochondrial Ca<sup>2+</sup> uptake, suppression of inactivating-phosphorylation of pyruvate dehydrogenase, and a modest increase of mitochondrial respiration. Inhibition of mitochondrial Ca<sup>2+</sup> uptake by genetic deletion of MCU markedly inhibited tumor growth of HEK293T cells and transformed fibroblasts in mouse xenograft models. Reduced tumor growth was primarily a result of a substantially reduced proliferative potential of tumorigenic cells *in vivo* and *in vitro*, with MCU particularly important for progression through S-phase of the cell cycle. MCU deletion inhibited cell invasion and cancer stem cell self-renewal *in vitro*. Surprisingly, mitochondrial matrix Ca<sup>2+</sup> content, membrane potential, global dehydrogenase activity, respiration, and ROS production were not altered by genetic deletion of MCU in transformed cells. In contrast, deletion of MCU elevated glycolysis and altered agonist-induced cytoplasmic Ca<sup>2+</sup> signals. Our results reveal a fundamental dependence of tumorigenesis *in vivo* and *in vitro* on mitochondrial Ca<sup>2+</sup> uptake by MCU, mediated by a reliance on mitochondrial Ca<sup>2+</sup> for cellular metabolism and Ca<sup>2+</sup> dynamics necessary for cell-cycle progression and cell proliferation.

## Morning Session II – Talk 8 (11:55 – 12:10)

### **The bitter taste of cancer: targeting bitter taste receptor 14 to kill head and neck squamous cell carcinoma**

Zoey A. Miller, Ray Z. Ma, Sahil Muthuswami, Derek B. McMahon, Ryan M. Carey & Robert J. Lee

Within head and neck squamous cell carcinomas (HNSCCs), oral and oropharyngeal squamous cell carcinomas (SCCs) affect ~34,000 people in the US each year. Patients face a 50% 5-year survival rate and overall decline in quality of life due to morbidities of current treatments. Novel targeted therapies are needed for SCCs to prolong survival and decrease off-target effects of treatment. Due to the oral localization of SCCs, bitter taste receptors (T2Rs) have sparked interest as potential therapeutic targets. T2Rs are a subset of G-protein coupled receptors (GPCRs) that induce an intracellular calcium ( $\text{Ca}^{2+}$ ) response when activated. T2R14, one of 25 T2R isoforms, has implications in breast, ovarian and pancreatic cancers. However, its role in HNSCCs remains unknown. Here, we show that T2R14 is expressed in immortalized HNSCC cell lines: SCC 47, FaDu, and RPMI 2650. We found that T2R14 agonists thujone, flufenamic acid, and lidocaine trigger intracellular  $\text{Ca}^{2+}$  release in HSNCC cells. 6-methoxyflavanone (6-MF), a T2R14 agonist, dampens this  $\text{Ca}^{2+}$  response. Furthermore, lidocaine in particular reduces NADH metabolism via XTT assay, indicative of reduced cellular health. It also depolarizes the mitochondrial membrane as measured by JC-1 dye. Stimulation with these compounds ultimately induces the cleavage of caspases 3 and 7, indicating apoptosis. Co-incubation with 6-MF inhibits apoptosis induction. Taken together, T2R14 agonists could function as alternative or complementary therapies due to their pro-apoptotic effects in HSNCC cells. Further work is warranted to understand T2R signaling in HNSCC and normal surrounding epithelia.

## Afternoon Session I – Talk 9 (1:15 – 1:30)

### **Hyperelastic continuum models for isotropic athermal fibrous networks**

Dawei Song<sup>1,2</sup>, Assad A. Oberai<sup>3</sup> & Paul A. Janmey<sup>1,2,4</sup>

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Many biological materials contain fibrous protein networks as their main structural components. Understanding the mechanical properties of such networks is important for creating biomimicking materials for cell and tissue engineering, and for developing novel tools for detecting and diagnosing disease [Song *et al.*; 2021]. Motivated by this, we develop continuum models for isotropic, athermal fibrous networks, for which the persistence length of individual fibers is much larger than the fiber's contour length. For this purpose, we combine a single-fiber model that describes the axial response of fibers, with network models that assemble individual fiber properties into overall network behavior. More specifically, we assume in the single-fiber model that the fiber can stiffen under axial tension and soften under axial compression. Moreover, we consider four different network models, including the affine, three-chain, eight-chain, and microsphere models, which employ different assumptions about network structure and kinematics. Further, to account for the compressibility of the network, we incorporate into our models a volumetric energy term that can describe nonlinear volumetric responses of the network. Thus, our work provides a generalization of popular network models from incompressible, thermal networks like rubbers to compressible, athermal networks like collagen and fibrin networks. Thereafter, we systematically investigate the ability of these models to describe the mechanical response of athermal collagen and fibrin networks, by comparing model predictions with experimental data available in the literature. We test how each model captures network behavior under three different loading conditions: uniaxial tension, simple shear, and combined tension and shear. We find that the affine and three-chain models can accurately describe both the axial and shear behavior, whereas the eight-chain and microsphere models fail to capture the shear response, leading to an unphysical zero shear moduli at infinitesimal strains. This is because the eight-chain and micro-sphere models describe fiber deformations using a single effective stretch, being insufficient to simultaneously capture the multi-axial response of the network. In contrast, the affine and the three-chain models, in which fibers along different directions deform differently, appear to have sufficient complexity to represent the macroscopic behavior of the networks with reasonable accuracy. Our study is the first to systematically investigate the applicability of popular network models for describing the macroscopic behavior of athermal fibrous networks, offering insights for selecting efficient models that can be used for large-scale, finite-element simulations of athermal networks.

## Afternoon Session I – Talk 10 (1:30 – 1:45)

### **A solution to the long-standing problem of actin expression and purification**

Rachel H. Ceron, Peter J. Carman, Grzegorz Rebowksi, Malgorzata Boczkowska, Robert O. Heuckeroth & Roberto Dominguez

Actin is the most abundant protein in the cytoplasm of eukaryotic cells and interacts with hundreds of proteins to perform essential functions, including cell motility and cytokinesis. Numerous diseases are caused by mutations in actin. There is therefore intense interest in studying the biochemistry of actin mutants, but a reliable method to obtain recombinant actin does not exist. Furthermore, biochemical studies have typically used tissue-purified  $\alpha$ -actin, whereas humans express six isoforms that are nearly identical but perform specialized functions and are difficult to obtain from natural sources in isolation. Here, we describe a solution to the problem of actin expression and purification. We obtain high yields of actin isoforms in human Expi293F cells. Experiments along the multi-step purification protocol demonstrate the removal of endogenous actin and the functional integrity of recombinant actin isoforms. Proteomics analysis confirms the presence of native post-translational modifications, including N-terminal acetylation achieved after affinity-tag removal using the actin-specific enzyme Naa80. The method described opens the way to studying actin under fully native conditions, including differences among isoforms and the effects of disease-causing mutations that occur in all six isoforms.

## Afternoon Session I – Talk 11 (1:45 – 2:00)

### **Actin' different? Distinct *in vivo* functions of cytoplasmic actin proteins**

Pavan Vedula, Marie Fina, Sergei S. Nikonov, Dawei W. Dong & Anna Kashina

Non-muscle actin is ubiquitously expressed and is an essential component of the cytoskeleton. Higher vertebrates express two non-muscle actins –  $\beta$ - and  $\gamma$ - cytoplasmic actins. These two proteins differ by only 4 conservative substitutions in their N-terminus, but have distinct functions at the gene level:  $\beta$ -actin is essential for embryogenesis, while  $\gamma$ -actin is dispensable. Prior data from our lab showed that these functional distinctions are encoded at the nucleotide level, leaving an open question whether the conserved amino acid differences between these two actins play a role in their *in vivo* functions. Here we used gene-edited mouse models lacking  $\beta$ -actin protein and expressing  $\gamma$ -actin both natively and off the nearly intact  $\beta$ -actin gene to investigate specific amino acid-dependent functions of the cytoplasmic actins. We find that microvilli, cytoplasmic actin-rich cellular protrusions present in many tissues throughout the body require  $\beta$ -actin protein for their maintenance. Lack of  $\beta$ -actin proteins leads to microvilli disorganization which impairs the structure and functions of different organs, including the small intestine and the retina, and likely affects the overall animal survival over time. In the retina, changes in cytoplasmic actins in the absence of  $\beta$ -actin protein led to progressive retina degeneration and decreased light sensitivity, likely due to disruption of F-actin organization and altered interaction with myosins. Our results demonstrate a crucial function for the evolutionarily conserved amino acid differences between  $\beta$ - and  $\gamma$ -actin in the proper structural organization and physiology.



# Poster presentations

Session I: Poster 1 & Session II: Poster 25

## **DEI Committee**

The Physiology Department Diversity, Equity, and Inclusion Committee advocates for equal access and support for all members of the department, ensuring the next generation of scientists are given every opportunity to succeed. Come learn about our exciting *At the Leading Edge* seminar series, our trainee organization *DOPAMINE*, opportunities to get involved, and more!

## Session I: Poster 2

### **FMRP granules are dynamically positioned along mitochondria to regulate fission in neurons**

Adam Fenton, Charles R. Bond, Melike Lakadamyali, Thomas A. Jongens & Erika L. F. Holzbaur

The Fragile X Mental Retardation Protein (FMRP) is a component of RNA granules whose absence causes Fragile X Syndrome. Loss of FMRP impairs mitochondrial homeostasis in neurons, resulting in fragmented mitochondria with impaired metabolic function. However, the mechanism by which FMRP supports the maintenance of mitochondrial function in neurons is not known. We find that FMRP granules form dynamic contacts with mitochondria in neurons that preferentially occur at the mitochondrial midzone and ends. Midzone-associated FMRP granules frequently mark sites of mitochondrial fission, where the FMRP granule stays associated with the mitochondrial end following fission. Functionally, mitochondria-associated FMRP granules serve as sites of local protein synthesis. Inhibition of protein synthesis reduces the frequency of mitochondrial fission at FMRP granules without affecting fission at sites lacking FMRP, indicating that a previously unidentified subclass of mitochondrial fission events in neurons, which are marked by FMRP, are dependent on protein synthesis. Lysosomes have previously been shown to associate with sites of mitochondrial fission and to deliver RNA granules to mitochondria in neurons. We demonstrate that FMRP granules are co-transported with lysosomes, which mediate stable FMRP-mitochondria contacts. Lysosomes position FMRP granules at mitochondrial fission sites in a Rab7 GTPase-dependent manner, suggesting that lysosomes deliver FMRP-positive RNA granules to mitochondria to locally promote fission. Mitochondrial fission is also facilitated by the Mitochondrial Fission Factor (MFF) protein, which recruits fission machinery to mitochondria. We find that the MFF mRNA is present in mitochondria-associated FMRP granules and that the MFF protein colocalizes with FMRP granules at sites of fission, but FMRP recruitment to the mitochondrial midzone precedes MFF. These findings suggest that FMRP is involved in the local translation of MFF at mitochondria. Taken together, our findings demonstrate a direct role for FMRP in the regulation of mitochondrial fission and suggest that FMRP granules promote the maintenance of neuronal mitochondrial homeostasis.

