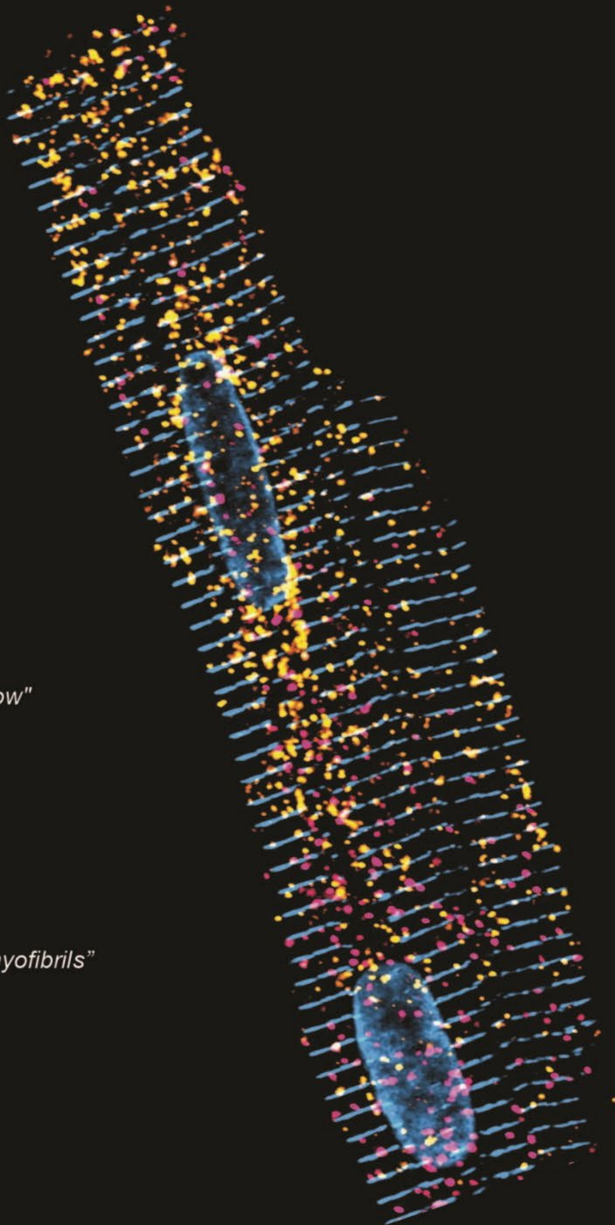
Pennsylvania Muscle Institute
Annual Retreat and Symposium

Perelman School of Medicine
University *of* Pennsylvania

Formation, Maintenance, and Repair of Skeletal and Cardiac Muscle



November 1, 2022

Arthur H. Rubenstein Auditorium
Smilow Center for Translational Research

Invited Lectures:

Leslie Leinwand, PhD
University of Colorado, Boulder
"Sarcomere dynamics vary between muscle and in health and disease"

Mary Baylies, PhD
Sloan Kettering Institute
"Regulation of the size, number, and function of sarcomeres during drosophila muscle growth"

Dylan Burnette, PhD
Vanderbilt University
"How does a heart grow? A cell biologist wants to know"

Izhak Kehat, MD, PhD
Technion Institute
"Localized translation in cardiomyocytes"

The Sanger Lab
Upstate Medical University
"Assembly and Dynamics of sarcomeric proteins in myofibrils"

William Roman, PhD
Stanford University
"Myofiber self-repair after exercise"

Local Lectures:

Keita Uchida (Prosser Lab)
University of Pennsylvania
"Dynamics of Cardiomyocyte Translation""

Nuoying Ma (Mourkioti Lab)
University of Pennsylvania
"Piezo1 is a key mechanosensor for muscle stem cell morphological state transition and function"

Quentin McAfee (Arany Lab)
University of Pennsylvania
"Truncated titin in human dilated cardiomyopathy"

Scan to see schedule

www.med.upenn.edu/pmi

Pennsylvania Muscle Institute Annual Retreat and Symposium 2022

"Formation, Maintenance, and Repair of Skeletal and Cardiac Muscle"

Tuesday, November 1, 2022

Location: Arthur H. Rubenstein Auditorium & Commons,
Smilow Center for Translational Research,
3400 Civic Center Blvd., Philadelphia, PA 19104

Organized by E. Michael Ostap and Benjamin Prosser
www.med.upenn.edu/pmi

Sponsored by the Physiological Society of Philadelphia and the Pennsylvania Muscle Institute

Registration

8:30 – 9:00am **Registration Check-in, Breakfast, Coffee, Poster Setup**
Location: Arthur H. Rubenstein Auditorium & Commons*
**Rubenstein Auditorium is located at the Northwest corner of the 1st floor of Smilow (up the escalator from the ground floor)*

9:00 – 9:15am **Welcome and Introduction**
E. Michael Ostap, PhD
Director, Pennsylvania Muscle Institute
Professor of Physiology

Andrew P. Somlyo Honorary Lectures

9:15 – 10:00am **Mary Baylies, PhD**
Sloan Kettering Institute
"Regulation of the size, number, and function of sarcomeres during drosophia muscle growth"

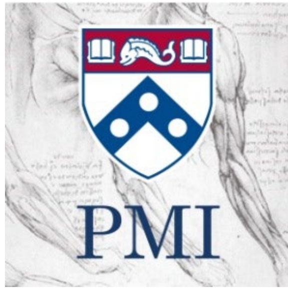
10:00 – 10:30am **PMI Trainees**
Keita Uchida (Prosser Lab)
University of Pennsylvania
"Dynamics of Cardiomyocyte Translation"

Nuoying Ma (Mourkioti Lab)
University of Pennsylvania
"Piezo1 is a key mechanosensor for muscle stem cell morphological state transition and function"

10:30 – 11:00am	**Coffee Break, Posters**
11:00 – 11:45am	**William Roman, PhD** Stanford University *"Myofiber self-repair after exercise"*
11:45am – 12:30pm	**Izhak Kehat, MD, PhD** Technion-Israel Institute of Technology *"Localized translation in cardiomyocytes"*
12:30 – 1:30pm	**Lunch, Posters**

Saul Winegrad Honorary Lectures

1:30 – 1:45pm	**Yale E. Goldman, MD, PhD** University of Pennsylvania *Remembering Professor Saul Winegrad*
1:45 – 2:30pm	**Leslie Leinwand, PhD** University of Colorado, Boulder *"Sarcomere dynamics vary between muscle and in health and disease"*
2:30 – 3:15pm	**The Sanger Lab** Upstate Medical University *"Assembly and Dynamics of sarcomeric proteins in myofibris"*
3:15 – 3:45pm	**Coffee Break, Posters**
3:45 – 4:00pm	**PMI Trainee** **Quentin McAfee (Arany Lab)** University of Pennsylvania *"Truncated titin in human dilated cardiomyopathy"*
4:00 – 4:45pm	**Dylan Burnette, PhD** Vanderbilt University *"How does a heart grow? A cell biologist wants to know"*
4:45 – 5:30pm	**Reception and Posters**



Pennsylvania Muscle Institute
Perelman School of Medicine
University of Pennsylvania

The Pennsylvania Muscle Institute (PMI) is an internationally renowned center for muscle and motility research supported by Penn Medicine with a mission to:

- Discover the mechanisms of muscle function, muscle disease and motile biological systems through innovative and cross-disciplinary research, and to apply these discoveries to new therapies,
- Develop state-of-the art technologies for the study of muscle and motile systems,
- Provide education and training in muscle biology and motility to scientists, physicians, and students.

Research is conducted by its more than 60 laboratories using biophysics, biochemistry, genetics, physiology and ultrastructure to understand cell migration and intracellular transport, molecular motors, cell division, muscle contraction and development, muscle pathologies and therapies targeted to muscle disease. We are prominent in technological and methodological development for these investigations especially in advanced light microscopy, structural spectroscopy, nanotechnology, biochemical kinetics, image processing, molecular biology, and viral gene targeting. Extramural grants, seminars, symposia, and journal clubs are uniquely initiated and supported by the PMI. Additionally, the PMI sponsors vigorous graduate and post-doctoral training activities, including a NIAMS-supported training program in "Muscle Biology and Muscle Disease."

For questions or inquiries about PMI membership, please contact:

E. Michael Ostap, Ph.D.
Director, PMI
Professor of Physiology
Email: ostap@pennmedicine.upenn.edu

Benjamin L. Prosser, Ph.D.
Associate Director, PMI
Associate Professor of Physiology
Email: bpros@pennmedicine.upenn.edu

Pennsylvania Muscle Institute (PMI)
Perelman School of Medicine at the University of Pennsylvania
700A Clinical Research Building
415 Curie Blvd. Philadelphia, PA 19104

Please visit our website: http://www.med.upenn.edu/pmi/

Guest Speaker Biographies

Mary Baylies, PhD
Full Member/Professor in the Developmental Biology Program in Sloan Kettering Institute at the Memorial Sloan Kettering Cancer Center; joint appointment at Weill Cornell School of Biomedical Sciences in the Cell and Developmental Biology Department

Mary Baylies is a Full Member/Professor in the Developmental Biology Program in Sloan Kettering Institute at the Memorial Sloan Kettering Cancer Center; she also holds a joint appointment at Weill Cornell School of Biomedical Sciences in the Cell and Developmental Biology Department. Her laboratory investigates muscle specification, differentiation, growth, homeostasis, and function, both during normal development and in disease contexts. Dr. Baylies earned her Ph.D. at Rockefeller University in 1991 in the lab of Michael Young, where she investigated molecular mechanisms underpinning circadian rhythms. She completed her postdoctoral work at the University of Cambridge with Michael Bate in 1997, researching muscle development, particularly with a view to understanding the intrinsic and extrinsic programs necessary to build a muscle cell. In 1997, Dr. Baylies joined the faculty at Sloan Kettering Institute, where she investigates the molecular machinery required in making and maintaining muscle fibers. Her research is conducted by developing and applying novel genetic, cell biological, imaging, molecular and mathematical approaches, using models including Drosophila and human iPSC-derived Skeletal myoblasts/myofibers. The ultimate aim of her research is to better understand skeletal muscle, and so contribute to the search for therapies for the treatment of muscle diseases including Rhabdomyosarcoma, Nemaline Myopathy, Centronuclear myopathies, and cancer cachexia. The current focus of her work is on how multinucleated muscle cells are formed, how individual muscles achieve particular sizes and shapes, and how muscle subcellular architecture changes during differentiation, muscle function and in diseases. The work from her group has provided new insights to muscle identity through interactions between signal transduction pathways, transcription factors and chromatin regulators; muscle size through identification of mechanisms involved in muscle fusion, growth, and nuclear scaling; and muscle function through study of genes and mechanisms responsible for myonuclear/organelle movement and positioning and sarcomere/ myofibril growth and maturation. Dr. Baylies has 25+ years' experience in muscle biology and has published over 75 papers with NIH funding.

William Roman, PhD
Stanford University

William Roman obtained his PhD from the Paris Descartes University and the Freie University of Berlin working on nuclear positioning during skeletal muscle development. He then performed his post-doctoral training in the laboratory of Pura Munoz in Barcelona studying how myofibers respond to discrete injuries. In parallel, William leads the tissue engineering MyoChip team in Lisbon aimed at supplementing neurons and a vasculature to in vitro muscle culture. He is currently at Stanford University to apply

imaged-based spatial genomic techniques to muscle specimens and will begin his laboratory on intercellular communication within the muscle organ at Monash University in Australia.

