abstracts: oral presentations

ASCB | EMBO
2017 meeting

Pennsylvania Convention Center

Philadelphia, USA | December 2-6

ascb-embo17.ascb.org | **#ascbembo17**

President: Pietro De Camilli
Program Co-Chairs: Laura Machesky
and **Tobias Walther**

Download the Meeting App
Search for "ascbembo17"

Sunday, December 3

E. E. Just Award Lecture

A1

Cell Signaling by Protease-activated Receptors.

J. Trejo[1];
[1]Pharmacology, University of California, San Diego, La Jolla, CA

Protease-activated receptors (PARs) are atypical G protein-coupled receptors (GPCRs) that have fundamental importance in vascular biology and cancer. PARs are activated through an unusual irreversible proteolytic mechanism and raises important questions of how signaling is regulated. Our research is focused on delineating novel regulatory mechanisms that control signaling by PAR1 and closely-related family members in normal physiology and disease. We showed previously that internalization and lysosomal sorting of GPCRs is important for the temporal and spatial dynamics of receptor signaling and appropriate cellular responses. Our work also led to the identification of a beta□-arrestin independent internalization pathway for PAR1 and PAR4, which require alternative clathrin adaptors and tyrosine-based sorting signals rather than beta□-arrestins and has important functions in the regulation of receptor signaling. This work provided the basis for uncovering a novel endosomal sorting mechanism for PAR1 and connections between receptor sorting and signaling that play important roles in inflammation and cancer. The central premise of our current research is that ubiquitination offers novel and diverse mechanisms for regulation of GPCR biology. A thorough understanding of the molecular mechanisms by which key regulators and mediators of ubiquitination regulate GPCR signaling and trafficking is essential for understanding dysregulated mechanisms in disease and identifying new targets for drug development. Two exciting outcomes of our recent work were the discovery that ubiquitination of PAR1 drives p38 mitogen-activated protein kinase (MAPK) signaling on endosomes and not lysosomal sorting. This work reveals a distinct function for ubiquitination of GPCRs in promoting p38 inflammatory signaling rather than receptor degradation. Another discovery was the identification of a new lysosomal sorting pathway for GPCRs that requires ALG-interacting protein (ALIX) and arrestin-related domain containing protein-3 (ARRDC3). The ALIX-dependent pathway bypasses the requirement for GPCR ubiquitination and is distinct from the canonical endosomal sorting complexes required for transport (ESCRT) pathway. Importantly, ARRDC3 expression is suppressed or lost in basal-like invasive breast cancer, a system where PAR1 displays dysregulated trafficking and persistent signaling. The goal of our research is to uncover the unappreciated role for ubiquitination and ARRDC3 in regulation of GPCR biology, not limited to PAR1 but also for other members of the GPCR superfamily.

ASCB Porter Lecture

A2

Sorting out protein traffic in the endocytic pathway.

S.D. Emr[1];
[1]Department of Molecular Biology and Genetics Weill Institute, Cornell University, Ithaca, NY

Our lab studies the molecular mechanisms responsible for the sorting and trafficking of proteins within the secretory and endocytic pathways. Our focus has been on the biogenesis, maintenance and function of the lysosome, a major site for macromolecular degradation. We have used the single-cell yeast Saccharomyces cerevisiae as a genetic model system to discover and isolate the complex machinery that

sorts and delivers proteins and enzymes to the lysosome including endocytic cargoes, plasma membrane (PM) proteins. PM proteins, such as signaling receptors, ion channels, and nutrient transporters, initially are synthesized at the ER, transported to the Golgi and then on to the PM. Maintenance of proper PM proteostasis is crucial to prevent loss of PM integrity and dissipation of essential ion and chemical gradients. As such, when PM resident proteins become damaged or misfolded, they must be recognized and delivered to the lysosome for degradation. The ubiquitin ligase Rsp5, the yeast homolog of human Nedd4, is a key mediator of protein quality control at the PM. We have shown that a family of Rsp5 adaptors, arrestin-related trafficking adaptors (or ARTs), target the Rsp5 ligase to specific PM proteins. Ubiquitin modification of these PM proteins serves as a signal for their endocytosis and subsequent sorting at the endosome en route to the lysosome. We have identified a series of ubiquitin-binding proteins, the ESCRT complexes (ESCRT-0, -I, -II, -III, and the Vps4 AAA ATPase), which recognize ubiquitinated membrane proteins at the endosome. ESCRT binding also depends on the presence of a specific phosphoinositide lipid in the endosomal membrane, PI3P (generated by the Vps34 PI3-kinase), which serves as an additional docking site for the ESCRT complexes. The ESCRT complexes then sort the ubiquitinated PM protein cargoes into vesicles that invaginate and bud into the lumen of the endosome (opposite topology to conventional vesicle budding reactions) forming multi-vesicular bodies, MVBs. The MVBs fuse with the lysosome delivering their internal vesicle cargo into the lumen of the lysosome where they are degraded. Importantly, other key cellular processes that shape membranes with similar topology also require the ESCRT machinery (e.g., cytokinesis, budding and release of HIV virus, PM wound repair, nuclear envelope reformation). I will discuss some of the molecular details underlying these processes.

Symp 1: Structure of the Cell

S1

Unraveling the spatial and temporal dynamics of subcellular organelles.