## Session I: Poster 3

### **Testing NAD<sup>+</sup> precursors in a progressive model of Friedreich's Ataxia**

Caroline Perry<sup>1</sup>, Sarah Halawani<sup>2</sup>, Melissa LaRocca<sup>1</sup>, Sarmistha Mukherjee<sup>1</sup>, Beishan Chen<sup>1</sup>, David Lynch<sup>1,2</sup>  
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Heart disease results in over 650,000 deaths per year in the United States, and nearly half of all adults are currently living with some form of cardiovascular disease, as a result of inherited genetic mutations or lifestyle and environment. Although medication and lifestyle changes are occasionally sufficient to manage the symptoms and progression of heart disease, many patients ultimately require a heart transplant for curative treatment. Thus, there is significant need for better therapeutic interventions. Heart failure can take years to develop and is ultimately characterized by a reduction in cardiac output that becomes insufficient to maintain flow throughout the body. A considerable body of research demonstrates maladaptive metabolic reprogramming of cardiomyocytes in the failing heart, which ultimately contributes to dysfunction and remodeling of the left ventricle. Among these observed changes is a decline in nicotinamide adenine dinucleotide (NAD<sup>+</sup>) at the tissue level during ischemic injury and in failing hearts. In the mitochondria, NAD<sup>+</sup> is a key regulator of several metabolic reactions, where it is needed not only as an electron carrier for the electron transport chain, but also to support metabolism of ketones, fatty acids, and glucose, which all feed into the TCA cycle. NAD<sup>+</sup> biosynthetic precursors have been found to be therapeutic for heart failure in multiple pre-clinical models, including pressure overload-induced hypertrophic cardiomyopathy. Given the functional role of NAD<sup>+</sup> in mitochondria and its observed decline, we set out to examine the connection between disruption of mitochondrial function and the development of heart failure. To accomplish this, we utilized a model of Friedreich's Ataxia (FA), a primary mitochondrial disease, where we have observed decline of mitochondrial function in the heart accompanied by the development of progressive hypertrophic cardiomyopathy. My preliminary data demonstrate that the NAD<sup>+</sup> precursors nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) prevented heart dysfunction in FA mice. Unexpectedly, this improvement does not appear to be dependent on a rescue of mitochondrial function, as complex respiration was partially recovered in NR-treated mice, but not in NMN-treated mice. Both precursors showed a trend of recovering NAD<sup>+</sup> in FA tissue. Taken together, these data suggest that boosting NAD<sup>+</sup> levels can be protective of heart function under mitochondrial stress, but suggest that the beneficial effect is at least partially independent of mitochondrial function per se.

Session I: Poster 4

## Session I: Poster 5

### **Mechanism of MIRO-mediated activity and recruitment of cytoskeletal effector TRAK in mitochondrial dynamics.**

Elana Baltrusaitis, Erika Ravitch & Roberto Dominguez

The disruption of mitochondrial movement, fission, and fusion can lead to improper cellular function and is linked to neurodegenerative disease. Driving mitochondrial dynamics are the Mitochondrial Rho GTPases (MIROs), which link the outer mitochondrial membrane to both the microtubule and actin cytoskeleton. Specifically, MIROs serve as adaptors for Trafficking Kinesin-binding proteins (TRAKs) which interact with kinesin-1 and the dynein-dynactin complex, therefore coordinating mitochondrial motility towards the plus and minus ends of microtubules. How are MIROs association with TRAKs regulated? MIROs are composed of two atypical GTPase domains at the N- and C-termini flanking two internal calcium ( $\text{Ca}^{2+}$ )-binding EF-hand domains, though it's unclear how functional these domains are and if their activities are intertwined.  $\text{Ca}^{2+}$  sensing and GTP hydrolysis are common ways proteins regulate their interactions with binding partners. Thus, it is speculated these domains control binding of TRAKs to MIROs. This study explores the role of  $\text{Ca}^{2+}$  sensing and GTP hydrolysis in the regulation of MIROs' interaction with TRAKs and takes a preliminary step towards mapping where these interactions occur. Using purified N- and C-termini of MIRO1, we demonstrate an interaction with a highly conserved region of TRAK1 that has not yet been shown to bind to other partners.

## Session I: Poster 6

### **Developing quantitative methods to define stoichiometry of large protein complexes using super-resolution microscopy**

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Single Molecule Localization Microscopy (SMLM) has been used to visualize many subcellular structures in exceptional detail, including cytoskeletal networks and chromatin. These studies have been used to illustrate the concept of nanodomains within the cell, wherein multiple proteins cluster together, such as T-cell receptors in the plasma membrane. Assertions made from studies of protein nanodomains using SMLM, however, have been called into question because these imaging techniques are prone to “clustering artifacts” that arise from the photo-physics of blinking fluorophores and the way in which fluorescent signal is localized and mapped. We are interested in overcoming the limitations set by clustering artifacts and making SMLM a more quantitative modality for the study of protein distribution. Previously, we developed a DNA origami calibration template for use with SMLM to serve as stoichiometric calibration. We found, however, that protein complexes with copy numbers greater than 6 were not reliably measured by the DNA origami nano-template due to the inability to functionalize the origami platform with more than 6 proteins of interest. Many cellular structures, however, contain far more than 6 components, therefore it was necessary to look for a new platform with qualities similar to DNA origami that could also be functionalized with greater numbers of proteins. Here, we seek to define the quantitative capacity of computationally-designed cage-like protein nanomaterials (protein nano-cages) for use as calibration templates in studies of large protein complexes.

## Session I: Poster 7

### **PINK1/Parkin mitophagy in astrocytes**

Julia Riley & Erika L.F. Holzbaur

Efficient turnover of damaged mitochondria is critical for long-term viability of nervous system cells. This includes astrocytes, a glial subtype. Impaired mitochondrial health in astrocytes has been linked to neurodegenerative diseases such as Parkinson's disease (PD). Several PD-causing mutations are demonstrated to impair neuronal mitophagy, an autophagic pathway in which damaged mitochondria are degraded following ubiquitination coordinated by the kinase PINK1 and E3 ligase Parkin. However, it is not established whether PINK1/Parkin mitophagy occurs in astrocytes, and whether it involves the same autophagy receptors as in neurons. Here, we use murine astrocytes, both in monoculture and co-cultured with human iPSC-derived neurons, to investigate proteins involved in astrocytic mitophagy. Preliminary data suggests that Parkin is recruited to mitochondria in astrocytes following whole-scale mitochondrial damage. This occurs in monocultured astrocytes and in astrocytes co-cultured with human neurons. Preliminary results also suggest partial recruitment of phosphorylated optineurin, an autophagy receptor involved in neuronal mitophagy, following mitochondrial stress in astrocytes. Future experiments will determine whether mitochondrial stress and impaired mitophagy elicit pro-inflammatory reactive phenotypes in astrocytes capable of instigating neuronal death.

## Session I: Poster 8

### **Gigantic polyacrylamide hydrogels for large-batch cell culture**

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The rigidity of cell culture substrate or extracellular matrix plays a vital role in regulating cell and tissue functions. Polyacrylamide (PA) hydrogel is one of the most widely used cell culture substrate that provides a physiologically relevant range of stiffness. However, it is still arduous and time-consuming to prepare PA substrates in large batches for high-yield or multi-scale cell cultures. Here we present a simple method to prepare PA hydrogels with less time cost and easily accessible materials. The hydrogel is mechanically uniform and supports cell culture in a large batch. We further show that the stiffness of the hydrogel covers a large range of Young's modulus and is sensed by cells, regulating various cell behaviors including cell morphology, cell proliferation, and cell contractility. Together, this method could be immediately adopted by start-up level of research laboratories and performed by entry-level of researchers and may be applied for mechanobiology research requiring large numbers of cells or experimental groups.



## Session I: Poster 9

### **Investigating the role of phosphatidylcholine in NAFLD/NASH-associated inflammation and fibrosis**

Jaclyn E. Welles<sup>1</sup>, Kahealani Uehara<sup>1</sup>, Michael V. Gonzalez<sup>2</sup>, James P. Garifallou<sup>2</sup> & Paul Titchenell<sup>1</sup>

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Phosphatidylcholine (PC) is a lipid macromolecule demonstrated to play an integral role in multiple intracellular processes (i.e., membrane integrity; VLDL-TAG secretion; lipid metabolism; lipid droplet dynamics). Notably, 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). Interestingly, hepatic PC production is also significantly decreased in patients with NASH, an advanced disorder which affects a subset of patients with NAFLD, and Type-2 Diabetes (T2D). Despite the association of hepatic PC production with the onset of hepatic steatosis, it still remains relatively unknown mechanistically, how the reduction of PC produced in the liver promotes the onset of NASH. 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 lacking PCYT1A expression in the liver, to address our hypothesis. PCYT1A is the gene that encodes for CTP: phosphocholine cytidyltransferase, alpha (CCT- $\alpha$ ), the rate-limiting enzyme required for ~70% of hepatic PC production via the Kennedy pathway. Given the complex nature of NASH pathogenesis, as well as the heterogeneity of the cells found within the liver, we used single-nuclei transcriptomics (RNA-Seq) to perform this study. Cluster analyses using SEURAT, highlighted ~8 clusters of nuclei consisting of ~35,000 nuclei, with distinct gene expression signatures between each experimental group: Control, L-PCYT1A/CCT- $\alpha$  KO, and LMCD-HFD. Notably, we observed changes to hepatocyte-driven ligand secretion to receptors expressed in downstream nonparenchymal cells (NPCs) (HSCs, ECs) in the livers with reduced PC biosynthesis. Moreover, in addition to a stellate cell cluster enriched for genes associated with extracellular matrix organization and fibrosis, the emergence of a novel cluster of nuclei enriched for genes associated with microtubule organization and stem cell differentiation, was also found exclusively in the livers of mice with abrogated PC production (genetic and dietary).