Izhak Kehat, MD, PhD

Associate Professor and the head of the Department of Physiology, Biophysics, and System Biology at the Ruth and Bruce Rappaport Faculty of Medicine, Technion – Israel Institute of Technology

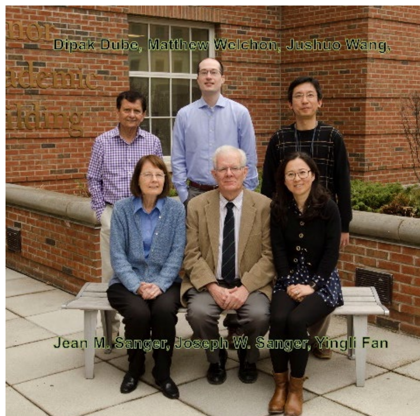
Izhak Kehat MD, PhD received his MD (1997, Suma cum laude) from the Faculty of Medicine, Technion – Israel Institute of Technology. During his PhD (2005), he worked on deriving cardiomyocytes from human embryonic stem cells at the Faculty of Medicine at Technion, under the supervision of Professor Lior Gepstein. He completed a residency in Internal Medicine (2006) at the Rambam Medical Center, Haifa, Israel, and a fellowship in Cardiology at the Rambam Medical Center (2011, in excellence). At Cincinnati Children's Hospital Medical Center (CCHMC, USA) (2006-2010), he was a postdoctoral fellow under the guidance of Prof. Jeffery Molkentin, where he investigated the signals and gene regulation of cardiac hypertrophy. On his return to Israel, he finished a fellowship in clinical Echocardiography, under the supervision of Prof. Yoram Agmon at Rambam Medical Center, department of physiology. Dr. Kehat is an associate professor and the head of the Dept. of Physiology, biophysics, and system biology, at the Ruth and Bruce Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, and a senior cardiologist at the Rambam Medical Center (Haifa, Israel) echocardiography unit. In his research, he aims to understand the structure and function of the sarcomere, the contractile "engine" of muscle and heart, as well as how its deterioration contributes to heart failure. Sarcomere protein components have a short life span; therefore, they need to be replaced constantly. The Kehat lab recently discovered that each sarcomeric 'engine' is maintained by a nano-scale 'factory' in which mRNA is localized, sarcomeric proteins are locally produced, and excess proteins are discarded. The discovery sheds light on the maintenance of sarcomeres and opens up new therapeutic possibilities. The Kehat lab also studies the process of vascular and valve calcifications. Prof. Kehat is a member of the ESC and the AHA, was the secretary of Israel Heart association myocardium and pericardium working group, and is the treasurer of the Israeli sub-section of the ISHR.

Leslie Leinwand, PhD

Molecular, Cellular, and Developmental Biology (MCDB) Distinguished Professor and the Chief Scientific Officer of the BioFrontiers Institute, University of Colorado, Boulder

Leslie Leinwand, PhD is a Molecular, Cellular, and Developmental Biology (MCDB) Distinguished Professor and the Chief Scientific Officer of the BioFrontiers Institute at the University of Colorado Boulder. She received her Bachelor's degree from Cornell University, her PhD from Yale University and did post-doctoral training at Rockefeller University. She joined the faculty at Albert Einstein College of Medicine in New York in 1981 and remained there until moving to Colorado in 1995. She co-founded Myogen, Inc. which was sold to Gilead Pharmaceuticals. More recently, she was a co-founder of Hiberna, Inc, and of MyoKardia, Inc., a

company founded to develop therapeutics for inherited cardiomyopathies. MyoKardia was acquired in 2020 by Bristol Myers Squibb. She is a Fellow of the AAAS, former MERIT Awardee of the NIH, Established Investigator of the American Heart Association and was recently elected to the American Academy of Arts and Sciences and the National Academy for Inventors. She has been honored by the American Heart Association with its Distinguished Scientist Award and its Braunwald Academic Mentorship Award. The interests of Dr. Leinwand's laboratory are the genetics and molecular physiology of inherited diseases of the heart and particularly how biologic sex affects the heart in health and disease. The study of these diseases has required multidisciplinary approaches, involving molecular biology, mouse genetics, mouse cardiac physiology, and the analysis of human tissues. She has also studied the biology of the Burmese python to uncover cardio-protective mechanisms.



The Sanger Lab
SUNY Upstate Medical University

Jean M. Sanger, PhD (Professor, Department of Cell and Developmental Biology); Joseph W. Sanger, PhD (Professor, Department of Cell and Developmental Biology); Yingli Fan, PhD (Research Associate, Department of Cell and Developmental Biology); Dipak K. Dube, PhD (Research Professor of Medicine, Adjunct Research Professor of Department of Cell and Developmental Biology); Matthew Welchon, MS; Jushuo Wang, PhD (Research Assistant Professor, Department of Cell and Developmental Biology).
Our research advances the understanding of fundamental molecular and biochemical mechanisms of cellular function and development. The aims of our training and educational programs are to apply biological knowledge to critical medical problems and empower the next generation of scientists, clinicians, and educators.

Dylan Burnette, PhD
Associate Professor of Cell and Developmental Biology,
Vanderbilt University School of Medicine

Dr. Dylan Burnette has been using high resolution microscopy to study cells for over 20 years. His laboratory at Vanderbilt University focuses on how cells grow and divide. He is interested in how these processes contribute to the function of heart muscle. He trained as a graduate student with Dr. Paul Forscher at Yale University and as a post-doctoral fellow with Dr. Jennifer Lippincott-Schwartz at the National Institutes of Health.

Honorary Lectures

Andrew P. Somlyo, M.D.

(1930 – 2004)

Professor of Physiology and Pathology and founding Director of the Pennsylvania Muscle Institute, Dr. Somlyo was a luminary in the field of smooth muscle physiology. His research (in collaboration with Dr. Avril Somlyo) played a key role in showing that actin-myosin interactions are responsible for force generation in smooth muscle. With colleagues at the University of Pennsylvania, Dr. Somlyo developed electron probe microanalysis to determine local ion concentrations in tissues at nanometer resolution. Additionally, his pioneering work in signaling revealed the mechanisms that regulate contraction of smooth muscle independently of the membrane potential – a process he termed pharmacomechanical coupling. Dr. Somlyo had a passion for science that is evident in the remarkable imprint that he left on the field of muscle physiology and on his students and colleagues. He was also a noted collector of Asian art. Dr. Somlyo left Penn Medicine in 1988 to chair the Department of Molecular Physiology and Biological Physics at the University of Virginia School of Medicine.

Saul Winegrad, M.D.

(1931 - 2020)

Professor Emeritus of Physiology at the Perelman School of Medicine, Dr. Winegrad was a founding member of the Philadelphia muscle-centric group known as the Myo-Bio Club, now the Pennsylvania Muscle Institute. A native Philadelphian, Dr. Winegrad received his BA in chemistry in 1952 and his MD in 1956, both from the University of Pennsylvania. After interning in Boston, Dr. Winegrad held a research fellowship at the NIH. Sir Andrew Huxley invited him to spend a year at University College London, after which Dr. Winegrad joined the Penn faculty as assistant professor of physiology and medicine, in 1962. Seven years later, he became a full professor. He founded and organized the Biomedical Graduate Studies program, which oversees all of Penn Perelman School of Medicine graduate groups. The Saul Winegrad Award for Outstanding Dissertation was established for the graduate groups upon Dr. Winegard's retirement. Dr. Winegrad's research interests were in cardiac muscle physiology at the sub-cellular level, specifically the role of myosin binding protein (MyBP-C) and its phosphorylation in the contractile function of the heart with particular attention to structure-function correlation and interaction with other myofibrillar proteins. He was recognized internationally for his research and was a Fulbright Fellow, National Science Foundation Fellow, Guggenheim Fellow and Fogarty-CNRS International Fellow. He served as vice president for research for the American Heart Association (AHA) and received the National Award of Merit from the AHA.

POSTER ABSTRACTS

1.

FMRP granules are positioned along mitochondria to regulate fission in neurons

Adam Fenton[1,2], Charles Bond[1], Melike Lakadamyali[1], Thomas Jongens[2], Erika Holzbaur[1]

[1]Department of Physiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA
[2]Department of Genetics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

The Fragile X Mental Retardation Protein (FMRP) is a component of RNA granules whose absence causes Fragile X Syndrome, a severe neurodevelopmental disorder. 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. Late endosomes and lysosomes have previously been shown to associate with sites of mitochondrial fission and to deliver RNA granules to mitochondria in neurons. We demonstrate contacts between FMRP and multiple markers of the endo-lysosomal system at mitochondrial ends and fission sites. FMRP positioning at mitochondrial fission sites is Rab7 GTPase-dependent, suggesting that late endosomes/lysosomes deliver FMRP-positive RNA granules to mitochondria to locally promote fission. Functionally, mitochondria-associated FMRP granules serve as sites of local protein synthesis. Inhibiting protein synthesis reduces the frequency of mitochondrial fission marked by FMRP, indicating that a subclass of mitochondrial fission events in neurons, which are marked by FMRP, are dependent on protein synthesis. Mitochondrial fission is also facilitated by the Mitochondrial Fission Factor (MFF) protein, which recruits fission machinery to mitochondria. We find that MFF marks mitochondrial ends and co-localizes with FMRP granules at sites of fission, but FMRP recruitment to the mitochondrial midzone precedes MFF. Further, we find evidence that MFF mRNA is present in mitochondria-associated FMRP granules and that MFF protein is translated at mitochondria-associated FMRP granules. Our findings demonstrate a role for FMRP in the control of mitochondrial fission in neurons and suggest that FMRP granules support neuronal mitochondrial homeostasis by serving as platforms to match local translation with mitochondrial function.

2.

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

Aleksandr Maiorov, Ekaterina V. Tarasovetc, Ekaterina L. Grishchuk

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

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 poster will describe our progress with reconstituting KMN recruitment via CENP-C and discuss future experiments.

3. **Mechanical regulation of chromosome loss in lung cancer spheroids**

Alisya Anlas, Brandon H. Hayes & Dennis E. Discher

Genomic instability is the inability of a cell to pass on its genetic information with fidelity and is a hallmark of cancer. About 85% of solid tumors exhibit an abnormal number of chromosomes, or aneuploidy, because of errors in DNA replication or mitosis. Changes in the tumor microenvironment during cancer progression can physically confine cancer cells and drive chromosome missegregation during mitosis. Here, using a chromosome reporter, we investigated whether a 3D tumor microenvironment contributes to aneuploidy. Specifically, we cultured lung adenocarcinoma spheroids to investigate how physical confinement regulates mitotic aberrations that could result in chromosome missegregation. Our data suggest that the cells at the surface of a spheroid display more mitotic events, but less chromosome loss, suggesting a role for mechanical confinement in the regulation of chromosome segregation in H23 lung adenocarcinoma spheroids.

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

Anastasia A. Masaltseva[1,2], Taisiya O. Shepelyuk[1], Fazoil A. Ataullakhanov[1,2,3,4,*], Dmitry Yu. Nechipurenko[2,3,4,*], Ekaterina L. Grishchuk[1,*]

[1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [3]Center for Theoretical Problems of Physicochemical Pharmacology, Russian Academy of Sciences, Moscow, Russia; [4]Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russia; [2]Department of Physics, Lomonosov Moscow State University, Moscow, Russia; *these authors contributed equally to developing and implementing this work.

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.

5.

Mechanisms of Nonmuscle Myosin II Turnover in the Cellular Contractile System

Anil Chougule and Tatyana Svitkina

Department of Biology, University of Pennsylvania, Philadelphia, PA, USA

Nonmuscle myosin II (NM II) is essential to generate cellular contractility in all eukaryotic cells, which express three isoforms: NM IIA, IIB, and IIC. All three paralogs employ a mix-and-match approach to copolymerize into homo- and heteropolymers to form the NMII bipolar filaments in cells. The most abundantly expressed NM IIA and IIB paralogs differ in their intrinsic motor activities and filament turnover rates, attributed to their N-terminal heads and C-terminal tails, respectively. Recent work in the lab has shown that NM IIA and NM IIB can copolymerize to form hybrid NM II filaments and that an ectopic expression of NMIIA in COS7 cells, which are devoid of NMIIA, induces re-distribution of the endogenous NMIIB from sharply defined stress fibers to interconnected stress fiber network and to scattered distribution depending on the NMIIA expression level. These data suggested a self-sorting model wherein indiscriminate copolymerization of NMIIA and NMIIB coupled with selectively faster dissociation of NMIIA subunits and retrograde actin flow leads to their polarized distribution in cells. However, it remains unclear which isoform-specific properties of NMII isoforms contribute to their subcellular distribution and organization of the cellular contractile system. To address this question, we express NMIIA mutants and NMIIA/NMIIB chimeras in COS7 cells and compare their effects to those of NMIIA. Our initial analysis using NM II chimeras revealed that the tail of NMII IIA controls the re-distribution of endogenous NM IIB in COS7 cells. We are validating these results by analyzing the NM IIA constructs lacking C-terminal nonhelical tailpiece or carrying mutated phosphorylation sites in the tail domain.

6.

Differential N-terminal processing of beta and gamma actin

Li Chen, Pavan Vedula, Hsin Yao Tang, Dawei W Dong, **Anna S Kashina**

Cytoplasmic beta- and gamma-actin are ubiquitously expressed in every eukaryotic cell. They are encoded by different genes, but their amino acid sequences differ only by four conservative substitutions at the N-termini, making it difficult to dissect their individual regulation. Here, we analyzed actin from cultured cells and tissues by mass spectrometry and found that beta, unlike gamma actin, undergoes sequential removal of N-terminal Asp residues, leading to truncated actin species found in both F- and G-actin preparations. This processing affects up to ~3% of beta actin in different cell types. We used CRISPR/Cas-9 in cultured cells to delete two candidate enzymes capable of mediating this type of processing. This deletion abolishes most of the beta actin N-terminal processing and results in changes in F-actin levels, cell spreading, filopodia formation, and cell migration. Our results demonstrate previously unknown isoform-specific actin regulation that can potentially affect actin functions in cells.

7.

Drug specificity and affinity are encoded in the probability of cryptic pocket opening among myosin isoforms

Artur Meller, Jeffrey M Lotthammer, Louis G Smith, Borna Novak, Lindsey A Lee, Lina Greenberg, Leslie A Leinwand, Michael G Greenberg, Gregory R Bowman

The design of compounds that can discriminate between closely related target proteins remains a central challenge in drug discovery. Specific therapeutics targeting the highly conserved myosin motor family are urgently needed as mutations in at least 6 of its members cause numerous diseases. Allosteric modulators, like the myosin-II inhibitor blebbistatin, are a promising means to achieve specificity. However, it remains unclear why blebbistatin inhibits myosin-II motors with different potencies given that it binds at a highly conserved pocket that is always closed in blebbistatin-free experimental structures. We hypothesized that the probability of pocket opening is an important determinant of the

potency of compounds like blebbistatin. To test this hypothesis, we used Markov state models (MSMs) built from over 2 milliseconds of aggregate molecular dynamics simulations with explicit solvent. We find that blebbistatin's binding pocket readily opens in simulations of blebbistatin-sensitive myosin isoforms. Comparing these conformational ensembles reveals that the probability of pocket opening correctly identifies which isoforms are most sensitive to blebbistatin inhibition and that docking against MSMs quantitatively predicts blebbistatin binding affinities (R^2=0.82). In a blind prediction for an isoform (Myh7b) whose blebbistatin sensitivity was unknown, we find good agreement between predicted and measured IC50s (0.67 µM vs. 0.36 µM). Therefore, we expect this framework to be useful for the development of novel specific drugs across numerous protein targets.

8.

Modulation of autophagic flux during mitochondrial stress in neurons

<u>Bishal Basak</u> and Erika L.F. Holzbaur

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

Quality control of neuronal mitochondria is mediated by both non-selective and selective autophagy (mitophagy). Defects in either pathway are associated with neurological disorders including Parkinson's Disease. Here we ask how mitochondrial damage regulates neuronal autophagy. We isolated cortical neurons from embryos of wild type and PINK1-/- mice, and cultured them in vitro. At days in vitro 8-11, mitochondrial depolarization was induced, and treated neurons were either imaged or analyzed by immunoblot. Upon mitochondrial depolarization, we observed degradation of two negative regulators of autophagy, Myotubularin related protein (MTMR)-2 and MTMR5. We also noted degradation of p62/SQSTM1, a marker of autophagic flux. In contrast, proteins that promote autophagosome biogenesis were unaffected. We show that degradation of MTMR2 and MTMR5 is not mediated by autophagy; instead they are degraded by the proteasomal pathway. Further, this is a graded response, dependent on the extent of mitochondrial insult, but independent of PINK1 expression. Our data suggests, that during mitochondrial damage, a stress response program is triggered, which promotes the proteasomal degradation of negative regulators of autophagy. Preliminary data suggest that this stress response program is specifically induced by mitochondrial damage; further, this response mechanism may be neuron-specific as we do not see a similar response in HeLa cells. Since these proteins negatively regulate autophagy, their degradation increases autophagic flux, hence we hypothesize this facilitates clearance of damaged mitochondria during stress. Future work will elucidate the mechanisms of how MTMR2 and MTMR5 regulate mitochondrial turnover, and the molecular basis of mitochondrial stress induced autophagic modulation.

9.

In vitro Study of Mitochondrial Motility and Myosin-19 Dynamics

<u>Cameron P. Thompson</u>, 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.

10.

Actin polymerization promotes invagination of flat clathrin-coated lattices in mammalian cells by pushing at lattice edges

Changsong Yang[1], Patricia Colosi[2], Siewert Hugelier[2], Daniel Zabezhinsky[1], Melike Lakadamyali[2] and Tatyana Svitkina[1]

[1] Department of Biology, University of Pennsylvania
[2] Department of Physiology, Perelman School of Medicine, University of Pennsylvania

Clathrin-mediated endocytosis (CME) requires energy input from actin polymerization in mechanically challenging conditions. The roles of actin in CME are poorly understood due to inadequate knowledge of actin organization at clathrin-coated structures (CCSs). Using platinum replica electron microscopy of mammalian cells, we show that Arp2/3 complex-dependent branched actin networks, which often emerge from microtubule tips, assemble along the CCS perimeter, lack interaction with the apical clathrin lattice, and have barbed ends oriented toward the CCS. This structure is incompatible with the widely held "apical pulling" model describing actin functions in CME. Arp2/3 complex inhibition or epsin knockout produce large flat non-dynamic CCSs, which split into invaginating subdomains upon recovery from Arp2/3 inhibition. Moreover, epsin localization to CCSs depends on Arp2/3 activity. We propose an "edge pushing" model for CME, wherein branched actin polymerization promotes severing and invagination of flat CCSs in an epsin-dependent manner by pushing at the CCS boundary, thus releasing forces opposing the intrinsic curvature of clathrin lattices.

11.

Micro-utrophin gene therapy is protective against acute cardiac injury in the mdx muscular dystrophy mouse

Coral Kasden, Christopher Greer, Ben Kozyak, Hansell Stedman

Duchenne muscular dystrophy (DMD) is caused by the genetic deficiency of the dystrophin protein, a cytoskeletal load-bearing rod that transmits intracellular forces across the sarcolemma during muscle contraction. Our lab has designed a gene therapy treatment using a miniaturized version of utrophin (micro-Utro; µUtro), a functional substitute for dystrophin. Systemic expression of AAV-µUtro rescues the dystrophic phenotype in skeletal muscle with undetectable immune responses to the transgene in dystrophin-deficient mouse and dog models. However, it remains untested whether µUtro is protective in the dystrophic heart. The *mdx* muscular dystrophy mouse model fails to recapitulate the early cardiac phenotype seen in human patients. Therefore, we are using an acute cardiac damage model in which we challenge µUtro-treated or untreated *mdx* mice with atropine and isoproterenol to increase heart contractility, then measure subsequent cardiac myofiber injury by cardiac troponin I (cTnI) levels in the blood, Evans blue dye (EBD) uptake into injured muscle fibers, and echocardiogram. We will also employ a chronic stress model in which isoproterenol is slowly released over time by osmotic minipump. These studies will stringently test therapeutic efficacy of the first immunologically safe DMD gene therapy in the dystrophic heart.

12.

Distinct Pathogenic Mutations in LRRK2 Disrupt Axonal Transport of Autophagosomes

Dan Dou, C. Alexander Boecker, Erika L.F. Holzbaur

Dysfunctional autophagy has been repeatedly implicated in Parkinson's disease (PD) pathogenesis. Leucine-rich repeat kinase 2 (*LRRK2*) mutations are the most common genetic cause of PD and result in hyperactive phosphorylation of the Rab family of GTPases. We hypothesized that LRRK2 hyperactivity contributes to autophagic disruption by causing deficits in autophagosome transport in

neuronal axons. To investigate, we live-imaged the autophagosome marker LC3B in axons of human iPSC-derived neurons with knock-in (KI) of the most common LRRK2 mutation, p.G2019S. In wild-type neurons, we observed smooth retrograde transport of autophagosomes, characteristic of physiologic axonal autophagy. In contrast, imaging p.G2019S KI neurons revealed striking increases in autophagosome pausing. Another PD-linked mutation, LRRK2-p.R1441H, has been reported to induce even greater magnitude of kinase hyperactivity than p.G2019S. In p.R1441H KI neurons, we observed more severe disruption of autophagosome transport, manifesting as increased pause duration and likelihood of stationary autophagosomes. Thus, magnitude of autophagosome transport deficits may scale with LRRK2 activity. In p.R1441H KI neurons, we also found that overexpression of the small GTPase Arf6, a motor protein regulator, partially rescued the deficits in axonal transport of autophagosomes. Together, our results lead us to propose a model where imbalance between Arf6 and LRRK2-phosphorylated Rabs induces aberrant recruitment of the scaffold protein JIP4 to autophagosomes. This results in an unproductive tug-of-war between molecular motors, disrupting the autophagosomal transport that is tightly linked to neuronal homeostasis. Importantly, autophagosome transport deficits were reversed by LRRK2 kinase inhibitor treatment, further reinforcing LRRK2's status as a promising therapeutic target.

13. The LINC complex mediates chromatin organization and mRNA localization in the adult cardiomyocyte

Daria Amiad Pavlov, Lauren Testa, Taryn Wilson, Alexey Bogush, Benjamin 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 chromatin organization demonstrates a spatial shift of chromatin from the lamina-associated domains towards the nucleoli-associated domains in the LINC perturbed cardiomyocytes, with overall increase in the H3K9me2 and H3K9me3 repressive chromatin marks both at the nuclear and nucleolar periphery. Furthermore, we demonstrate that perturbing the LINC complex results in mRNA backup within the nucleus due to the loss of perinuclear kinesin motors which are required for mRNA transport. Overall, our findings help dissect the role of the LINC complex in cytoplasmic to nuclear strain transfer, chromatin organization, and mRNA localization, in the adult cardiomyocyte which are essential to understand the adaptive and pathologic response of the heart to changing mechanical loads.

14. Clustering of scaffolding protein CENP-T activates recruitment of Ndc80 complexes to assemble functionally active kinetochore-like particles

Ekaterina V. Tarasovetc[1], Gunter B. Sissoko[2], Iain M. Cheeseman[2], Ekaterina L. Grishchuk[1]

[1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
[2]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, including the Ndc80 complex, the major microtubule-binding component. 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 Ndc80 and other outer kinetochore proteins; moreover, they accumulate at spindle poles, suggesting that they can engage in productive interactions with spindle microtubules. To directly examine their functional activity, we isolated T-particles from mitotic cells and visualized their interactions with microtubules *in vitro* using fluorescence microscopy. T-particles assembled on clusters of GFP-CENP-T^{1-242}, but not the control GFP-containing clusters, displayed robust binding to stabilized microtubules, processive motions at the dynamic microtubule tips, microtubule-wall diffusion, and occasional plus-end-directed motility. Thus, T-particles exhibit both, the composition and functional features that are characteristic of real kinetochores and, therefore, they represent a powerful tool to gain insights into the molecular and biophysical principles of human kinetochore assembly. To investigate whether multimerization of CENP-T is sufficient to activate Ndc80 recruitment, we asked if this phenomenon can be recapitulated using only recombinant components. We expressed in *E. coli* the GFP-CENP-T^{1-242} clusters with phosphomimetic substitutions at the two Ndc80 binding sites within the CENP-T N-termini. Purified GFP-CENP-T clusters recruited recombinant Ndc80 Bonsai complex but not the Ndc80 Broccoli that lacks the CENP-T binding domain. Up to two Ndc80 Bonsai molecules can be recruited per CENP-T molecule within a cluster, consistent with previous studies *in vitro*. Strikingly, under the same conditions, binding of Ndc80 to monomeric GFP-CENP-T^{1-242} is significantly reduced. Thus, the enhancement of Ndc80 recruitment is an intrinsic feature of CENP-T clusters, as our *in vitro* system lacks any other kinetochore components or regulatory enzymes. We propose that formation of supramolecular assemblies of the scaffolding components is a distinct regulatory mechanism that promotes efficient recruitment of outer kinetochore components specifically at the centromere loci, while avoiding interactions between the soluble components in the cytoplasm.