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## Session I: Poster 10

### **Myosin-IIs promote the growth of actin ‘comet tails’**

Mengqi Xu, Luther W. Pollard, Grzegorz Rebowksi, Malgorzata Boczkowska, Roberto Dominguez & E. Michael Ostap

Myosin-IIs are single-headed, membrane associated members of the myosin superfamily that participate in crucial cellular processes related to membrane morphology and trafficking. Recent studies show that myosin-II isoforms frequently concentrate on membranes in areas of active Arp2/3 complex-mediated actin polymerization, promote actin assembly and drive membrane bending. To investigate how myosin-II isoforms affect actin assembly, we performed a “comet tail assay” where branched actin filament networks were nucleated by Arp2/3 complex from a bead surface coated with a nucleation promoting factor (NPF). We site-specifically coupled a range of densities of myosin-II to the bead surface and assessed their effects on actin symmetry breaking, polymerization, and network architecture. We found that high myosin densities prevented comet tail formation due to the newly formed actin network being pushed away from the bead by myosin. Decreasing the myosin density resulted in the formation of a polarized comet tail, which elongated from the beads at a faster rate and frequently showed smooth, rather than pulsatile motion. Myosin also changed the architecture of actin networks in comet tails. Compared with the coherent actin networks from non-myosincoated beads, actin networks emerging from myosin-coated beads were sparser with more disordered actin distribution, which might be due to the gliding and rotating effect of myosin. Strikingly, under low capping protein concentrations, where actin symmetry breaking was completely inhibited due to the high elasticity of the network, myosin-coated beads were able to overcome this inhibition, break symmetry and form a comet tail. These studies show synergy between myosin activity and actin polymerization to power morphological changes at the cell membrane.

Session I: Poster 11

## Session I: Poster 12

### **Reconstitution of the CENP-C-dependent kinetochore assembly pathway using CENP-C multimers *in vitro***

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Previous studies established the structural framework of human outer kinetochore, which contains a scaffolding protein CENP-C. CENP-C links in phosphorylation-dependent manner the chromatin-associated CENP-A nucleosome with the microtubule-binding KMN complex, containing KNL1, Mis12 and Ndc80 complexes. We have previously showed that while in mitotic HeLa cell extracts binding between CENP-C and CENP-A nucleosomes can be successfully reconstituted, the recruitment of native KMN components to the nucleosome-bound CENP-C was poor, suggesting partial inhibition of this interaction. To gain more insight into the CENP-C-activation mechanisms we developed a novel *in vitro* approach that relies entirely on recombinant components. A fragment of CENP-C containing Mis12-binding site was fused with SpyTag and conjugated to the SpyCatcher-containing 60-mer particles, which represent a simplified model of kinetochore-localized CENP-C. Interactions between the GFP-labeled CENP-C particles and recombinant Alexa647-Mis12 complex were monitored using real-time TIRF microscopy that enables accurate control of system components, as well as accurate measurement of reaction kinetics and stoichiometry. Our preliminary results show that Mis12 becomes stably recruited to CENP-C clusters, but unexpectedly neither the number of bound molecules nor the kinetics of binding were affected by Mis12 phosphorylation. Although we detected recruitment of Ndc80 to the CENP-C/Mis12 clusters, Ndc80 binding was substoichiometric, with only 0.1 Ndc80 molecules bound per one CENP-C. This outcome could not be improved by using Mis12 complexed with a fragment of KNL1, suggesting that some important components or post-translational modifications are missing. The work will describe our progress with reconstituting KMN recruitment via CENP-C and discuss future experiments.

## Session I: Poster 13

### **mCLIFY: monomeric, bright, circularly-permuted yellow fluorescent protein**

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The discovery of the genetically expressible green fluorescent protein (GFP) has afforded the cell biological sciences with a well-equipped rainbow tool box to study chemical and mechanical properties of proteins and the life-sustaining processes dependent on them. To effectively use these tools, it is of critical importance to characterize their biophysical and photophysical properties to understand how they interact with other proteins and respond within the confines of their *in vivo* or *in vitro* environments. The popular bright FP in the green-yellow spectrum, YPet (Yellow fluorescent Protein for Energy Transfer), has been extensively used, but has been a challenge to characterize and exists as a weak dimer with a  $K_D = 3.4 \mu\text{M}$ . We have been able to further structurally characterize YPet by introducing a monomeric (m) and circularly permuted (CP) derivative of YPet, mCLIFY (Monomeric Circularly permuted long Lifetime Intense Fluorescent Yellow protein), with high quantum yield (0.76), extinction coefficient ( $137,600 \text{ M}^{-1}\text{cm}^{-1}$ ), long fluorescence lifetime (3.48 ns), low pH sensitivity (pKa 5.58), favorable photostability and among the brightest monomeric FPs. As the first structurally solved YPet derived variant, the crystal structure of mCLIFY reveals details of this FP's brightness and stability. Steady state fluorescence lifetime imaging and time resolved anisotropy experiments have suggested that HOMO-FRET, especially for YPet, and possibly inner filtering effects may interfere with data interpretation in the cell. With an excitation wavelength in the blue-green spectrum and emission in the green-yellow spectrum, mCLIFY is not only useful as an excellent tag for monitoring protein movement and localization within cells, and also as a structurally sound and high performing photostable donor or acceptor for FP based bio-sensors without compromising the quantification of biophysical signals due to dimerization.

## Session I: Poster 14

### **Discovering TRPV2 channel molecular function via proximity proteomics and electron microscopy**

Pamela Gallo<sup>1</sup>, Elaine Mihelc<sup>1</sup>, Gary A. Bradshaw<sup>2</sup>, Robyn J. Eisert<sup>2</sup>, Marian Kalocsay<sup>2</sup> & Vera Moiseenkova-Bell<sup>1</sup>

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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.

## Session I: Poster 15

### **Positive feedback loop in platelet dense granule secretion promotes rapid formation of a thrombus shell**

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Thrombus formation at a site of vascular injury is triggered by thrombin, and is also regulated by other clotting activators. A growing thrombus comprises a core of tightly packed platelets activated by thrombin, and a shell of loosely associated platelets that are activated by ADP and other factors. Interestingly, highly concentrated ADP is stored in platelets in the form of dense granules, which become secreted upon platelet activation. It is well established that secretion of dense granules is required for proper shell formation, but determining how the granules are secreted in a growing thrombus is a daunting experimental task. Previously, we visualized dense granule secretion in individual platelets, and found that secretion is enhanced by the addition of soluble fraction of platelets pre-incubated with thrombin. However, significance of this activation for thrombus growth and generation of shell is unknown. To address this question, we developed a bottom-up computational model of thrombus formation. Platelets, modelled as two-dimensional discs, adhere to an injury site and each other in a blood flow within a narrow channel. Thrombin and ADP are modelled explicitly; they activate platelets by strengthening cell-cell interactions and inducing secretion of dense granules. The model incorporates a positive feedback loop whereby granule secretion is enhanced by the soluble factors that they release. First, we validated this framework by applying this model to reproduce secretion in individual cells. We then examined structural features of the growing thrombus in silico. Thrombi formed with both a core and a shell, however, analogous model lacking positive feedback loop predicts much slower shell formation with reduced size owing to the decreased ADP concentration. Thus, positive feedback loop in dense granule secretion within the growing thrombus is required for normal shell formation.

## Session I: Poster 16

### **Subcellular localization patterns and protein-protein interaction networks of wild-type human TRPML1 and mild and severe Mucopolipidosis type IV-causing mutations**

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Transient receptor potential mucolipin 1 (hTRPML1) is a ubiquitously expressed, nonselective, cation-permeable ion channel from the TRP channel super-family with polymodal activation and localization to the membranes of late endosomes and lysosomes (LEL), where it mediates calcium (Ca<sup>2+</sup>) flux from the lysosomal lumen to the cytosol to coordinate membrane trafficking events, endocytosis, autophagy, and lysosomal biogenesis and exocytosis. Dysfunctional hTRPML1 activity underlies numerous neurodegenerative disorders due to the accumulation of lysosomal cargo, compromised heavy metal homeostasis, and reactive oxygen species formation, therefore it serves as a potential therapeutic target. Specifically, loss-of-function mutations in the TRPML1 gene, *MCOLN1*, causes Mucopolipidosis type IV (MLIV) disease, an autosomal recessive neurodegenerative lysosomal storage disease. To further the current understanding of TRPML1 endogenous function, we studied the consequences of a mild (F408Δ) and severe (F465L) MLIV-causing mutations on wild-type hTRPML1 by exploiting the dual-function of APEX2, an ascorbate peroxidase, which was engineered as a tag to generate local contrast for transmission electron microscopy and to covalently biotinylate proximal proteins for proteomic screens. Our results identified TRPML1 localization patterns within intimate interorganelle contact sites and revealed previously known protein-protein interactors of TRPML1 as well as novel proteins that could provide insight into MLIV-disease progression and other neurodegenerative diseases with similar cellular pathogenesis.



## Session I: Poster 17

### **The mitochondrial calcium uniporter in pancreatic cancer growth, proliferation, and metastasis**

Jillian S. Weissenrieder<sup>1</sup>, Jason R. Pitarresi<sup>2</sup>, Ben Z. Stanger<sup>3,4</sup>, Anil K. Rustgi<sup>5</sup> & J. Kevin Foskett<sup>1</sup>

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Cancer is one of the top causes of death in the United States. While outcomes have improved in many cancer types with advances in targeted therapies and immunotherapies, prognosis remains poor for pancreatic ductal adenocarcinoma (PDAC), an effect which is at least partially attributable to early metastasis contributing to mortality. Our lab has previously shown that cancer cell lines may be “addicted” to constitutive uptake of  $\text{Ca}^{2+}$  by mitochondria through the mitochondrial calcium uniporter (MCU) at endoplasmic reticulum-mitochondria contact sites. The resultant mitochondrial  $\text{Ca}^{2+}$  ( $\text{mCa}^{++}$ ) influx through MCU may contribute to cancer development, proliferation, and metastasis in PDAC. Here, we note that high MCU expression associates with both KRAS mutations, the most common driver of PDAC, and poor survival outcomes. Employing the Pdx1cre; Kras<sup>LSL-G12D/+</sup>; p53<sup>fl/+</sup>; Rosa26<sup>LSL-YFP/LSL-YFP</sup> (KPCY) murine model of PDAC  $\pm$  Mcu<sup>fl/fl</sup> alleles, we isolated tumor tissues and leveraged CRISPR/Cas9 techniques and stable re-expression to generate isogenic cell lines with or without MCU expression for further analysis. KPCY-Mcu<sup>KO</sup> pancreatic cell lines (via Cre or CRISPR) lacked  $\text{mCa}^{2+}$  uptake in a manner rescued by stable re-expression of MCU. MCU expression increased wound healing, self-renewal capacity, and proliferation rate over MCU<sup>KO</sup> isogenic lines. MCU<sup>KO</sup> also reduced tumor growth and metastasis as compared to MCU<sup>+</sup> cells in both immunocompetent and immunocompromised orthotopic models of PDAC, utilizing both Cre- and CRISPR- mediated isogenic lines. Transcriptomic profiling identified key differences in gene expression related to specific pathways, including epithelial to mesenchymal transition and metabolism. Given these findings, MCU may contribute to growth and metastasis in PDAC by contributing to  $\text{mCa}^{++}$  flux, potentially supporting EMT and pro-growth metabolic activity. We conclude that MCU-mediated  $\text{mCa}^{2+}$  uptake contributes significantly to PDAC growth, proliferation, and metastasis and may present a therapeutic target for cancer treatment.