15.

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 (Ca^{2+})-binding EF-hand domains, though it's unclear how functional these domains are and if their activities are intertwined. 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 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.

16.

The Role of the Nuclear Receptor Co-regulator RIP140 (Nrip1) in the Control of Muscle Fitness

Elizabeth Pruzinsky[1], Tsunehisa Yamamoto[1], Tejvir Khurana[2], Kirill Batmanov[1], Daniel P. Kelly[1]

[1]Cardiovascular Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania
[2]Pennsylvania Muscle Institute, University of Pennsylvania, Philadelphia, Pennsylvania

Skeletal muscle metabolic fitness is a key factor in regulating systemic metabolism and overall health. Poor muscle metabolic fitness, largely due to physical inactivity, contributes to several disease states such as obesity and diabetes, cardiovascular disease, primary muscle diseases, and age-related cachexia. Receptor Interacting Protein 140 (RIP140) is a nuclear receptor co-regulator that has been shown to

repress genes involved in oxidative metabolism. To investigate the role of RIP140 in skeletal muscle, striated muscle-specific RIP140 KO mice (str-RIP140$^{-/-}$) were generated and characterized. RNA-sequencing revealed broad upregulation of skeletal muscle genes involved in mitochondrial oxidative metabolism including fatty acid oxidation, oxidative phosphorylation, branched-chain amino acid degradation, and the TCA cycle. In addition, str-RIP140$^{-/-}$ mice display a remarkable endurance performance phenotype. Specifically, str-RIP140$^{-/-}$ mice run significantly longer on an endurance treadmill regimen and have increased VO_{2max}. Respiratory Exchange Ratio (RER) is significantly decreased during exercise in str-RIP140$^{-/-}$ mice, indicative of increased utilization of fatty acids. Oxidative fiber type gene expression is increased in str-RIP140$^{-/-}$ mice with a concomitant decrease in fast glycolytic fiber type expression. Lastly, str-RIP140$^{-/-}$ mice can be exercise trained to a greater VO_{2max} compared to control trained mice. We conclude that RIP140 serves as a co-repressor of a network of skeletal muscle genes involved in mitochondrial energy transduction and contractile function. Genetic RIP140 loss-of-function enhances skeletal muscle oxidative capacity and endurance performance in pre-clinical models. These results suggest that RIP140 could prove to be a target for novel metabolic therapies aimed at cardiac and skeletal muscle diseases. This work was supported by NIH grants R01DK045416 (D.P.K.), R01HL128349 (D.P.K.) and 5T32AR053461-15 (E. P.).

17. RNAs are localized and translated at discrete cardiomyocyte microdomains

Emily A. Scarborough[1], Keita Uchida[1], Izhak Kehat[2,3], and Benjamin L. Prosser[1]

[1]Department of Physiology, University of Pennsylvania, Philadelphia, Pennsylvania
[2]The Rappaport Institute and the Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel
[3]Department of Cardiology and the Clinical Research Institute at Rambam, Rambam Medical Center, Haifa, Israel

Introduction: Long-lived cardiomyocytes (CMs) must maintain proteostasis while also retaining the ability to rapidly increase protein content, particularly during times of cardiac growth. One conserved mechanism of translational control relies on subcellular localization of mRNA near sites of its protein's function. Our data suggest there may be subdomains within the CM where discrete mRNAs are locally translated, including at the intercalated disc (ICD), sarcomere and perinuclear region. Yet the molecular components and regulation of such domains remain unknown.

Objectives: We aim to investigate 1) the subdomains of mRNA localization in the CM, particularly at the ICD, 2) whether mRNA localization dictates local translation, and 3) how mRNA localization changes during hypertrophy.

Methods: To visualize mRNA and protein, we couple super-resolution immunofluorescence (IF) and single molecule RNA- FISH with quantitative image analysis. To assay local translation, CMs are methionine-depleted and treated with a methionine-analogue, which is then chemically linked to biotin; a proximity ligation assay detects interactions between the protein of interest and actively translating peptides (i.e. biotin) as a proxy for translation.

Results: We find evidence of at least 3 microdomains in the CM, defined as discrete localization and local translation of a specific mRNA. We find that some, but not all, ICD proteins localize their mRNA to the ICD, with desmoplakin (*Dsp*) transcripts highly enriched both *in vivo* and *in vitro*. A mouse model of acute hypertrophy increases transcript abundance for most targets (even non-muscle specific genes), and specifically reinforces *Dsp* ICD enrichment. We find that *Dsp* localization to the ICD is microtubule-dependent and that ICD-localized transcripts are stabilized relative to *Dsp* within the cytosol. Translation of *Dsp* is enriched at the ICD and enhanced under hypertrophic conditions, mirroring its mRNA localization.

Conclusions: The ICD is a key translational hub in the CM. Only some ICD proteins use mRNA localization as a form of translational regulation, and *Dsp* represents a model mRNA to study subcellular localization in the CM. Our data suggests the proper localization of mRNAs may increase their stability and dictate their local translation. Ongoing work is focused on the molecular mechanisms by which mRNAs are localized to microdomains, as well as how these translational hubs are regulated to facilitate cardiac remodeling.

18.

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

Faviolla A. Baez-Cruz, E. Michael Ostap

Unconventional class-I myosins (myosin-Is) are single-headed, actin-based motors that link membranes to the cytoskeleton and participate in a range of cellular functions. *Drosophila* has two myosin-I isoforms, myosin-1C (Myo1C) and myosin-1D (Myo1D), which have roles in establishing organismal chirality during development, likely through the interaction with E-cadherin and β-catenin at sites of cell-cell adhesion. Remarkably, ectopic expression of either Myo1C or Myo1D in non-chiral organs (e.g., epithelium and trachea) result in generation of organs with distinct chirality, depending on the myosin-I isoform expressed. The activity of the myosin-I motor domains was shown to be crucial for this induced chirality, with the handedness of chirality depending on which motor domain was expressed. To better understand the motor properties of these myosins, we performed a detailed biochemical investigation of recombinant, full length Myo1C and Myo1D obtained by expression in Sf9 cells using baculovirus. The steady-state ATPase activities of the motors are actin-activated, with Myo1D having a Vmax 12.5-fold larger than Myo1C, consistent with slower actin gliding speeds of Myo1C found via *in vitro* motility assay. ATP binding is fast and non-rate-limiting in both myosins. The maximum rate of actin-activated phosphate release from Myo1C (0.4 s^{-1}) is similar in magnitude to its ADP release rate (1 s^{-1}). However, the rate of ADP release from Myo1D (8 s^{-1}) is substantially slower than the rate of phosphate release (28 s^{-1}). Notably, the ADP affinities for both Myo1C (89 nM) and Myo1D (44 nM) are among the tightest measured for any myosins, and will likely result in substantial steady-state population of the force-bearing AM.ADP states under physiological nucleotide concentrations. This work was supported by NIGMS Grant (GM057247).

19.

Bridging mechanism between Arp2/3 complex and actin filaments explains Cortactin synergy with nucleation promoting factors

Fred E. Fregoso, Peter J. Carman, Malgorzata Boczkowska, Trevor van Eeuwen, and Roberto Dominguez

Branched actin networks, nucleated by the Arp2/3 complex, provide the structural foundation of lamellipodia - a cellular architecture at the leading edge of motile cells where a concentration of proteins that regulate Arp2/3 complex function reside. Cortactin, the focus of this study, localizes to the cortex of lamellipodia and have been shown to synergize with NPFs. Structural insights on how cortactin interacts with Arp2/3 complex and the mechanism of Cortactin/NPF synergy has remained ambiguous. We show through F-actin cosedimentation assays that Arp2/3 complex associates more with filaments when Cortactin contains the F-actin binding repeats, while NTA alone poorly or if at all promotes this interaction. Cryo-EM structure of the NTA region bound to Arp2/3 complex shows that the interaction is primarily made through 10 residues, and cross-linking assay that captures the transition of the Arps into the activated filament-like conformation shows that NTA does not drive conformational changes within Arp2/3 complex alone or with NPF. Cosedimentation and pyrene-actin polymerization assays show that Cortactin NTA constructs that harbor either the first four or last four repeats display different affinities to F-actin and indicates synergy activity with NPF. Lastly, synergy of NTA with dimeric NPF was abolished when a conserved helix was removed. Here, we provide structural and biochemical work that both recapitulates prior observations with robust methodology, while revealing a novel role of a priorly uncharacterized structural region within the NTA.

20.

tRNA localization in myocytes is a microtubule-dependent process

Jennifer M. Petrosino[1,3]; Keita Uchida[1,3]; Vasiliki Courelli[2,3], Kenneth B Margulies[1,3], Michael Ibrahim[2,3], Benjamin L. Prosser[1,3]

[1]Department of Physiology, Pennsylvania Muscle Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [2]Division of Cardiovascular Surgery, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA; [3]Penn Cardiovascular 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 $tRNA^{Gly\text{-}GCC}$, $tRNA^{Gly\text{-}CCC}$, and $tRNA^{Pro\text{-}AGG}$, 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 $tRNA^{Gly\text{-}GCC}$ 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 their proper localization, in the mammalian heart.

21.

An essential telomere protein for muscle stem cell function and regeneration during homeostasis, disease, and aging

Ji-Hyung Lee[1] and Foteini Mourkioti[1,2,3]

[1]Department of Orthopaedic Surgery, [2]Department of Cell and Developmental Biology, [3]Penn Institute for Regenerative Medicine, Musculoskeletal Program, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Skeletal muscle exhibits extraordinary regeneration after muscle injury and this ability exclusively depends on the activity of residential stem cells called muscle stem cells (MuSCs). Disruption of MuSC action leads to impaired muscle regeneration and it is observed in several muscle disease such as muscular dystrophies and muscle aging. Telomeric repeat binding factor 2 (TRF2) is a DNA binding element of shelterin complex, which protects telomere from undesired DNA damage responses and recombination. TRF2 has been reported to have both telomeric and extra- telomeric functions with cell type and tissue specific manner. However, nothing has been studied about the TRF2 as a regulator of MuSCs behavior. We found that TRF2 expression was dynamically changed in MuSCs during muscle regeneration. Intriguingly, TRF2 was downregulated in both dystrophic and aged MuSCs without affecting the expression of other shelterin component, TRF1. MuSC-specific removal of TRF2 severely impaired muscle regeneration and DMD progression deteriorated by TRF2 deletion in MuSCs of mdx mice. In vivo imaging of Pax7-positive cells illustrated that MuSC population was declined by TRF2 deletion in MuSCs. However, acute cell death was not observed in TRF2 knockout MuSCs implying that stem cell depletion is independent from cellular apoptosis. Proliferation test showed that loss of TRF2 inhibits MuSCs propagation after muscle injury. These results demonstrate that TRF2 is an an

indispensable factor for adequate MuSC function and muscle regeneration. Findings from this study propose TRF2 as an intrinsic gatekeeper of appropriate MuSC function and provide new strategies to overcome skeletal muscle disease.

22.

YPet Through the Eyes of mCLIFY

Him Shweta[2,3] Kushol Gupta[4], Yufeng Zhou[2], Xiaonan Cui[1], Selene Li[1], Yale E. Goldman[1,2,3] and **Jody A. Dantzig[1,2]**

[1]Pennsylvania Muscle Institute, [2]Department of Physiology, [3]Center for Engineering and MechanoBiology, [4]Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

The popular bright fluorescent protein (FP), YPet (**Y**ellow fluorescent **P**rotein for **E**nergy **T**ransfer), a weak dimer, has been extensively used, but has been a challenge to characterize. We have been able to further characterize YPet by introducing a monomeric (m) and circularly permuted (CP) derivative, mCLIFY (**M**onomeric **C**ircularly permuted long **L**ifetime **I**ntense **F**luorescent **Y**ellow protein), now among the brightest monomeric FPs, with high quantum yield (0.76), extinction coefficient (137,600 $M^{-1} cm^{-1}$), long fluorescence lifetime (3.48 ns), low pH sensitivity (pKa 5.56), and favorable photostability. As the first 1.9 Å-structurally solved YPet variant, the crystal structure of mCLIFY reveals a remarkably well-aligned π-interaction and a water-mediated network of hydrogen bonds which supports the chromophore. To better understand the self-association properties of YPet, sedimentation velocity analytical ultracentrifugation (SV-AUC) determined a monomer-dimer dissociation constant K_D of 3.4 μM, whereas mCLIFY did not dimerize up to 40 mM. Size-exclusion chromatography in-line with synchrotron small-angle X-ray scattering and multi-angle light scattering (SEC-SAXS-MALS) confirmed these findings. SAXS studies show that mCLIFY correlates well to the solution monomer, whereas YPet exists as an antiparallel dimer in solution. The fluorescence anisotropy of YPet showed significant decrease with increasing concentration presumably due to HOMO-FRET/or trivial reabsorption of emitted photons, whereas the anisotropy of mCLIFY did not show much change even at high concentration. With an excitation wavelength in the blue-green spectrum and emission in the green-yellow spectrum, mCLIFY is 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.

23.

CFL1-Mediated Actin Remodeling:
A Potential Right Ventricular Failure-Specific Therapeutic Target

Jonathan Edwards, MD; Gen Uy; Spencer Williams; Jeffrey Brandimarto, MS; Joshua Rhoades, MS; Ling Lai MD, PhD; Joanna Griffin MS; Kyle Barrie; Kenneth Bedi; Kenneth Margulies, MD; Zoltan Arany, MD, PhD

Background: Right ventricular failure (RVF) is the leading cause of death in patients with pulmonary hypertension and doubles the mortality risk in left ventricular failure (LVF). There are no proven therapies for RVF, and our understanding for RVF molecular mechanism that could yield therapeutic targets is limited.

Methods: RNAseq was performed separately on matched nonfailing (NF, n=16) and dilated cardiomyopathy (DCM, n=33) RVs and LVs. Fisher's Meta-Analysis was performed using MetaboAnalyst to compare RV and LV DCM vs NF differential gene expression (DGE). SomaLogic peptidomics was performed on arterial and coronary sinus blood in a separate cohort of individuals with reduced LV function with (n=7) or without (n=14) concomitant RV dysfunction. Pulmonary artery banding (PAB, n=6) or sham (n=4) was performed on 9-week old C57/BL6 (50% female) and sacrificed after 10 days.

Results: RNAseq revealed 1008 genes with RV-specific DGE in DCM (Bonferroni adjusted $p<0.05$). PantherDB gene set enrichment analysis revealed broad downregulation for Actin remodeling among RV-specific DGE, including *CFL1, PFN1, CAP2*, and *WASF3* (all, adj $p<5x10^{-4}$). In parallel, peptidomics of arterial and coronary sinus blood revealed that the actin depolymerizing factor CFL1 was consistently excreted by the heart only in the context of RV dysfunction (adj $p<0.05$). In RV DCM with preserved RV function, RV myocardial CFL1 was increased 3.2-fold and CFL1 phosphorylation—a marker of CFL1 inhibition—was decreased 3.9-fold. In contrast, in RVF, CFL was reduced 3.9-fold without a significant change in phosphorylation levels (all $p<0.05$). Surprisingly, the filamentous-globular actin ratio was 2.2-fold lower in RVF compared to preserved RV function. In mice, PAB led to RV hypertrophy (RV/tibia length 1.94 vs 1.28 mg/cm, p=0.006), increase in fetal Myh7 (32-fold, $p<0.001$), increased CFL1 (2.6-fold, p=0.002), and reduced CFL1 phosphorylation (3.5 fold, $p<0.001$).

Discussion: Actin remodeling for sarcomeric thin filament maintenance may be uniquely disrupted in RV DCM, being enhanced in preserved RV function and compromised in RVF. Further work optimizing the mouse PAB RVF as a platform to interrogate the therapeutic implications of this pathway is necessary.

24.

Impact of MYO3A Motor Properties on Actin Protrusion Length and Dynamics

Joseph A. Cirilo, Jr.[1], X. Liao[2], B. J. Perrin[2], C. M. Yengo[1]

[1]Penn State Univ/Milton S Hershey Medical Ctr, Hershey, PA, [2]Indiana University-Purdue University Indianapolis, Indianapolis, IN

Vertebrate class III myosins exist in two isoforms, MYO3A and MYO3B, and localize to the tips of stereocilia in the inner ear. Mutations within the motor domain of MYO3A that alter its intrinsic motor properties have been associated with non-syndromic hearing loss, suggesting that the motor properties of MYO3A are critical for its function within stereocilia. Indeed, class III myosin transgenic knockout mouse models display dysregulated stereocilia length and morphology. In this study, we aimed to understand how the motor properties of MYO3A are fine-tuned for stereocilia elongation and length regulation. To address this question, we generated chimeric MYO3A constructs that lack the kinase domain (ΔK) and replace the MYO3A motor and first two IQ motifs with the motor and first two IQ motifs of other myosins (5A, 10, 15A, 1A, NM2A, and 7A). The resulting chimeras contained an N-terminal GFP, followed by the new motor and 2IQ of the other myosins and the MYO3A tail. Transfection into COS7 cells revealed duty ratio as a critical motor property that dictates the ability to tip localize within filopodia, while *in vitro* actin gliding velocities correlated with filopodial extension velocities. Filopodia length was unaltered, suggesting membrane tension becomes a dominant opposing force at longer lengths. Furthermore, we utilized similar constructs for two MYO3A hearing loss mutants, H442N and L697W. Interestingly, we found that H442N demonstrated an increase in tip localization within filopodia and an increase in filopodia extension velocity, while maintaining similar filopodial lengths. However, L697W resulted in similar tip localization, slower extension velocity, and shorter filopodia. Taken together, our data suggests a model in which tip-localized myosin motors exert force that slides the membrane tip-ward, which, along with actin polymerization forces, combat membrane tension to lead to protrusion elongation. We propose that both "gain of function" and "loss of function" mutations in MYO3A can impair stereocilia length regulation, which is crucial for stereocilia formation during development and normal hearing.

25.

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

Juliet Goldsmith, Alban Ordureau, C. Alexander Boecker, Madeleine Arany, J. Wade Harper, Erika Holzbuar

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 from mouse brain tissue and used proteomics to identify the major cargos engulfed within. At baseline, in wildtype mature adult mice brains, primary hippocampal neurons and human iPSC-derived neurons, we found that mtDNA and nucleoid-associated proteins were significantly enriched compared to other mitochondrial proteins, such as components of the electron transport chain. This pathway is distinct from the selective removal of damaged mitochondria by mitophagy. We believe that this engulfment may help to balance a healthy and functional population of mitochondria with the increased risk of inflammation and reactive oxygen species that each additional mitochondria provides. We also compared the autophagy cargo from wildtype mice to two different genetic mouse models of Parkinson's disease: LRRK2^{G2019S} and PINK1$^{-/-}$. We found that PD associated mutations upregulate compensatory pathways in order to maintain the removal of basal cargo, including mitochondria. When autophagosome acidification is impaired by the hyperactive LRRK2^{G2019S} mutations, secretory pathways are increased. We observed increased secretion of markers of EVs, the pathological precursor of Lewy bodies α-synuclein, and the mitochondrial nucleoid associated protein TFAM, from both primary cortical neurons, and in the serum of LRRK2^{G2019S} mice. When the selective removal of damaged mitochondria is impaired by complete loss of PINK1, compensatory receptors and the initiation machinery for autophagosomes are increased. Proteomics identified higher levels of the autophagy receptors BNIP3 and BCL2L13, which recruit mitochondria to the autophagosome, in the autophagosomes. We also observed higher levels of WIPI2 in the brain, which we think is in part due to changes in the autophagic degradation of components in the spermidine and nitric oxide production pathway. What is regulating the sensing and upregulation of different pathways, and how this creates vulnerabilities in the neurons that contribute to Parkinson's disease progression which may be targeted by novel therapeutics are exciting avenues of current research.

26.

Tension-suppressed degradation of collagen controls tissue stiffness scaling with fibrillar collagen

Karanvir Saini[1], S. Cho[1], M. Tewari[1], A. Jalil[1], M. Wang[1], M. Vashisth[1], A. Kasznel[1], K. Yamamoto[2], D. Chenoweth[1] and D. E. Discher[1]

[1]University of Pennsylvania, Philadelphia, PA 19104, USA; [2] University of Liverpool, UK

Extremely soft tissues such as developing hearts or adult brain contain far less collagen than highly stiff adult tissues such as tendons, but cell and molecular mechanisms for such homeostatic differences remain unclear. We hypothesized that cell-generated or exogenous forces combine with tension-suppressed collagen degradation in order to sculpt extracellular matrix (ECM) collagen levels in tissues. For various mature mice tissues and beating embryonic chick hearts, we find collagen-sensitive second harmonic generation (SHG) image intensity scales non-linearly versus tissue stiffness, aligning well with the results from cellularized gels of collagen. Chick hearts beating at ~5% strain maintain collagen levels until their contractile strain is suppressed by myosin-II inhibition and endogenous matrix metalloproteinases (MMPs) then degrade collagens within ~30-60 minutes – based on SHG and mass spectrometry proteomics measurements. Although tendons composed of oriented collagen fibrils exhibit heterogeneous strain distributions upon deformation, the addition of exogenous MMP or bacterial collagenase suppresses collagen degradation for strains within physiological limits (i.e., up to ~5-8%). Sequestration of collagen cleavage sites by tissue strain is a likely mechanism because molecular permeation and mobility prove strain-independent whereas artificial collagen cross-links accelerate strain-dependent collagen degradation via collagen molecular unfolding. Tension-suppressed degradation of collagen thus underlies tissue stiffness scaling.

27.

Translational Activation of Single Cardiomyocytes

Keita Uchida, Emily Scarborough, Jennifer Petrosino, Benjamin Prosser

Department of Physiology, University of Pennsylvania, Philadelphia, PA

In response to hypertrophic stimuli, cardiomyocytes augment protein synthesis to promote hypertrophic growth. While many aspects of hypertrophy have been extensively studied, nearly all available studies have focused on tissue-level translational regulation. How individual cardiomyocyte translational activity is controlled remains unclear. To measure new protein synthesis, isolated adult rat cardiomyocytes (aRCMs) treated with various pharmacological agents were metabolically labeled with the methionine analog, L-Homopropargylglycine (HPG) followed by a click chemistry reaction to fluorescently label and image nascent proteins. In some experiments, cells and cardiac tissues from animals injected with phenylephrine (PE) or vehicle were stained and imaged for phosphorylated ribosomal protein S6 (p-S6) to indicate activated ribosomes. Surprisingly, indicators of active translation (both HPG labeling and p-S6 staining) were highly heterogeneous in aRCMs and cardiac tissues. After 30 minutes of HPG incubation, many cells have HPG fluorescence levels similar to cells treated with the translation inhibitor cycloheximide, suggesting they were translationally silent during this time. Others show robust staining, indicating recent translational activity. When the HPG incubation period is extended to 60 minutes, the proportion of translationally active cells nearly doubles, indicating stochastic translational activation over this time window. aRCMs treated with PE and/or Isoproterenol do not increase their maximal HPG fluorescence, but instead a larger fraction of cells become translationally active. The magnitude of HPG incorporation strongly correlates with p-S6 staining in aRCMs . In tissue sections, p-S6 labeling shows striking cell-to-cell heterogeneity indicating active and quiescent cells, similar to that observed in aRCMs . However, within 4 hrs of hypertrophic stimulation, p-S6 labeling is more uniform throughout the myocardium, suggesting rapid induction of translational activation. Overall, the data suggests that protein synthesis in individual cardiomyocytes behaves in an ON or OFF manner. Hypertrophic stimuli augment global translation in the heart not by increasing translational activity of all cells, but by recruiting more cells into a translationally active state from a silent state.