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## Session I: Poster 18

### **Electrical stimulation human mesenchymal stem cells on conductive chitosan-polyaniline substrates promotes neural priming**

Behnaz S. Eftekhari, Dawei Song & Paul A. Janmey

Electrical stimulation (ES) within conductive polymer substrates has been suggested to promote the differentiation of stem cells toward a neuronal phenotype. The use of conductive scaffolds in tissue regeneration provides a unique and attractive new option to control the amount and location of ES delivery. Besides, scaffold stiffness has been shown as another important regulator of stem cells behavior and fate. Therefore, to improve stem cell-based regenerative therapies, it is essential to characterize the simultaneous effects of electroconductive substrate stiffness and electric field stimuli on stem cell fate processes. In this study, biodegradable electroconductive substrates based on chitosan-polyaniline (CS-g-PANI) were fabricated with different stiffnesses. Human mesenchymal stem cells (hMSCs) seeded on these scaffolds were electrically stimulated for 14 days with 100 mV/cm (20 min every day). For hMSCs cultured on soft conductive scaffolds, a morphological change with significant filopodial elongation was observed after 2 weeks of electrically stimulated culture. Compared with stiff conductive CS-g-PANI scaffold and non-conductive CS scaffolds, for soft conductive CS-g-PANI scaffolds microtubule-associated protein 2 (MAP2) and neurofilament (NF-H) expression increased after application of ES. At the same time, there was a decrease in the expression of the glial markers, the glial fibrillary acidic protein (GFAP) and vimentin after ES. Furthermore, the elevation of intracellular calcium [ $Ca^{2+}$ ] during spontaneous, cell-generated  $Ca^{2+}$  transients further suggested that, electric field stimulation of hMSCs cultured on conductive CS-g-PANI substrates can promote a neural-like phenotype. Our findings propose that the combination of the soft conductive CS-g-PANI substrate and ES is a promising new tool for enhancing nerve tissue engineering outcomes.

## Session I: Poster 19

### ***In vitro* study of mitochondrial motility and myosin-19 dynamics**

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

Mitochondrial motility is vital to cellular health and function. This is especially true in neurons, where mitochondria are transported long distances, while errors in this process have been implicated in neurodegenerative diseases. Intriguingly, mitochondrial motility relies on both microtubule- and actin-based transit. Reconstituting mitochondrial motility *in vitro* permits more accurate dissection of the mechanisms regulating transport, and how microtubule-based and actin-based motors may contribute. Construction of such a model first requires isolation of both organelle and motor protein components; here, a mitochondrial isolation approach has been adapted to collect mitochondria from cultured cells. Initial mitochondrial-actin interaction experiments suggest that the organelle interacts with actin, but no filament motility was observed despite evidence that the mitochondrial-bound Myosin-19 is present. Super-resolution microscopy indicates that myosin-19 motors are dispersed along the outer mitochondrial membrane (Coscia et al., in prep) and may act as a tether rather than a motor. To understand Myosin-19 function more thoroughly, photobleaching experiments were performed. Results suggest that while most of the population of Myosin-19 is monomeric, there is a significant population of dimer. Because Myosin-19 is a high-duty-ratio, single-headed myosin, two motors must be present for processive motility to occur. If a dimeric subpopulation exists, then it is possible that Myosin-19 could function as both a tether and motor, opening more avenues for regulation of mitochondrial motility through Myosin-19.

## Session I: Poster 20

### **Striking the balance of mitochondrial quality control between selective and non-selective autophagy**

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Accumulation of damaged mitochondria or its products have been associated with the progression of several neurodegenerative disorders. Therefore, clearance of dysfunctional mitochondria by selective autophagy, also termed as mitophagy, serves critical to preserving neuronal health. While PINK1 and Parkin mediated mitophagy remains the most well characterized pathway for damaged mitochondrial clearance, what happens when this pathway is perturbed? Absence of any striking phenotype in mammalian knock-out models of PINK1 and Parkin hints towards the existence of complementary pathways for mitochondrial clearance. We find that LAMP-1 and total LC3 protein levels are elevated in brains of PINK1 <sup>-/-</sup> mice, implying that one such alternate mechanism of quality control can be the upregulation of general non-selective autophagy. Thus, it is essential to understand how the balance between selective autophagy (mitophagy) versus non-selective autophagy is maintained in neurons. However, neurons are resistant to conventional methods of autophagy induction. A recently identified gene (Myotubularin related 5, mtmr5) has been identified to be essential for conferring this neuronal resistance to Torin1 mediated autophagy induction [Chua et al., BioRxiv, 2021]. Our initial results show that MTMR5 remains stably associated with autophagosomes implying it can play a role in both selective and non-selective autophagy. Taken together, our work aims to understand how MTMR5 regulates autophagy broadly, and in turn verify if it can compensate for defects associated with PINK1/Parkin dependent mitophagy.

## Session I: Poster 21

### **Mapping PTBP splicing in human brain identifies targets for therapeutic splice switching including *SYNGAP1*.**

Jennine M. Dawicki-McKenna\*, Alex J. Felix\*, Elisa A. Waxman, Congsheng Cheng, Defne A. Amado, Paul T. Ranum, Alexey Bogush, Lea V. Dungan, Elizabeth A. Heller, Deborah L. French, Beverly L. Davidson & Benjamin L. Prosser

\*These authors contributed equally to this work.

Alternative splicing of neuronal genes is controlled in part by the coordinated action of the polypyrimidine tract binding proteins (PTBP1 and PTBP2). While PTBP1 is ubiquitously expressed, PTBP2 is predominantly neuronal, controlling the expression of such targets as *DLG4*, which encodes PSD95, a protein important in synaptic function whose deficiency causes neurodevelopmental disorders. Here, we fully define the PTBP2 footprint in the human transcriptome using both human brain tissue and neurons derived from human induced pluripotent stem cells (iPSC-neurons). We identify direct PTBP2 binding sites and define PTBP2-dependent alternative splicing events, finding novel targets such as *STXBP1* and *SYNGAP1*, which are synaptic genes also associated with neurodevelopmental disorders. The resultant PTBP2 binding and splicing maps were used to test if PTBP2 binding could be manipulated to increase gene expression in PTBP-targeted genes that cause disease when haploinsufficient. We find that PTBP2 binding to *SYNGAP1* mRNA promotes alternative splicing and non-sense mediated decay. Antisense oligonucleotides that disrupt PTBP binding sites on *SYNGAP1* redirect splicing and increase gene and protein expression. Collectively, our data provide a comprehensive view of PTBP2-dependent alternative splicing in human neurons and human cerebral cortex, guiding the development of novel therapeutic tools that may benefit a range of neurodevelopmental disorders.

## Session I: Poster 22

### **Super-resolution imaging of Alzheimer's disease hyperphosphorylated tau aggregates**

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Alzheimer's disease (AD) is a neurological disorder characterized by the accumulation of microtubule-associated protein tau into many abnormal intraneuronal aggregates detected at distinct brain regions. Their emergence strongly correlates with the progressive severity of AD diagnosed patients' clinical symptoms and neuropathological features. These observations suggest tau aggregation plays a crucial and toxic role in AD. Tau is a monomeric highly soluble protein that maintains the assembly and stability of microtubules. Tau's function is regulated through post-translational modifications of a specific number of residues, primarily phosphorylation. Previous studies indicate that the tau proteins that make up polymorphous insoluble tau aggregates (e.g., neurofibrillary tangles (NFTs)) have abnormal hyperphosphorylation. Based on these observations, it is suggested that ubiquitous hyperphosphorylation of tau promotes its aggregation in disease. However, the precise link between the pattern and degree of tau hyperphosphorylation with aggregation is unclear. Furthermore, how a heavily molecularly modified protein can form a wide range of morphologically diverse aggregates within one disease is not well-understood. These questions have not been investigated thoroughly due to the diffraction limit of conventional light microscopy (~250nm), in which aggregates of a size well below this limit are not resolvable. By labeling human postmortem AD brain tissues with phospho-tau antibodies that target disease relevant hyperphosphorylated residues (phospho-Thr231 (Thr231), phospho-Ser202/ phospho-Thr205 (AT8)) and super-resolution imaging, we have been able to identify tau oligomers (20-30 nm), linear fibrils (30-250 nm), branched fibrils (50-350 nm), and NFTs (>1 $\mu$ m). Moreover, by using a semi-supervised shape classification strategy, we demonstrate that each of these morphologically distinct tau aggregates have unique phosphorylation patterns. Preliminary results reveal that phospho-Ser202/phospho-Thr205 are enriched in linear fibrils, whereas phospho-Thr231 is enriched in branched fibrils. In addition, using dual-color super-resolution microscopy, we show that linear fibrils, as well as NFTs, consist of a heterogeneous distribution of hyperphosphorylated tau oligomers. Our work suggests that unique patterns and degree of tau hyperphosphorylation may lead to tau aggregate heterogeneity found in AD.

## Session I: Poster 23

### **Targeting miRNAs using antisense oligonucleotides as a novel therapeutic strategy for STXBP1 encephalopathy**

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<sup>#</sup>These authors contributed equally to this work.