28. Arginylation Regulates Cytoskeleton Organization and Cell Division and Affects Mitochondria in Fission Yeast

Li Chen and Anna Kashina

Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Protein arginylation mediated by arginyltransferase Ate1 is a posttranslational modification of emerging importance implicated in the regulation of mammalian embryogenesis, the 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 pathways regulated by arginylation. Here, we conducted a global analysis of cytoskeleton-related arginylation-dependent phenotypes in Schizosaccharomyces pombe, a fission yeast species that shares 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 the total Sc. 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*.

29. Ensembles of human Myo19 bound to calmodulin and regulatory light chain drive multi-micron transport

Luther W. Pollard[1,3], Stephen M. Coscia[1,2,3], Nicholas J. Palmer[1,3,4], Erika L. F. Holzbaur[1,3], Roberto Dominguez[1,3,5], and E. Michael Ostap[1,3,5]

[1]Department of Physiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA; [2]Cell and Molecular Biology Graduate Group, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA; [3]Pennsylvania Muscle Institute, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA; [4]Biochemistry and Molecular Biophysics Graduate Group, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

Myosin-19 (Myo19) controls the size, morphology, and distribution of mitochondria but the underlying role of Myo19 motor activity is unknown. Complicating mechanistic *in vitro* studies, the identity of Myo19's light chains (LC) remains unsettled. Here, we show by coimmunoprecipitation, reconstitution, and mass spectrometry-based proteomics that the three IQ motifs of human Myo19 expressed in a human cell line (Expi293) bind both cytoplasmic regulatory light chain (RLC12B) and calmodulin (CaM). Overexpression of Myo19 in HeLa cells results in an increased recruitment of both Myo19 and RLC12B to mitochondria, suggesting cellular association of RLC12B with the motor. Further experiments reveal that RLC12B binds IQ2 and is flanked by two CaM. *In vitro*, robust motility at the maximal rate (~350 nm/s) occurs when Myo19 is supplemented with CaM, but not RLC12B, suggesting a preference of CaM for weak-affinity-binding IQs. The addition of 100 µM calcium slows actin gliding (~200 nm/s) without an apparent effect on CaM affinity. Small ensembles of Myo19 motors attached to quantum dots can undergo processive runs over several microns. Calcium reduces the attachment frequency and run length of Myo19. Together, our data are consistent with a model where a few single-headed Myo19 molecules attached to a mitochondrion can sustain prolonged motile associations with actin in a CaM- and calcium-dependent manner. Based on these properties, Myo19 can possibly function in mitochondria transport along actin filaments, tension generation on multiple randomly-oriented filaments, and/or pushing against branched actin networks assembled near the membrane surface.

30. Unraveling differences in copy number variation (CNV) between liquid and solid tumors & tissue models

Wang, Mai., Hayes, B., Phan, S and Discher, D. E.

Genomic instability is considered a hallmark of cancer and is relevant to some cancer immunotherapies, but chromosome copy number variation (CNV) or aneuploidy is much more common in *solid* tumors than in *liquid* tumors for unknown reasons. Here, we first quantify and confirm these trends among the 33 solid and liquid cancers in The Cancer Genome Atlas (TCGA) database, showing proportional levels of CNV and loss of heterozygosity (LOH) for one parent-specific chromosome. Experiments then focus on a widely used liquid culture leukemia line that lacks p53, which should allow genetic changes to be tolerated in, and single cell and bulk clonal sequence analyses are used to quantify levels of genetic aberration, including LOH. Single cell results on average resemble bulk profiles of relevance in TCGA, but individual cells also exhibit a reproducible heterogeneity. Drugs and conditions that perturb mitosis and increase aneuploidy in solid tumor cells also lead to more variation with initially suppressed growth, but any diversification is certainly far below that of most solid tumor types. Rescue of p53 expression is ongoing, but these liquid tumor results have implications for tumor evolution and therapies of solid as well as liquid tumors.

31. Targeting Co-Chaperones of HSP70 To Regulate Sarcomeric and Z-disc Protein Expression

Wagner, Marcus J.[1], Branscom, G.[2], Alcaro L.[1], Yob, J[1]. and Sharlene M. Day, MD[1]

[1]Division of Cardiovascular Medicine and Cardiovascular Institute, Perelman School of Medicine,
[2]College of Arts and Sciences, University of Pennsylvania

Background: Hypertrophic Cardiomyopathy (HCM) is a genetically inherited cardiovascular disease that affects 1 in 500 people. Pathogenic sarcomeric variants are the genetic basis for familial HCM with half of patients exhibiting pathogenic variants in *MYBPC3,* resulting in a 40% reduction in MyBP-C protein. Two recent GWAS reports identified non-sarcomeric allelic variants that associate with an increased risk of developing HCM. Three of the top 10 HCM risk alleles are localized near or in gene loci that code for co-chaperones (BAG3, DNAJC18, and HSPB7) of HSP70, implicating protein quality control as a polygenic modifier of HCM pathophysiology.

Hypothesis: Co-chaperones of HSP70, BAG3, DNAJC18, and HSPB7, regulate the expression and stability of sarcomeric and z-disc proteins.

Methods and Results: Human iPSC-CMs were transduced with adenovirus driving shRNA against BAG3, DNAJC18, or HSPB7. Adenoviral transfection efficiency surpassed 70% ($p<0.0001$) and resulted in a $\geq$50% decrease ($p<0.001$) of co-chaperone protein expression. Sarcomeric and Z-disc protein expression was assessed 4 days following knockdown. BAG3 knockdown decreased MyBP-C expression ($p<0.0001$) and most other profiled proteins. Utilizing a cycloheximide (CHX) chase assay to inhibit protein synthesis, MyBP-C expression was reduced by 81% with BAG3 knockdown compared to 43.5% with scrambled shRNA at 24 hours post the addition of CHX($p<0.0001$). DNAJC18 knockdown had minimal effects on steady state protein expression, while HSPB7 knockdown increased MyBP-C ($p<0.01$), myosin ($p<0.01$), tropomyosin ($p<0.05$), and myosin light chain 2 ($p<0.05$) protein levels compared to scrambled shRNA. DNAJC18 or HSPB7 knockdown each increased BAG3 expression ($p<0.01$), while BAG3 knockdown reduced DNAJC18 protein ($p<0.0001$) but had no effect on HSPB7.

Conclusion: BAG3, DNAJC18 and HSPB7 are important for maintaining stability of sarcomeric and z-disc proteins or regulating each other's expression. The HSP70 co-chaperone network may be a novel therapeutic target to promote stability of MyBP-C and other sarcomeric proteins in patients with HCM.

32.

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

<u>Xu Mengqi</u>, Pollard LW, Rebowski G, Boczkowska M, Dominguez R, Ostap EM

Myosin-Is 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-I isoforms frequently concentrate on membranes in areas of Arp2/3 complex-mediated actin polymerization that affect membrane shape and dynamics. To investigate how myosin-Is affect actin assembly, we performed a "comet tail assay" where branched actin networks were nucleated by Arp2/3 complex from a bead surface coated with a nucleation promoting factor (NPF). Actin filaments first formed a cloud around the bead, which transitioned into a polarized comet tail after symmetry breaking. We site-specifically coupled a range of densities of myosin-Is to the bead surface and assessed their effects on actin polymerization, network architecture, and symmetry breaking. We found that high myosin densities prevented comet tail formation. Instead they created an extremely sparse actin cloud surrounding the bead. Decreasing the myosin density resulted in an actin network that broke symmetry more rapidly and formed a polarized comet tail. This actin comet tail was able to elongate from the bead 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-myosin-coated beads, actin networks emerging from myosin-coated beads were sparser and more disordered, which might be a result of the gliding and rotating effect of myosin. Strikingly, under low capping protein concentrations, where bead was embedded in a highly-dense actin shell and symmetry breaking was completely inhibited, myosin-coated beads were able to overcome this inhibition to 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

33.

Gaussian curvature dilutes the nuclear lamina, favoring nuclear rupture, especially at high strain rate

Michael P. Tobin, Charlotte R. Pfeifer, Irena L. Ivanovska, Dennis E. Discher

The nuclear lamina serves as the key mechanoprotective barrier for a cell nucleus. While deficits and defects in lamin filaments are known to elevate nuclear rupture, physical determinants of this rupture are less understood. We show here that curvature acts as a major determinant of rupture, as lamin-B filaments stably interact with the nuclear membrane at regions of low Gaussian curvature whereas they dilute at high curvature nuclear poles. Such loss of lamin-B can lead to rupture, and indeed the probability of nuclear rupture for cells within intact chick embryonic hearts shows a strong dependence on nuclear curvature. Lamin-B behavior at sites of varying curvature and its correlation to rupture were further characterized using both live imaging of lamin-B1 gene-edited cancer cells as well as fixed imaging in IPS-derived progeria patient cells. Notably, rupture of progeria cell nuclei is only weakly rescued by myosin-II inhibition, making it clear that the curvature dependence of nuclear rupture dominates myosin stress modulation. Similar curvature-dependent lamin filament behavior is observed in cells containing lipid droplets— whose high interfacial tension enables the organelle to impose Gaussian deformation onto the nucleus and lamina. Data collected fits a simple, single semi-flexible filament model which binds or not to a curved surface. Altogether, these results point to high Gaussian curvature as a driver of lamina distortion and nuclear envelope rupture, undercovering the possibility that curvature-sensing mechanisms of proteins at plasma membranes, including Piezo 1, might apply at nuclear membranes.

34.

Role of Skeletal Muscle AKT Signaling in the Regulation of Glucose Homeostasis

Natasha Jaiswal[1], Matthew Gavin[1], Louise Lantier[2], David H. Wasserman2 and Paul M. Titchenell[1,3]

[1]Institute for Diabetes, Obesity, and Metabolism, Perelman School of Medicine, University of Pennsylvania [2]Vanderbilt Mouse Metabolic Phenotyping Center, Nashville, TN
[3]Department of Physiology, Perelman School of Medicine at the University of Pennsylvania

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 PDPK-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.

35.

Characterizing the mechanisms and role of acetate generation during skeletal muscle glucose disposal

Nora Yucel[1], Ioana Soaita[1], Megan Blair[1], Zoltan Arany[1]

[1]Division of Cardiovascular Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA

The mammalian pathways of acetate generation, and the physiological function of this poorly characterized metabolite remain understudied. Skeletal muscle, which makes up for ~40% of body mass, has been shown to release acetate under conditions of excess acetyl-CoA production. We show that this acetate release is concomitant with changes in total histone acetylation, and is dependent on glucose uptake. In particular, insulin stimulation of skeletal muscle myotubes increases glucose-derived acetate, while increasing histone acetylation. Unlike previous studies, which demonstrated that acetate is primarily generated via non-canonical PDH activity in cancer cell lines, we further show that histone deacetylation is a significant contributor to both released, and intracellular acetate content in myotubes. Finally we propose that acetate, which is not oxidized in the TCA cycle, serves to limit depletion of free CoA during stimulated glucose uptake and generation of glucose-derived acetyl-CoA, thus preserving free CoA for fatty acid transport. Ultimately we hypothesize that acetate generation and release may potentially play a critical role in skeletal muscle metabolic switching.

36.

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

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

Non-muscle actin is ubiquitously expressed and is an essential component of the cytoskeleton. Higher vertebrates express two non-muscle actins – β- and γ- cytoplasmic actins. These two proteins differ by only 4 conservative substitutions in their N-terminus, but have distinct functions at the gene level: β-actin is essential for embryogenesis, while γ-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 β- actin protein and expressing γ-actin both natively and off the nearly intact β-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 β-actin protein for their maintenance. Lack of β-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 β-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 β- and γ-actin in the proper structural organization and physiology.

37.

Cryo-EM structures of actin filaments

Peter J. Carman, Kyle R. Barrie, Rachel H. Cerón, Malgorzata Boczkowksa, Grzegorz Rebowski, Roberto Dominguez

Actin is the most abundant protein in the cytoplasm of most eukaryotic cells. It plays an essential role in multiple cellular functions, including cytokinesis, vesicular trafficking, and the maintenance of cell shape and polarity. There are six isoforms of human actin, each with a distinct set of cellular functions, localizations, and cell-specific expression. Mutations in all actin isoforms are associated with disease. The length of actin filaments must also be tightly regulated to effectively perform cellular processes. Capping proteins slow the addition or loss of actin monomers at the ends (Tmod at the pointed end, CapZ at the barbed end). While the structure at the middle of actin filaments is known, the ends are

unknown. Here, we solve the cryo-EM structures of wild-type and R258C mutant gamma-smooth muscle actin filaments. We also solve the structure of the ends of the actin filament, including the free pointed end, the CapZ-bound barbed end, and the Tmod-bound pointed end.

38. **Truncated titin in the myocardial sarcomere in human dilated cardiomyopathy**

Quentin McAfee[1], Christina Chen, Yifan Yang, Matthew Caporizzo, Michael Morley, Apoorva Babu, Sunhye Jeong, Jeffrey Brandimarto, Kenneth C. Bedi Jr, Emily Flam, Joseph Cesare, Thomas P. Cappola, Kenneth Margulies, Benjamin Prosser[2], Zolt Arany[1]

[1]Cardiovascular Institute, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, PA, USA
[2]Department of Physiology, Pennsylvania Muscle Institute, Perelman School of Medicine,University of Pennsylvania, PA, USA

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 TTNtvs, 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 eiptopes. 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 straited 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.

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

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

Actin is the most abundant protein in eukaryotic cells and plays essential roles in muscle contraction, cell motility, cytokinesis, and other cellular processes. Humans express six non-interchangeable actin isoforms, and mutations in all isoforms cause devastating diseases. Most studies of actin biochemistry use tissue-purified alpha skeletal actin, and there is currently no reliable method for producing pure recombinant human actin in its native form. Previously published purification strategies for recombinant actin failed to address folding concerns, demonstrate proper post-translational modification, and

eliminate contamination with other highly similar actin isoforms. We developed a method to obtain high yields of recombinant actin in human cells that addresses the shortcomings of previous methods. We use a combination of experimental approaches to rigorously demonstrate the removal of highly homologous endogenously expressed actin and the functional integrity of recombinant actin for multiple isoforms of actin. Proteomics analysis confirms the presence of native post-translational modifications and proper affinity-tag removal. With this method, we can now study actin under fully native conditions to investigate differences among isoforms and the effects of disease-causing mutations.

40.

Dysregulated macrophage phenotype in fibrotic VML injury

Ricardo C. Whitaker and Kara Spiller

Volumetric muscle loss (VML) is a debilitating condition resulting from the loss of a large portion of soft muscle due to injury or disease. Macrophages play a crucial role in VML outcome; however, a lot is still unknown about macrophage phenotype and interplay with other cells. We have identified systemic and local changes in immune cell trafficking, macrophage behavior and cytokine secretion in fibrotic and non-fibrotic VML injuries.

Fibrotic injuries were generated by removing a portion of muscle tissue (quadriceps) from C57BL/6 mice via a 4mm biopsy punch, while non-fibrotic injuries were created using a 2 mm biopsy punch. At the site of injury, macrophages from fibrotic injuries expressed higher levels of inflammatory markers CD9, CD38 and CXCR4 at earlier timepoints, and lower levels of anti-inflammatory markers CD163 and CD301b at later timepoints, as seen by flow cytometry analysis.

To further characterize macrophage phenotype, multiplex gene expression analysis (NanoString) for a custom panel of >220 genes was performed on FACS-sorted macrophages on Days 1 and 3 post-injury. At Day 1, genes related to inflammation, such as *IL1a, CD9 and CD86* were upregulated in macrophages in fibrotic injuries compared to non-fibrotic injuries. Surprisingly, at Day 3, macrophages in fibrotic injuries expressed lower levels of most genes evaluated.

Nanostring performed at the whole tissue level at Day 1, revealed 25 significantly different genes between fibrotic and non-fibrotic injuries. More notably, there was a significant decrease in *IGF1,* important in satellite cells maturation, and increase in *CXCL2* and *CXCL3*, crucial for neutrophil recruitment, in the fibrotic group compared to non-fibrotic.

Immune cell accumulation at the site of injury was markedly different between fibrotic and non-fibrotic injuries as well. Fibrotic injuries presented a significantly higher accumulation of neutrophils at Days 1 and 3 post injury, accompanied by an increase in systemic G-CSF levels. In addition, macrophage accumulation in the fibrotic injuries is significantly higher on Days 3 and 28 post injury.

Immune cell trafficking at main myeloid reservoirs (blood, bone marrow and spleen) showed little differences between fibrotic and non-fibrotic injuries at the first 7 days post injury. Bone marrow macrophages showed no phenotypic differences between fibrotic and non-fibrotic injuries. On the other hand, splenic macrophages in the fibrotic group consistently presented higher levels of inflammatory markers compared to non-fibrotic injuries.

Here we demonstrate that macrophage phenotype and presence at the site of injury is markedly different between fibrotic and non-fibrotic injuries. Our data suggests that macrophage phenotype in fibrotic injuries is more complex than simply exacerbated inflammation or high presence of M2 markers, as often suggested. Finally, we also elucidated that those changes in macrophage phenotype, potentially leveraging fibrosis, occur at early timepoints. The early changes in macrophage phenotype, accompanied by high levels of neutrophils and systemic G-CSF, may indicate macrophage-neutrophil crosstalk as a potential interaction orchestrating fibrosis.

41.

Membrane curvature governs the distribution of Piezo1 in live cells

Shilong Yang, Xinwen Miao, Steven Arnold, Boxuan Li, Alan T. Ly, Huan Wang, Matthew Wang, Xiangfu Guo, Medha M. Pathak, Wenting Zhao, Charles D. Cox, Zheng Shi

Piezo1 is a *bona fide* mechanosensitive ion channel ubiquitously expressed in mammalian cells. The distribution of Piezo1 within a cell is essential for various biological processes including cytokinesis, cell migration, and wound healing. However, the underlying principles that guide the subcellular distribution of Piezo1 remain largely unexplored. Here, we demonstrate that membrane curvature serves as a key regulator of the spatial distribution of Piezo1 in the plasma membrane of living cells. Piezo1 depletes from highly curved membrane protrusions such as filopodia and enriches to nanoscale membrane invaginations. Quantification of the curvature-dependent sorting of Piezo1 directly reveals the *in situ* nano-geometry of the Piezo1-membrane complex. Piezo1 density on filopodia increases upon activation, independent of Ca^{2+}, suggesting flattening of the channel upon opening. Consequently, the expression of Piezo1 inhibits filopodia formation, an effect that diminishes with channel activation.

42.

The role of telomeric protein repeat binding factor 2 (TRF2) in cardiac development and disease

Sienna Pyle[1,2], Foteini Mourkioti[1,2,3]

[1] Department of Orthopaedic Surgery, Perelman School of Medicine, The University of Pennsylvania, Philadelphia, PA, USA.
[2] Bioengineering Graduate Program, The University of Pennsylvania, Philadelphia, PA, USA.
[3]Department of Cell and Developmental Biology, Penn Institute of Regenerative Medicine, Musculoskeletal Regeneration Program, Perelman School of Medicine, The University of Pennsylvania, Philadelphia, PA USA.

Cardiomyopathy refers to any disease of the heart muscle that impairs the ability of the heart to deliver blood to the body. Interestingly, telomere dysfunction has been associated with multiple modes of cardiomyopathy, yet the involvement of telomeric proteins such as telomeric repeat binding factor 2 (TRF2) in cardiac physiology and disease is currently unknown. We found attenuated levels of TRF2 in cardiac tissue from human patients with cardiomyopathy, suggesting that this protein has an important role in human cardiac diseases. To investigate the cardiac role of TRF2, we generated mice with cardiomyocyte specific deletion of both copies ($TRF2^{CardiacKO}$) or one copy ($TRF2^{CardiacHet}$) of TRF2 by crossing $TRF2^{Fl/Fl}$; $Ai9^{Fl/Fl}$ mice with αMyHC-Cre mice. We found TRF2 to be essential for cardiac development, with $TRF2^{CardiacKO}$ resulting in embryonic lethality. TRF2 was also found to be haploinsufficient for heart function and survival into late adulthood. There were no significant changes in cardiomyocyte proliferation and death, while telomere length remained unaltered in both $TRF2^{CardiacKO}$ and $TRF2^{CardiacHet}$ mice, showing the unique function of TRF2 independent of telomeres in cardiomyocytes. Recent studies implicate the importance of epigenetic mechanisms in cardiomyocyte function, and cardiac development and remodeling during heart failure. Interestingly, we demonstrated that, besides its role as a telomeric protein, TRF2 also has extra-telomeric functions in cardiomyocytes via interactions with lamin, promoting optimal expression of genes associated with cardiac contractility. Our findings define a previously unrecognized role associated with TRF2 in developing and adult diseased hearts, and link how TRF2 guides chromatin reorganization in cardiomyocytes.

43.

Interplay between secretory autophagy and macroautophagy in neurons

Sierra Palumbos, Juliet Goldsmith, Erika Holzbaur

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? Recent findings suggest that autophagy-dependent degradation and autophagy-dependent secretion can act in coordination to regulate cellular homeostasis in non-neuronal cell types. When autophagosome maturation is impeded, autophagy-dependent secretion of extracellular vesicles (EVs) can be initiated as a mechanism to unburden the degrative machinery. Whether neurons similarly extrude damaged material via autophagy dependent secretion is unclear. We propose that stressed neurons engage autophagy-dependent secretion as an alternate quality control mechanism to dispel cellular waste. This is supported by several preliminary observations. First, we observe that LRRK2 mutant neurons, which exhibit strained degradative autophagy, shunt cargo toward a secretory fate. Additionally, we observe that treating cultured neurons with Bafilomycin A1, effectively blocking lysosomal fusion, prompts the upregulation of secretion. Together, our data suggest that chronically or acutely straining macroautophagy leads neurons to upregulate secretory autophagy. These observations have important implications in neurodegenerative diseases where autophagy is strained, and the expulsion of damaged proteins or dysfunctional organelles could heighten systemic inflammatory responses. Future directions will further describe interplay between cell-autonomous autophagy and secretory autophagy.

44.

Dilated cardiomyopathy mutation (E525K) in human beta-cardiac myosin enhances actin-activated phosphate release but stabilizes the auto-inhibited super relaxed state

Skylar Bodt, David V. Rasicci, Rohini Desetty, and Christopher M. Yengo

Mutations in beta-cardiac myosin (M2β) are a common cause of inherited cardiomyopathies including dilated (DCM) and hypertrophic cardiomyopathy (HCM). We investigated the impact of a DCM mutation (E525K) in the human M2β motor domain, which has been shown to stabilize both the interacting heads motif (IHM) and auto-inhibited super relaxed (SRX) state via head-tail electrostatic interactions in 15 heptad heavy meromyosin (M2β HMM). These findings suggest E525K may reduce muscle force and power by triggering an increase in the IHM/SRX state. However, it is unclear how E525K impacts the intrinsic motor properties of M2β. Thus, we introduced the mutation into single-headed human cardiac myosin subfragment 1 (M2β-S1), unable to form the IHM, and examined its impact on the ATPase mechanism. We revealed that E525K induces a 2.8-fold increase in maximum steady-state actin-activated ATPase activity (k_{cat}), a 6-fold decrease in the actin concentration at which ATPase is half maximal (K_{ATPase}), and a 3.4-fold increase in the fast phase of actin-activated phosphate release. Glutamate 525 is located in the conserved activation loop that is proposed to trigger phosphate release by interacting with the negatively charged N-terminus of actin. We propose the mutation may enhance the rate of rotation of the lower 50 kDa domain, movement of the relay helix/switch II, and lever arm rotation associated with the power stroke. Thus, we will directly examine the impact on the actin-activated power stroke utilizing a FRET approach that measures lever arm rotation. Our results suggest the E525K mutation triggers an increase in intrinsic motor properties, while its ability to stabilize the SRX/IHM state in the myosin dimer likely dominates the molecular mechanism that leads to decreased muscle force and power and DCM pathogenesis.