STXBP1 encephalopathy is caused by mutations in the *STXBP1* gene. In neurons, STXBP1 plays an essential role in neurotransmitter release from synaptic vesicles. The reduction of functional STXBP1 protein impairs neurotransmitter release, which leads to uncontrolled neuronal activation, epilepsy, intellectual disability and motor impairments. In cases of loss-of-function mutations in STXBP1 leading to haploinsufficiency, the upregulation of functional STXBP1 can be therapeutic. Since the majority of human mRNAs are at least partly repressed by miRNAs, blocking the interaction between miRNAs and STXBP1 mRNA could upregulate STXBP1 expression and presumably provide a neuroprotective therapeutic effect. Here, we performed comprehensive *in silico* analyses and identified the top predicted miRNAs that may be repressing STXBP1 through binding to its 3'UTR. We cross-referenced this information with our miRNA-sequencing data generated from STXBP1 patient iPSC-derived neurons and identified several miRNAs that are both predicted to bind to STXBP1-3'UTR and highly expressed in STXBP1 patient iPSC-neurons. We demonstrated that miR-218, miR-148b and miR-3175 repress endogenous STXBP1 protein expression in HeLa cells, and that inhibiting either miR-218 or miR-424 is sufficient to upregulate STXBP1 mRNA and protein in human neuroblastoma cells. In iPSC-neurons, we showed that blocking miR-15b, miR-148b, miR-218, and miR-3175 using antagomirs led to STXBP1 mRNA upregulation, demonstrating that STXBP1 is repressed by these particular miRNAs in a neuronal context. Finally, we identified a group of antisense oligonucleotides targeting different miRNA binding sites in STXBP1-3'UTR that showed a dose-dependent upregulation of STXBP1 mRNA. Overall, these studies establish several miRNAs as promising targets for STXBP1 encephalopathy.

## Session I: Poster 24

### **High-throughput screen to identify NAD<sup>+</sup> boosting compounds**

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Nicotinamide adenine dinucleotide (NAD<sup>+</sup>) is a redox co-factor for more than 400 chemical reactions in key metabolic pathways and a signaling co-factor required to maintain stress response, chromosome stability, DNA repair, and epigenetic regulation. NAD<sup>+</sup> levels have been observed to be decreased in a myriad of pathological states, including aging, obesity, liver regeneration, and muscular dystrophy. Therefore, increasing NAD<sup>+</sup> through precursor supplementation has been suggested as a therapeutic strategy for many other conditions characterized by NAD<sup>+</sup> decline. The Baur lab previously demonstrated that reducing NAD<sup>+</sup> levels by deleting Nampt (the rate-limiting enzyme in the NAD<sup>+</sup> salvage pathway) in skeletal muscle is sufficient to drive degeneration and muscle dysfunction. Consistently, this phenotype can be reversed by administering the NAD<sup>+</sup> precursor nicotinamide riboside (NR). Similar improvement in muscle function by NR supplementation has been reported in the *mdx* mouse model of Duchenne Muscular Dystrophy (DMD) with reduced NAD<sup>+</sup> levels. Despite the consistent demonstration of the benefits of NAD precursor supplementation in rodent disease models, there is limited evidence that the lower doses that can be tolerated in humans are sufficient to raise NAD or have clinical benefits. Thus, there is a compelling need to identify alternative strategies to improve NAD<sup>+</sup> metabolism. We hypothesize that screening for small molecules that increase NAD<sup>+</sup> may have different pharmacokinetics and mechanisms and could be more effective in treating human diseases than the currently available supplements. For that purpose, Baur, Khurana, Huryn, and Schultz collaborate to develop a high-throughput screening method to identify possible compound candidates that increase NAD<sup>+</sup> levels. We screened 148,000 molecules from different chemical libraries for their ability to boost NAD<sup>+</sup> level in C2C12, a skeletal muscle myoblast cell line, and identified 182 hits. The potential implications of identified candidates to boost NAD<sup>+</sup> levels will be verified in multiple cell lines, and dose-response curves will be established. Promising candidates will be evaluated *in vivo* to establish their effect on tissue NAD<sup>+</sup> content and evaluate pharmacokinetics. In parallel, we will work with chemists to structurally modify the compounds to improve the magnitude of the effect on NAD<sup>+</sup>. Thus, pursuing alternative molecules that enhance NAD<sup>+</sup> levels can ultimately improve human health.



## Session II: Poster 26

### **Truncated titin in the myocardial sarcomere in human dilated cardiomyopathy**

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Truncating variations in the titin gene (*TTNtv*) have long been known to cause dilated cardiomyopathy (DCM) but work thus far has failed to provide convincing evidence to support either haploinsufficiency or dominant negative hypotheses, leaving the pathomechanisms of *TTNtv* in DCM open to controversy. To address this, we performed proteomic, transcriptomic, and biochemical analysis of a collection of 184 post-transplant human hearts, 22 with pathogenic *TTNtv*s, to interrogate pathogenic mechanisms of *TTNtv* in DCM. We show evidence supporting both dominant-negative gain of toxic function due to truncated titin protein expression and association with the Z-disc and thick filament, as well as haploinsufficiency due to lack of sufficient full-length titin. Combining whole exome sequencing data with proteomics, we proteomically mapped the expression of both truncated and non-truncated titin using multiple heterozygous missense variations uniquely found in the *TTN* gene of several patients. We orthogonally confirm the presence of *TTNtv* protein in patient myocardium using *TTN* epitope specific multichannel immunoblotting, wherein *TTNtv* protein bands appear at the genetically predicted molecular weight, and with the expected epitopes. Immunoblots of these same samples also show clear haploinsufficiency of full length *TTN* with respect to total and other sarcomeric proteins. To determine if *TTNtv* proteins are integrating with the sarcomere, we showed that *TTNtv* sediment with the sarcomere bearing insoluble fraction of human myocardium but are more weakly attached to the sarcomere than full length titin, consistent with their reduced number of thick filament binding sites and entirely absent M-line attachment sites. Using a patient specific antibody against an amino acid sequence variation found in a single patient, we detect *TTNtv* localized to the myocardial sarcomere in a striated pattern flanking the M-line. When stretched, these striations do not elongate with respect to the thick filament, but do elongate across I-band, indicating that in this case that *TTNtv* integrate into the sarcomere, attach to the thick filament, and stretch with the sarcomere. This evidence—the direct observation of titin haploinsufficiency along with the detection of *TTNtv* as well as direct observation of *TTNtv* mechanical integration into the sarcomere, support a combined dominant negative and haploinsufficient mechanism of pathogenesis of *TTNtv* induced DCM.

## Session II: Poster 27

### **Single-molecule studies of a translational readthrough inducing drug (TRID) effect on eukaryotic termination**

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Translational readthrough-inducing drugs (TRIDS) are one class of novel therapies developed to treat premature stop codon (PSC) diseases. As yet, only one TRID, ataluren, has been approved for clinical use. In recent publications [Ng, M.Y. *et al.*, 2021; Huang, S. *et al.*, 2022], employing an *in vitro* eukaryotic PURE-LITE system in conjunction with the Cricket Paralysis Virus-Internal Ribosome Entry Site, we studied the mechanism of ataluren read-through. We found that ataluren induces readthrough of a nonsense codon solely via inhibition of termination activity by the release factor complex (RFC, eRF1.eRF3.GTP). We identified three sites of ataluren binding within the PURE-LITE system by using photoaffinity labeling and RNA-seq analysis, two within rRNA, proximal to the decoding center (DC) and the peptidyl transfer center (PTC) of the ribosome, respectively, directly responsible for ataluren inhibition of termination activity, and one within eRF1. We used single-molecule TIRF and ensemble measurements to demonstrate that ataluren is a competitive inhibitor of productive RFC binding to the pre-termination complex. We further showed that RFC binding to the pre-termination complex results in comparatively rapid hydrolysis of peptidyl-tRNA, followed by much slower, but similar rates of nascent peptide and tRNA release from the ribosome. Here we present new results using an sm-FRET assay that utilizes a Cy3-labeled peptidyl-tRNA bound in the P-site adjacent to the UGA stop codon and Cy5-labeled human eRF1 (heRF1). Upon RFC binding we observe a transient FRET efficiency value of  $E = \sim 0.25$  between Cy5-heRF1 and Cy3-tRNA, consistent with successful accommodation of eRF1 within the ribosomal A site. Following peptidyl-tRNA hydrolysis, the dissociation times of eRF1 and tRNA are strongly correlated, unlike the weak correlation between the release of tRNA and peptide. We also found that ataluren and an added near-cognate suppressor tRNA each have a major inhibitory effect on the apparent rate of RFC arrival time to the ribosome, which contrasts with minor effects on tRNA and RFC dissociation. 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.

## Session II: Poster 28

### **ZSF-1 obese rats develop features of HFpEF**

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Heart failure with preserved ejection fraction (HFpEF) represents a major unmet clinical need with few therapies available to patients. A limiting factor in the development of new therapeutics is the lack of representative animal models. The ZSF-1 obese rat, which has two mutations for leptin, naturally develops obesity, diabetes, and hypertension, commonly associated comorbid conditions with HFpEF. We aimed to characterize the progression of the HFpEF phenotype of ZSF-1 obese rats versus the lean control.

ZSF-1 obese (o) and lean (l) rats underwent echocardiography assessment at 16 and 20 weeks. Left ventricular (LV) fractional shortening was similar between groups at 16 and 20 weeks, representing preserved systolic function. LV mass was significantly higher in obese animals vs. lean at 16 weeks (1429 mg  $\pm$  63, n=6 vs. 1231mg  $\pm$  30, n=6; P=0.028), and continued to increase at 20 weeks (1588 mg  $\pm$  68, n=6 vs. 1283 mg  $\pm$  37, n=6; P=0.001). E/e' was similar in both groups at 16 weeks, but significantly higher at 20 weeks in obese animals compared to lean (22.0  $\pm$  1.5, n=6 vs. 15.5  $\pm$  1.7, n=6; P=0.007), suggestive of progressive diastolic dysfunction. Invasive hemodynamic studies at 20 weeks of age corroborated diastolic dysfunction, with observed increases in left ventricular end-diastolic pressure (14.9 mmHg  $\pm$  2.6, n=3 vs. 8.2 mmHg  $\pm$  0.5, n=3). Exercise intolerance is a hallmark feature of clinical HFpEF. To assess this, exercise testing was performed at 20 weeks of age using a graded treadmill protocol. Rats ran at 9 meters/minute for 2 minutes, then the speed of the treadmill was increased by 3 meters/minute every minute until animals showed signs of exhaustion. Obese rats ran significantly less than the lean controls (58.7 meters  $\pm$  5.8, n=2 vs. 150.7 meters  $\pm$  3.6, n=3; P<0.001) and had a lower exercise workload (39.1 kg\*m  $\pm$  4.4, n=2 vs. 74.7kg\*m  $\pm$  3.8, n=3; P=0.009).

ZSF-1 rats develop structural and functional features of clinical HFpEF that progress with age. Phenotyping of animals at later time points will provide more context to the extent of the development of the HF phenotype.

## Session II: Poster 29

### **Using smFRET to understand structural dynamics of sex-biased RNA helicases DDX3X and DDX3Y**

Amber Yanas, Clark Fritsch, Hui Shen, Him Shweta, Michael C. Owens, Yale E. Goldman & Kathy Fange Liu

Sex differences are pervasive in human health and disease. The most striking differences lie in the sex chromosomes, which encode a group of sex-specific protein homologs. Although the functions of the X chromosome proteins are well appreciated, how they compare to their Y chromosome homologs remains elusive. DDX3X and DDX3Y are one such pair of sexually dimorphic non-processive ATP-dependent RNA helicases. Herein, we demonstrate that DDX3Y forms larger RNA-dependent, phase separated liquid condensates compared to DDX3X, which is possibly due to differences in kinetics and dynamic interactions with RNA. By a malachite green phosphate assay and MESG continuous phosphate assay, we find that DDX3X has higher ATPase activity than DDX3Y. We used single-molecule FRET of immobilized Cy3- and Alexa647-labeled RNA duplexes, and report that DDX3X and DDX3Y show different binding and possibly different unwinding activities. The addition of ATP to either DDX3X or DDX3Y shifted FRET efficiency from  $E = 0.8$  to  $E \cong 0$ . The low FRET population was larger for DDX3X than for DDX3Y. Data at three protein concentrations suggest cooperative interaction of the proteins with RNA. Additionally, DDX3X showed a larger proportion of dynamic FRET recordings upon addition of ATP compared to DDX3Y. Freely diffusing complexes were studied by multiparameter confocal spectroscopy to obtain unwinding, anisotropy, diffusion coefficient, and FRET of the RNA duplex upon addition of the helicases in the presence and absence of ATP. Collectively, the kinetics and smFRET data support that DDX3Y has weaker ATPase activity, leading to the less dynamic RNA-DDX3Y complexes. Decreased dynamics, in turn, may contribute to the weaker disassembly of DDX3Y condensates upon addition of ATP compared to DDX3X condensates. Comparison of sex chromosome-encoded protein homologs may provide insights into sexual differences in RNA metabolism and human diseases.

## Session II: Poster 30

### **Mechanism of capping protein regulation by CARMIL**

Kyle R. Barrie, Peter J. Carman, Małgorzata Boczkowska, Grzegorz Rebowski & Roberto Dominguez

CARMIL is a 1371-amino-acid cytoskeletal scaffold that has crucial roles in cell motility and tissue development through interactions with cytoskeletal effectors and regulation of capping protein (CP) at the leading edge. However, the molecular mechanism by which CARMIL regulates CP is unknown. I used cryo-EM to resolve the first structure of a CARMIL dimer, revealing the molecular mechanism of dimerization and inviting further speculation into its role in CP regulation at the plasma membrane. I further used cryo-EM to obtain a preliminary structure of the CARMIL dimer bound to CP. Additional data will resolve the high-resolution structure of this complex and provide a clearer picture of CP regulation. These structures will be used to inform mutagenesis of key residues involved in both CP regulation and plasma membrane binding, which will be tested using a variety of biochemical, biophysical, and cell-biological approaches.

## Session II: Poster 31

### **Membrane lipids as an integral gating component of the large-conductance Ca<sup>2+</sup>- and voltage-gated Slo1 BK channel**

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Large-conductance Ca<sup>2+</sup>- and voltage-gated K<sup>+</sup> Slo1 BK channels play numerous physiological roles and their dysfunction is implicated in disease states. The functional versatility of the channel is enabled by the multi-tier allosteric gating mechanism encompassing its intracellular Ca<sup>2+</sup> sensors, voltage sensors, and the ion conduction gate. The physicochemical nature of the gate, unlike in other canonical K<sup>+</sup> channels, remains obscure. This is a critical knowledge gap; for instance, many modulators of the channel exert their effects by affecting the closed-open transitions of the gate. We showed previously that the Slo1 channel forms and breaks interactions its nearby membrane lipids during each gating cycle [Tian *et al.*, 2019]. Here we performed all-atom molecular dynamics simulations of the hSlo1 channel under low and high open probability (P<sub>o</sub>) conditions: with Ca<sup>2+</sup>/Mg<sup>2+</sup> bound, without Ca<sup>2+</sup>/Mg<sup>2+</sup>, -400 mV, 0 mV, and 400 mV. Machine learning approaches were deployed to reveal those characteristics uniquely associated with the low and high P<sub>o</sub> conditions. The network analysis was utilized to reveal the allosteric pathways. The results from multiple independent systems, totaling >25 μs collectively, show that under low P<sub>o</sub> conditions, the acyl tail groups of select membrane lipids extend into the channel vestibule/cavity, probably interfering with K<sup>+</sup> movement, and that under high P<sub>o</sub> conditions the acyl groups move out of the cavity. Further, in the high-to-low P<sub>o</sub> jump simulations, we observed membrane lipids entering the cavity. The simulation results will be validated with the bilayer electrophysiology with defined membrane lipids of different types.

## Session II: Poster 32

### **Arginylation regulates cytoskeleton organization, cell division, and mitochondria function in fission yeast**

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Protein arginylation, mediated by arginyltransferase ATE1 is a posttranslational modification of emerging importance implicated in regulation of mammalian embryogenesis, cardiovascular system, tissue morphogenesis, cell migration, neurodegeneration, cancer, and aging. ATE1 deletion results in embryonic lethality in mice, but does not affect yeast viability, making yeast an ideal system to study the molecular effects of arginylation. Here, we conducted a global analysis of cytoskeleton-related arginylation-dependent phenotypes in *S. pombe*, a fission yeast species that share many fundamental features of higher eukaryotic cells. Our studies revealed roles of ATE1 in cell division, cell polarization, organelle transport, and interphase cytoskeleton organization and dynamics. We also found a role of ATE1 in mitochondria morphology and maintenance. Furthermore, targeted mass spectrometry analysis of total *S. pombe* arginylome identified a number of arginylated proteins, including those that play direct roles in these processes; lack of their arginylation may be responsible for ATE1 knockout phenotypes. Our work outlines global biological processes potentially regulated by arginylation and paves the way to unraveling the functions of protein arginylation that are conserved at multiple levels of evolution and potentially constitute the primary role of this modification *in vivo*.

## Session II: Poster 33

### **NAD precursors as a treatment for acetaminophen-induced liver injury and acute alcohol-induced liver toxicity**

Beishan Chen, Sarmistha Mukherjee & Joseph Baur

Drug-induced and alcohol-induced liver injuries are the leading causes of acute liver failure worldwide. Acetaminophen (APAP) is a commonly used analgesic drug and it is safe to use at a therapeutic dose. However, overdose of APAP induces acute liver injury, and sometimes results in transplantation or death. Similarly, binge consumptions of alcohol can induce acute liver toxicity and often leads in liver cirrhosis and inflammation, which will lead to transplantations at the final stage. There has always been a substantial amount of interest on finding therapies post injuries for these two types of poisoning. A study showed that nicotinamide (NAM) has both pre- and post-injury preventative effects on APAP-induced liver disease [Shi *et al.*; 2012]. Another study confirmed that supplementing nicotinamide riboside(NR) attenuates short-term chronic alcohol-induced liver injury [Wang *et al.*; 2018]. Other studies have also demonstrated that maintaining and replenishing cellular NAD<sup>+</sup> level is crucial for the recovery in both injuries [Assiri *et al.*; 2019, Liao *et al.*; 2022]. Here, I am aiming to investigate the post-injury rescuing effects of various NAD<sup>+</sup> precursors using both models.



## Session II: Poster 34

### **Exploring the effects of RNA modifications on eukaryotic translation elongation and termination**

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Epigenetic gene regulation has been linked to pivotal roles in human diseases through altering gene expression. Epigenetic marks not only include DNA and histone modifications, but also RNA, defined as Epitranscriptomics. Epitranscriptomic studies have linked RNA modifications to influencing RNA stability dynamics and translation kinetics and efficiency by modulating elongation and termination steps of translation, thereby altering gene and protein expression. Recent studies have begun revealing mechanistic and functional interactions between modifications of different RNA species; however, the scope of such interactions remains to be determined. We are investigating the individual and functional interplay of mRNA and tRNA modifications in elongation and termination by studying the most abundant modification in mRNA, m<sup>6</sup>A, and the tRNA mcm<sup>5</sup>s<sup>2</sup>U wobble position modification. Such wobble modifications significantly affect the versatility of anticodon recognition. We have established an efficient method for obtaining mRNA specifically modified with a single m<sup>6</sup>A, using splint ligation. Several designs containing various coding sequences were ligated, and their corresponding translation activities in programming eukaryotic ribosomes were determined. Sequences containing multiple phenylalanine repeats were disfavored compared to those containing other combinations of amino acids. At present we have selected one design for further studies using single-molecule TIRF imaging to provide mechanistic details as to how single m<sup>6</sup>A modifications modulate translation. We will next prepare tRNAs with and without modification to elucidate single and combined effects of m<sup>6</sup>A and mcm<sup>5</sup>s<sup>2</sup>U in *in vitro* translation assays. We expect such assays to enhance our understanding of the impact of RNA modifications on translation in cells.

## Session II: Poster 35

### **mTORC1 controls postprandial hepatic glycogen deposition via *Ppp1r3b***

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Whole-body glucose homeostasis is essential for organismal survival. Insulin signaling is the central regulator of hepatic glucose metabolism. Insulin suppresses hepatic glucose production while simultaneously promoting the uptake and storage of glucose as glycogen. Canonically, insulin signals through AKT to promote glycogen synthase (GS) activation and stimulate glycogen synthesis. However, recent studies indicate that AKT-dependent phosphorylation of glycogen synthase kinase (GSK) is not required for the induction of glycogen storage, suggesting a mechanism in addition to AKT-mediated GSK phosphorylation. Downstream of AKT remains the mechanistic target of rapamycin complex 1 (mTORC1), as essential regulator of cellular metabolism. Human loss-of-function mutations in tuberous sclerosis complex (TSC) present with excess glycogen deposition, correlating with increased mTORC1 activity, suggesting a role for mTORC1 as a regulator of glycogen accumulation. To test the requirement of mTORC1 for postprandial glycogen storage in the liver, we employed an acute, liver-specific knockout model of Raptor (L-Raptor-KO), the main component of mTORC1. Here we find that mTORC1 activity is required for feeding-induced glycogen storage. Inhibition of hepatic mTORC1 resulted in a defect of postprandial GS activation. Mechanistically, hepatic mTORC1 inhibition blocked the induction of *Ppp1r3b*, an essential phosphatase required for activation of GS and glycogen synthesis. Collectively, we identify a new role of mTORC1 in controlling glycogen synthesis.