45.

Recruitment of small heat shock proteins to aggregates in myopathy model propagates contractile dysfunction

Srikar Donepudi, Anhelina Volchok, Vedasri Madala, Jana Smuts, Tali Gidalevitz

Protein aggregate myopathies (PAMs) are characterized by accumulations of protein aggregates containing misfolded structural myofilament proteins. Small heat shock proteins (sHSPs) are response chaperones that stabilize aggregation prone proteins to facilitate their subsequent refolding or degradation and are found in aggregates in PAMs. Despite this, sHSPs' role in PAM's pathogenesis remains unknown. It is unclear whether recruitment of sHSPs to aggregates represents detoxification of protein aggregates or byproducts of being titrated away from their endogenous physiological substrate(s). Here, we explore the role of sHSPs using various *C. elegans* models of PAMs that express

specific aggregates in body wall muscle cells. We find that in healthy animals, a muscle-specific worm sHSP, HSP-12.6, selectively binds to myofilaments. In certain PAM models, such as in worms expressing ASyn, HSP-12.6 still binds filaments and does not bind aggregates. In contrast, in animals expressing amyloid peptides, ADan and ABri, HSP-12.6 is preferentially found in aggregates over myofilaments. Interestingly, overexpression of HSP-12.6 provides strong protection against ASyn toxicity, while not being protective against amyloid toxicity, preliminarily suggesting that binding to the myofilaments is a prerequisite of its protective function. To directly test this, we used two ways to destabilize myofilaments. We found that a mutation in a structural myofilament protein that causes misfolding and aggregation results in the titration of HSP-12.6 from myofilaments to aggregates; in these animals, HSP-12.6 overexpression is not protective. In contrast, when we express a mutation in a myosin chaperone that results in functional but unstable filaments, HSP-12.6 is protective. Together, these results suggest a role in which the sHSP, HSP-12.6, protects functional units of myofilaments through binding, but when titrated to aggregates, such as in the case of myopathies, this protection is lost.

46.

An interphase actin wave promotes mitochondrial content mixing and homeostasis

Stephen M. Coscia, Andrew S. Moore, and Erika L. F. Holzbaur

Mitochondrial dynamics are regulated via a cycling wave of actin polymerization/depolymerization. In metaphase, F-actin assembly propels mitochondria in a comet-tail mechanism but in interphase the wave promotes mitochondrial fission. We hypothesize that here fission is induced because the force of the actin wave is resisted by mitochondrial tethering to microtubules. In support, upon microtubule depolymerization wave-associated mitochondria ceased to fragment and instead displayed comet-tail motility. Next, we focused on the machinery driving actin-cycling, which was blocked by CDK1 inhibition. We found the putative CDK1-substrate and F-actin nucleator/elongator FMNL1 to be required for cycling, as the wave was blocked by FMNL1 knock-down and expression of a non-phosphorylatable mutant. FMNL1-depleted interphase cells exhibited impaired mitochondrial health: decreased mitochondrial polarization/oxygen consumption, decreased cellular ATP, and increased cellular ROS. Mechanistically, we demonstrate that interphase actin-cycling promotes mitochondrial content-mixing. Thus, we propose that interphase actin-cycling enhances mitochondrial content-mixing and thus complementation to maintain mitochondrial homeostasis.

47.

Constructing Synthetic Organelles for Mammalian Cellular Engineering

Wentao Wang, Mikael Garabedian, Rachel Welles, Matthew Good

Introduction: Intrinsic disordered proteins (IDPs) form macromolecular membraneless compartments through phase separation to regulate cellular process. Previously, we built designer compartments function as hubs to modulate cellular behaviors by targeted sequestration and insulation of native proteins[1]. We achieved up to 90% of targeted enzymes to synthetic condensates via genomic tagged high-affinity dimerization motifs. We further validated our system in yeast by targeting endogenous yeast protein to induce control over yeast budding and proliferation. Additionally, we developed optogenetic strategy for controlled cargo release from condensates to switch cells between functional states. However, transformation of the current system from yeast to human cells was missing several crucial components, including inducible expression, uniform condensates formation, controlled reversibility, and proof of functional knockout. By integrating state of the art CRISPR genome editing, drug inducible promoter, lentiviral transduction and optogenetic release technology, we built a synthetic condensates system functioning in human cells featuring high specificity and consistency, decent efficiency and optogenetic induced reversibility. Together, our new results reveal the potential of synthetic organelles in modulating cellular signaling and behaviors through targeting and sequestering

endogenous proteins. These organelles offer a powerful and generalizable approach to modularly control cell decision-making with broad applications for human cellular engineering.

Method: Genes encoded synthetic scaffolds were cloned into either pcDNA3.1 vector (Addgene) for transient expression or 3rd gen lentiviral vector pLJM1 (Addgene) for lentiviral transduction. The inducible versions of scaffold were generated by swapping the constitutive promoter to either Tet-on 3G promoter (Takara Bio) or COMET promoter (gift from Joshua Leonard Lab). Transient transfections were performed through lipofection via lipofectamine 2000 (Invitrogen). Lentivirus were produced by lipofection in HEK293T cells. Both cell lines (HEK293T and U2OS) used in this study were obtained from ATCC. Cells were maintained in EMEM as described previously[1]. Cell lines with CRISPR tagged dimerization motifs at C-terminus of endogenous Rac1, Erk1 and Par6 were generated in previous study[1]. For stable double knock-in cell lines and scaffold expression cell lines, cells were FACS single-sorted into 96 well plates and expanded from single cell. Evaluation of condensates formation, recruitment, enrichment, and client partitioning were primarily done by data acquisition from confocal microscopy followed by analysis using ImageJ as described previously[1]. For evaluating efficiency of inducible systems, induced and uninduced cells were run through flow cytometry, followed by data analysis on FlowJo. Optogenetic release of client were done by 10 sec 405nm light pulse in a 40 sec acquisition cycle for consecutively 11 cycles.

Results and Discussion: We observed up to 80% of scaffold proteins and up to 60% of endogenous client ended up in the synthetic organelles via cognate recruitment motifs and no recruitment with non-matching recruitment motifs, demonstrating efficient and specific client recruitment. Lentiviral generated cells showed slightly weaker scaffold and client partitioning with greater consistency compared to transient transfection. After comparing Tet-on 3G system and COMET systems, we found Tet-on 3G system had highest inducibility and expression level with modest background expression while one of COMET system had lowest background expression. To maximize client partitioning, Tet-on 3G system is selected for future studies. The optogenetic controlled scaffold shows more than 50% of client release within the first 10 minutes, showing robust reversibility of the system controlled by light. Erk1 and Par6 double knock-in cell lines were screened and selected from single colony expansion and validated by PCR. We observed similar scaffold and client partitioning in the double knock-in cell lines with indications of phenotypical changes.

Conclusion: Our synthetic organelles have shown highly specific and efficient recruitment of endogenous clients with optogenetic controlled reversibility, reveals its potentials as a generic tool kit for cellular engineering. Furthermore, we successfully implement our platform with lentivirus, broadening its application to therapeutic development involving primary cells and stem cells. The future direction for this project will be continuing to enhance client partitioning, evaluating and improving its safety profile as a therapeutic approach and validating its efficacy in pre-clinical model.

Reference: [1]Garabedian, M.V. et al. (2021) 'Designer membraneless organelles sequester native factors for control of cell behavior', Nature Chemical Biology, 17(9), pp. 998–1007. doi:10.1038/s41589-021-00840-4.

48.

The role of a RhoGEF-anillin module in septin ring disassembly after cytokinesis

Xi Chen and Erfei Bi

Septins are a group of GTP-binding, filament-forming proteins that play essential roles in cytokinesis and many other processes in eukaryotes. In the budding yeast *Saccharomyces cerevisiae*, the septins form an hourglass at the division site from bud emergence to late anaphase, which is then converted into a double ring at the onset of cytokinesis. The septin hourglass scaffolds the actomyosin ring assembly during anaphase whereas the septin double ring acts as a diffusion barrier during cytokinesis. After cytokinesis and cell separation, the mother and daughter each inherits a septin ring at the old division site that disassembles while a new septin ring forms next door to control bud morphogenesis and cytokinesis during the next cell cycle. Previously, we have shown that the RhoGEF Bud3 and the anillin-

like protein Bud4 associate with the septin hourglass and double ring and play a collectively essential role in the hourglass-to-double ring (HDR) transition. In this study, we found that both Bud3 and Bud4 undergo the F-box protein Grr1-mediated degradation in late G1, which leads to our hypothesis that the cell cycle-dependent degradation of Bud3 and Bud4 triggers septin ring disassembly at the old cell division site. Indeed, we found that ectopic septin rings, together with Bud3 and Bud4, were stabilized at the old division sites, and deletion of either *BUD3* or *BUD4* abolished the ectopic rings. Thus, the Bud3- Bud4 module is required not only for the HDR transition but also for the maintenance and timely disassembly of the septin ring in post-cytokinesis cells.

49. **Investigating the mechanism of APC-mediated branched actin formation at the microtubule tip**

Xingyuan Fang[1], **Tatyana M. Svitkina**[1]

[1]Department of Biology, University of Pennsylvania

Cell migration depends on the pushing and pulling force generated by the actin cytoskeleton. The branched actin network in lamellipodia at the cell leading edge generates driving force for cell protrusion. Orientation of the protrusion is a key element of directional cell migration. On the other hand, microtubules largely control directed migration, but the mechanism remains to be fully understood. Previous study in the lab showed that branched actin at the leading edge of hippocampal growth cones grows directly from the microtubule tip. This observation suggests a direct mechanism of microtubule-controlled cell navigation through local formation of branched actin network and subsequent leading edge protrusion. Our lab also found that Adenomatous Polyposis Coli (APC), a protein known both as a tumor suppressor and a cytoskeletal protein, is directly responsible for this microtubule-dependent assembly of branched actin network. The aim of this project is to explore the molecular mechanism of this APC-mediated actin-microtubule crosstalk.

Using platinum-replica electron microscopy, we show that branched actin is also formed at the microtubule tips at the leading edge of Ref52 rat fibroblasts. Knocking-down APC in Ref52 cells suppressed formation of long cell processes and increased cell circularity, suggesting that similar to neuronal growth cones APC in Ref52 cells is responsible for branched actin formation at the microtubule tip and subsequent membrane protrusion. We hypothesize that the Armadillo repeats at the N-terminus of APC activate the Asef-Cdc42-N-WASP-Arp2/3 pathway to induce cellular processes. Indeed, overexpression of the N-terminus of APC in Ref52 cells promoted formation of long cell processes and decreased cell circularity, which was opposite to the effect of APC knockdown and indicated that the N-terminus of APC is mainly responsible for APC-induced process formation in Ref52 cells. Further experiments will test whether Cdc42, N-WASP and Arp2/3 complex are downstream effectors of the N-terminus of APC that lead to branched actin formation at the microtubule tip, and subsequent process formation.

50. **Understanding the function and mechanism of sex-specific RNA helicases DDX3X and DDX3Y using smFRET**

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 □ 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.

NOTES

Sponsored by the
Physiological Society of Philadelphia
and the *Pennsylvania Muscle Institute*

Pennsylvania
Muscle Institute
University of Pennsylvania

www.med.upenn.edu/pmi