## Session II: Poster 36

### **Insulin's acute regulation of hepatic glucose uptake and utilization**

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Insulin resistance is a prevalent metabolic condition of unknown cause. It is characterized by persistent hyperglycemia postprandially despite increased insulin secretion and appropriate activation of insulin signaling pathways in muscle, adipose tissue, and liver. Liver glucose uptake is significantly decreased in patients with insulin resistance and T2DM, which may contribute to hyperglycemia and progression of insulin resistance. The mechanism by which insulin stimulates glucose uptake in muscle and adipose tissue is well understood, but unknown in the liver. Glucose diffuses down its concentration gradient into hepatocytes and glucokinase (GCK) phosphorylates it to prevent its efflux. Insulin acutely increases kinase activity of GCK in the presence of glucose by promoting its translocation from the nucleus to cytoplasm, where it is catalytically active, through an unknown mechanism. We hypothesized that insulin increases hepatic glucose uptake by acutely activating GCK. Using immunofluorescence, we found that pharmacological inhibition of AKT, a downstream effector of insulin signaling, prevented insulin-mediated GCK cytoplasmic localization. Cytoplasmic GCK binds to 6-phosphofructokinase/fructose bisphosphatase-2 (6PFK2/FBPase2), whose PFK2 domain catalyzes formation of an allosteric activator of glycolysis. 6PFK2/FBPase2 contains two AKT consensus phosphorylation motifs at Ser469 and Ser486. Using western blotting, we found that 6PFK2/FBPase2 phosphorylation at Ser486 is increased by insulin treatment of primary rat hepatocytes and mice in an AKT-dependent manner. Based on these preliminary data, we will test the hypothesis that insulin promotes hepatic glucose uptake and utilization through AKT-mediated phosphorylation of 6PFK2/FBPase2 to increase binding to GCK, which might stabilize GCK in the cytoplasm and increase its activity. These experiments will elucidate how insulin acutely increases hepatic glucose uptake, which may inform novel strategies for controlling hyperglycemia in insulin resistant patients.

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***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-I<sub>s</sub>) 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 b-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 ( $0.4 \text{ s}^{-1}$ ) is similar in magnitude to its ADP release rate ( $1 \text{ s}^{-1}$ ). However, the rate of ADP release from Myo1D ( $8 \text{ s}^{-1}$ ) is substantially slower than the rate of phosphate release ( $28 \text{ s}^{-1}$ ). 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.

## Session II: Poster 38

### **Human Myo19 ensembles bound to calmodulin and regulatory light chain drive multi-micron transport**

Luther W. Pollard, Stephen M. Coscia, Nicholas Palmer, Erika L. F. Holzbaur, Roberto Dominguez & E. Michael Ostap

Myosin-19 (Myo19) promotes the normal size, morphology, and distribution of mitochondria but how Myo19 functions as a motor at the molecular scale is unknown. Complicating functional studies *in vitro*, Myo19's light chains (LC) remain controversial. Here, we show by coimmunoprecipitation, reconstitution, and mass spectrometry that the Myo19 expressed in a mammalian cell line (Expi 293) binds both cytoplasmic regulatory light chain (RLC12B) and calmodulin (CaM). Overexpression of Myo19 in HeLa cells resulted in an increase in both Myo19 and RLC around mitochondria, suggesting cellular association of RLC12B with the motor. RLC12B was predominantly located on the second (IQ2) of the three IQ motifs. Robust motility at the maximal rate (~350 nm/s) occurred when motility assays were supplemented with CaM, but not RLC, in solution, suggesting a preference of CaM over RLC for weak-binding sites. The effect of CaM on motility was unchanged in the presence of 100  $\mu$ M free calcium, with a modest reduction in overall speed. Finally, we show that small ensembles of Myo19 motors attached to quantum dots can undergo processive runs over several microns and that calcium weakly reduces attachment frequency and run length. Together, our data indicate that a few single-headed Myo19 molecules attached to a mitochondrion would be capable of prolonged motile associations with actin in a CaM-dependent manner which is atypically modulated by calcium. Based on these properties, conceivably Myo19 could function in transport along actin filaments, produce tension on multiple randomly-oriented filaments, and/or push against branched actin networks assembled near the membrane surface.

## Session II: Poster 39

### **Proteomic profiling identifies distinct mechanisms for the autophagic clearance of nucleoid-enriched mitochondria in Parkinson's disease**

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Neurons depend on autophagy to maintain cellular homeostasis, while defective autophagy and impaired mitochondrial maintenance are pathological hallmarks of neurodegenerative diseases. In order to better understand the role of basal autophagy in maintaining neuron health, and identify changes to autophagy in neurodegenerative disease, we established a reliable method for enriching autophagic vesicles (AVs) from mouse brain and used proteomics to identify the major cargos engulfed within autophagosomes under basal conditions. In AVs isolated from the brains of wild type adult mice, we found that mtDNA and nucleoid-associated proteins including TFAM were significantly enriched compared to other mitochondrial proteins such as components of the electron transport chain. Similar results were observed in AVs isolated from human iPSC-derived neurons. Next, we asked how autophagy is altered in neurodegeneration by comparing the cargos in brain-derived AVs from two different genetic models of Parkinson's disease (PD). Our proteomic analysis suggests there is an upregulation of distinct compensatory pathways in order to maintain the removal of basal cargo, including mitochondria and mtDNA. Autophagosome acidification is impaired by the expression of the LRRK2<sup>G2019S</sup> mutation, and we noted changes to proteins required for the formation of extracellular vesicles (EVs) within autophagosomes. This correlates with increased secretion of EV markers,  $\alpha$ -synuclein, and TFAM, in both conditioned media from LRRK2<sup>G2019S</sup> primary cortical neurons and in the serum of LRRK2<sup>G2019S</sup> mice. In contrast, when the selective removal of damaged mitochondria is impaired by the loss of PINK1, there is evidence for compensatory replacement by the PINK1/Parkin-independent mitophagy receptors BNIP3 and BCL2L13. We also observed higher expression of the essential autophagosome biogenesis protein WIPI2 in the brain of PINK1<sup>-/-</sup> mice. Importantly, engulfment of TFAM is maintained, and total brain levels of TFAM are in fact lower in PINK1<sup>-/-</sup> mice. We propose that the autophagic clearance of mitochondrial nucleoids is required to mitigate the pro-inflammatory risk posed by cytosolic release of mtDNA, and thus this pathway is highly protected and important for understanding the progression of neurodegenerative disease.

## Session II: Poster 40

### **Pulmonary endothelial activation with COVID-19: possible role of reactive oxygen species**

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**Introduction:** Recent research suggests that endothelial activation plays a role in COVID-19 pathogenesis by promoting a pro-coagulative and pro-inflammatory state. However, the mechanism by which the endothelium is activated in COVID-19 is unclear. **Objective:** Investigate the mechanism by which COVID-19 activates the pulmonary endothelium.

**Hypothesis:** The pulmonary endothelium generates reactive oxygen species (ROS) upon exposure to the “inflammatory load” of the systemic circulation. **Methods:** COVID-19 was recreated in vitro by exposing human lung endothelial cells to serum from COVID-19 affected subjects (sera were acquired from patients with COVID-19 infection admitted to the Intensive Care Unit of the Hospital at the University of Pennsylvania). This was followed by assessment of ROS (fluorescent dye, CellROX) and intercellular adhesion molecule (ICAM-1) by fluorescence labeling and imaging. **Results:** Both endothelial activation (as monitored by ROS production) and pro-inflammatory phenotype (as assessed by ICAM-1), were significantly higher with COVID-19 as compared to normal subjects. **Conclusions:** The endothelium is activated with COVID-19 via ROS production; the ROS thus produced drives a pro-inflammatory phenotype by inducing the expression of ICAM-1, a pivotal marker of endothelium inflammation. As ROS mediates endothelial activation and inflammation during COVID-19, ROS blockade could have therapeutic implications in maintaining vascular health.

## Session II: Poster 41

### **Glucagon blockade can prevent and treat stress-induced hyperglycemia**

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Stress-induced hyperglycemia (SIH) is a state of elevated blood glucose after injury or illness that is associated with mortality across disciplines including trauma, sepsis, and COVID-19. SIH is frequently attributed to insulin resistance as insulin infusions can improve outcomes. However, overtreatment is also associated with mortality, highlighting a need for novel therapies. Patients with SIH exhibit increased glucagon, a hormone that signals through PKA to increase hepatic glucose production. Excess glucagon is implicated in diabetes pathophysiology, thus we hypothesized that glucagon drives SIH. We used an established mouse model of SIH known as fixed pressure trauma and hemorrhage (TH) that includes soft-tissue injury and 90 minutes of hemorrhage-induced hypotension. At procedure's end (TH90), mice exhibited hyperglycemia and hyperinsulinemia compared to baseline consistent with SIH ( $p < 0.001$ ) as well as increased hepatic PKA signaling and serum glucagon ( $p < 0.01$ ). We found hepatic PKA signaling was required for SIH as mice bearing liver-specific dominant negative inhibitory mutation in PKA no longer exhibited SIH at TH90 ( $p < 0.01$ ). This was glucagon specific as both mice lacking the hepatic glucagon receptor and mice pretreated with a small molecule glucagon receptor antagonist (GRA) were protected from SIH ( $p < 0.05$  and  $p < 0.01$ ). To test if GRA's could treat SIH, we treated mice that exhibited SIH at 30 minutes into the TH protocol (glucose  $> 200$ mg/dL) with GRA and found the drug lowered glucose superior to vehicle ( $p < 0.01$ ). This demonstrates that genetic and pharmacological glucagon blockade can prevent and treat SIH. Future work will determine if treating SIH with a GRA improves survival.



## Session II: Poster 42

### **Substrate stiffness, but not the absence of a vimentin cage, modulates the mechanical properties of the nucleus**

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Previous studies using atomic force microscopy (AFM) have shown that cells are able to modulate the stiffness of their nuclei in response to changes in the stiffness of their environment. However, these measurements, made *in situ*, can be complicated due to the close interactions between the nucleus and cytoskeletal elements such as the vimentin cage. To address this issue, we isolated nuclei from mouse embryo fibroblasts (MEFs) that were cultured on glass as well as soft and hard polyacrylamide gels using a relatively gentle, non-detergent method combining treating cells with cytochalasin D and centrifugation. This method produces nuclei that are enveloped by the donor cell's plasma membrane as well as a thin layer of cytoplasm. We show that nuclei isolated from MEFs grown on soft substrates have a similar size but lower stiffness than nuclei from cells grown on stiffer substrates as determined by AFM. Furthermore, to determine how changes to the intracellular micro-environment may affect the mechanical properties of the nucleus, we compared the stiffness of nuclei that were isolated from wild type MEFs with those isolated from vimentin null MEFs that lack a vimentin cage around the nucleus. Interestingly, while the absence of a vimentin cage decreased the amount of force required to isolate nuclei from vimentin null MEFs, no differences were observed in the size or mechanical properties of these nuclei. These data further elucidate how the mechanical properties of the nucleus can be affected by the external and internal environment of the cell.

## Session II: Poster 43

### **Dynamic nuclear-sarcomeric coupling in the beating cardiomyocytes**

Daria A. Pavlov, Lauren Testa & Benjamin L. Prosser

The adult cardiomyocyte is subjected to substantial mechanical forces that are transferred from the actomyosin, microtubule, and intermediate filament networks into the nucleus through the linker of the nucleoskeleton and cytoskeleton (LINC) complex. Human mutations in various LINC components are associated with cardiomyopathies, and disrupting the LINC complex may be cardioprotective in myopathy driven by variants in Lamin-A, the key structural component of the nucleoskeleton. Despite this growing translational relevance, the nuclear mechanotransduction pathway in the adult cardiac myocyte is not well established. Here we investigated the pathway of mechanical force transfer into the nucleus by analyzing the dynamic sarcomere-nucleus strain coupling during stimulated contraction of isolated adult rat cardiomyocytes. We demonstrate that perturbing endogenous LINC complexes results in diminished strain transfer into the nucleus during myocyte contraction, while disrupting microtubules has the opposite effect of increased dynamic nuclear strain. Further 3D analysis of the chromatin demonstrates reduced peripheral chromatin density, despite an increase in the repressive H3K9me2 mark, in the LINC perturbed cardiomyocytes. Interestingly we also observe increased interaction between Lamin B and H3K9me3 proteins at the nuclear periphery, using the proximity ligation assay. These changes in protein localization and interactions at the nuclear periphery might have implication on the regulation of gene expression and help dissect the pathways of mechanical signal transfer into the nucleus and their downstream consequences in highly contractile cells, which are essential to understand the adaptive and pathologic response of the heart to changing mechanical loads.

## Session II: Poster 44

### **Savory signaling: T1R umami receptor modulates ER calcium store content and calcium release in airway epithelial cells**

Derek B. McMahon, Jennifer Jolivert, Li Eon Kuek, Zoey A. Miller, Nithin D. Adappa, James N. Palmer & Robert J. Lee

Chronic inflammation due to disease, smoking, or severe chronic rhinosinusitis or asthma causes remodeling of the airway epithelium, including squamous and basal cell metaplasia and loss of multiciliated cells. In addition to clearing pathogens, motile ciliated airway cells are important for nitric oxide (NO) production to kill pathogens. Inflammatory airway diseases are characterized by decreased airway NO. Boosting airway NO production is thought to be a strategy to improve innate defense. To understand how epithelial function is altered during obstructive airway diseases, we studied basal and squamous nasal and bronchial epithelial cells. We previously showed that these cells express bitter taste receptors (taste family 2 receptors, or T2Rs) that regulate nuclear  $Ca^{2+}$  to control proliferation and cell death. We also detected expression of taste family 1 umami receptor (T1R) receptor components T1R1 and T1R3. Umami agonists monosodium glutamate and inosine monophosphate decreased ER store  $Ca^{2+}$  content and reduced T2R agonist-induced  $Ca^{2+}$  release, while T1R3 antagonist lactisole increased both ER  $Ca^{2+}$  content and release, likely through cAMP/EPAC signaling. Lactisole increased T2R-stimulated cytosolic  $Ca^{2+}$  release but neither nuclear nor mitochondrial  $Ca^{2+}$  increased, and the rate of T2R induced apoptosis did not increase either. The cytosolic  $Ca^{2+}$  release was associated with increased NO production. Thus, a T1R antagonist like lactisole may be useful coupled with bitter compounds (for example, in a nasal rinse) to increase NO production in patients suffering from chronic inflammatory diseases.

## Session II: Poster 45

### **Impact of the mitochondrial NAD transporter SLC25A51 on respiratory complexes of the electron transport chain**

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The mitochondrial electron transport chain (ETC) consists of four complexes, Complex I through IV, that transfer electrons and pump protons across the inner mitochondrial membrane. The electrochemical gradient generated as a result, provides potential energy for ATP synthesis by complex V. Complex I function is NAD-dependent, as it requires oxidation of NADH as a source of electrons to fuel proton pumping. The fundamental question of how NAD enters the mitochondria remained a mystery for more than a century. Work from our lab and two others independently identified SLC25A51 as a mitochondrial NAD transporter. SLC25A51 deficiency reduces mitochondrial NAD and renders complex I nonfunctional. Surprisingly, our preliminary data indicate that the protein levels of various respiratory complexes are reduced in the SLC25A51 knockout cell line. Thus, in this study, we tested the role of SLC25A51 on the respiratory capacity of different complexes using high-resolution respirometry using isolated mitochondria and intact cells. We found that the mitochondria isolated from the SLC25A51 knockout cell line have a higher response to complex IV substrate than mitochondria from wild-type cell lines. Contrastingly, oxygen consumption, induced by complex II and III substrates, is unaltered in the SLC25A51 knockout cells compared to the wild type. In addition, the SLC25A51 knockout cell line continues to proliferate despite the >90% decrease in mitochondrial NAD and lack of complex I function. We hypothesize that the knockout cell lines are metabolically rewired to support cell proliferation. We found that the SLC25A51 knockout cells are resistant to complex I inhibitor rotenone-induced cell death but equally susceptible to complex II and complex IV inhibitors. However, treatment of cells with 2-deoxy-D-glucose, an inhibitor of glycolytic enzymes, profoundly affected the proliferation of SLC25A51 knockout cells but not wild-type cells. These findings suggest that glycolysis is required to sustain proliferation in the absence of SLC25A51. We are currently testing whether the proliferation of SLC25A51 KO cells could be rescued using alternative metabolic substrates, including succinate and proline. This study will lay the groundwork to identify molecules that can be used to maintain survival in pathological diseases with compromised complex I function, such as Parkinson's disease and mitochondrial myopathies.

## Session II: Poster 46

### **Global-run on sequencing in liver identifies *Gm11967* as an Akt-dependent long noncoding RNA involved in insulin sensitivity**

Dominic Santoleri, Hee-Woong Lim, Matthew J. Emmett, Julian Stoute, Matthew J. Gavin, Jaimarie Sostre-Colón, Kahealani Uehara, Jaclyn E. Welles, Kathy Fange Liu, Mitchell A. Lazar & Paul M. Titchenell

Akt and FoxO1 are key regulators of the hepatic transcriptional response that occurs in response to insulin and during feeding, and while much research has demonstrated the importance of the Akt/FoxO1 axis on hepatic transcription, the full extent of their regulation has not been fully outlined. We performed global run-on sequencing (GRO-seq) to measure nascent transcription in the liver of fasted and refeed control mice as well as in genetic mouse models lacking Akt-FoxO1 signaling specifically in hepatocytes. The ability of GRO-seq to measure nascent transcription enable it to read lowly or transiently expressed transcripts such as enhancer RNAs (eRNAs) or long noncoding RNAs (lncRNAs), making it a more powerful tool for global RNA profiling than methods such as RNA-seq or microarray analysis. We identified 599 feeding regulated gene transcripts out of over 7,000, among which were 37 lncRNAs as well as over 6,000 feeding regulated eRNAs. We furthered this analysis by mapping their dependency on Akt/FoxO1 signaling using mice lacking Akt or both Akt and FoxO1 in their livers. From this study, we identified the feeding and Akt/FoxO1 regulated lncRNA, *Gm11967*. *Gm11967* shares a transcriptional start site with glucokinase, a robust insulin-dependent transcriptional target in liver. Restoring *Gm11967* expression in mice lacking hepatic Akt signaling led to a partial improvement in insulin sensitivity and stimulated hepatic glucokinase translation. These data suggest that Akt-dependent control of *Gm11967* contributes to the translational control of glucokinase, which helps mediate insulin sensitivity. This new genome-wide dataset we developed to define the feeding and Akt-dependent changes in hepatic nascent transcription will be a powerful guide to further research into the discovery and understanding of how feeding and insulin regulate hepatic transcription.

## Session II: Poster 47

### **Interplay between secretory autophagy and macroautophagy in neurons**

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Unlike most cell types, neurons are largely resistant to autophagy induction following starvation or stress. Instead, autophagy primarily functions as a homeostatic mechanism, constitutively degrading damaged proteins and organelles. This reduced capacity for autophagy induction could leave neurons particularly vulnerable to stress, especially considering neurons are post-mitotic, precluding them from clearing damaged proteins and organelles through cell division. Do neurons mobilize additional pathways to discard of damaged material when their capacity for autophagy is overwhelmed? Our observations suggest neurons shunt material toward a secretory fate when autophagy capacity is reduced. Using cultured neurons, we find that offloading of material is increased when the autophagy pathway is pharmacologically or genetically strained. This has important implications in neurodegenerative diseases, such as Parkinson's Disease, where neuronal autophagy is strained, and aggregated proteins propagate throughout the brain via autophagy-dependent mechanisms that remain poorly understood. Future directions will investigate interplay between cell-autonomous autophagy and secretory autophagy.

## Session II: Poster 48

### **Development of microfluidic flow chambers to study blood clotting *ex vivo***

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Upon blood vessel injury platelets quickly accumulate at a damaged site to build a thrombus. During this process platelets become activated and secrete granules, which are known to significantly affect thrombus formation. However, spatiotemporal dynamics of granule secretion within a growing thrombus is still elusive. Our work is aimed to develop microfluidic flow chambers for real-time observation of dense granules secretion in individual platelets and platelets inside the thrombus. Our chambers are made of Polydimethylsiloxane (PDMS) cubes with several openings for flow channels attached to a glass coverslip and flow tubes. To mimic an injury site, we incubate various adhesive proteins and coagulation activators on a glass coverslip treated with Oxygen plasma. These systems allow us to choose and control complex and diverse conditions such as blood flow parameters, type and geometry of activator area etc. for studying thrombus formation and granules secretion interplay.

To study dense granules secretion in non-activated platelets we created a method based on flow chambers in which PECAM-1 antibody was used as an adhesive coating for fixing platelets on a glass surface. This poster will describe current progress and preliminary results for this method, as well as show detailed procedures to prepare flow chambers and visualization of antibody surface coating for immobilization of platelets and further analysis of dense granule secretion. In future this method can be applied to study single platelets adhesion and activation as well as thrombi growth dynamics using different activators or adhesive proteins such as Tissue Factor, Collagen, various antibodies etc.