J. Lippincott-Schwartz[1];
[1]Janelia campus, HHMI Janelia Research Campus, Ashburn, VA

Emerging visualization technologies are playing an increasingly important role in the study of numerous aspects of cell biology, capturing processes at the level of whole organisms down to single molecules. Here, I will discuss developments in probes and microscopes that are dramatically expanding productive imaging. These approaches are overcoming prior roadblocks from 1) fluorophore bleed-through, which limits the number of fluorophores that can be simultaneously imagined, 2) imaging speeds that are too slow, and 3) labeling densities that are too low for deciphering fine subcellular architecture. To surmount fluorophore bleed-through, we combined excitation-based spectral unmixing and lattice light sheet microscopy to visualize up to six organelles (i.e., ER, Golgi, mitochondria, lysosomes, peroxisomes and lipid droplets) simultaneously within cells. This allowed us to track these organelles through time and analyze their inter-organelle contacts, providing a systems-level map of the organelle interactome and how it is perturbed under different physiological conditions. To increase temporal resolution during imaging, we employed total internal reflection fluorescence combined with structured illumination microscopy to visualize organelle dynamics at very high temporal-spatial resolution. Examining the ER, we observed that many peripheral ER sheets seen using diffraction-limited imaging are actually highly perforated structures comprised of tightly latticed groups of dynamic tubules. Within the latticed ER tubule meshwork, subdiffraction-limited holes were observed (~150-250 nm diameter) having transient lifespans (~250 msec). Viewed at higher resolution using lattice light sheet microscopy combined with

point accumulation for nanoscale topology (PAINT), the peripheral ER sheets represented a complex meshwork of tightly cross-linked ER tubules.

S2

Cryo-Electron Tomography: Opportunities and Challenges of Structural Biology in situ.

W. Baumeister[1];
[1]Molecular Structural Biology, Max-Planck-Institute of Biochemistry, Martinsried, Germany

Traditionally, structural biologists have approached cellular complexity in a reductionist manner by characterizing isolated and purified molecular components. This 'divide and conquer' approach has been highly successful, as evidenced by the impressive number of entries in the PDB. However, awareness has grown in recent years that only rarely can biological functions be attributed to individual macromolecules. Most cellular functions arise from their acting in concert. Hence there is a need for methods developments enabling studies performed *in situ*, i.e. in unperturbed cellular environments. Sensu stricto the term 'structural biology *in situ'* should apply only to a scenario in which the cellular environment is preserved in its entirety. Cryo electron tomography has unique potential to study the supramolecular architecture or 'molecular sociology' of cells. It combines the power of three-dimensional imaging with the best structural preservation that is physically possible. (Trends Cell Biol. 26(11), 825-837 (2016).

S3

The role of ER membrane contact sites in regulating the structure of other organelles.

G.K. Voeltz[1];
[1]MCD Biology, University of Colorado, Boulder, Boulder, CO

Membrane-bound organelles have characteristic shapes and dynamic behaviors that allow them to perform their specific set of functions. These shapes are so conserved throughout eukaryotes that organelles are easy to identify even in textbook cartoon form. A handful of the proteins that regulate the shape and dynamics of the major cellular organelles have been identified. These membrane shaping proteins are usually members of larger protein families, are ubiquitous and highly conserved, and importantly most can be linked to some human disease state. My lab studies how the ER an organelle that has an elaborate and dynamic structure regulates the shape and dynamics of other organelles through membrane contact sites (MCSs). Over the past few years, we have discovered that ER MCSs with mitochondria and sorting endosomes, define the position where these organelles undergo constriction and division. Our current research goal is to unravel the mechanism by which ER MCSs regulate the biogenesis of contacting organelles. Specifically, we are studying 1) how ER tubules are recruited, positioned and then tethered to other organelles at MCS, 2) how ER tubules MCS can constrict and divide other organelles and 3) what signals trigger different MCS activities. In addition, we are probing the connections between ER shape and dynamics, MCS formation, and human disease.

Symp 2: Metabolism

S4

mTOR signaling in growth and metabolism.

M.N. Hall[1];
[1]Biozentrum, University of Basel, Basel, Switzerland

TOR (target of rapamycin) is a highly conserved serine/threonine kinase that controls cell growth and metabolism in response to nutrients, growth factors, and cellular energy. TOR was originally discovered in yeast but is conserved in all eukaryotes including plants, worms, flies, and mammals. The discovery of TOR led to a fundamental change in how one thinks of cell growth. It is not a spontaneous process that just happens when building blocks (nutrients) are available, but rather a highly regulated, plastic process controlled by TOR-dependent signaling pathways. TOR is found in two structurally and functionally distinct multiprotein complexes, TORC1 and TORC2. The two TOR complexes, like TOR itself, are highly conserved. Thus, the two TOR complexes constitute an ancestral signaling network conserved throughout eukaryotic evolution to control the fundamental process of cell growth. As a central controller of cell growth, TOR plays a key role in development and aging, and is implicated in disorders such as cancer, cardiovascular disease, obesity, and diabetes.
While the role of TOR in controlling growth of single cells is relatively well understood, the challenge now is to understand the role of TOR signaling in disease and in coordinating and integrating overall body growth and metabolism in multicellular organisms. This will require elucidating the role of TOR signaling in individual tissues. Data on the role of mammalian TORC1 (mTORC1) and mTORC2 in controlling cellular processes and in specific tissues will be presented.

S5

Human Genetics Provides Molecular Handles to Elucidate Pathogenesis of Fatty Liver Disease.

H.H. Hobbs[1,2], J.C. Cohen[1,2];
[1]Howard Hughes Medical Institute , Dallas, TX, [2]MCDermott Center, UT Southwestern, Dallas, TX

Fatty liver disease (FLD) is a burgeoning health problem in Western countries due to the increasing prevalence of obesity and diabetes. The disorder is characterized by the accumulation of triglyceride (TG) in lipid droplets of hepatocytes (so-called “bland” steatosis). In a subset of individuals, liver disease progresses, resulting in steatohepatitis, cirrhosis and hepatocellular carcinoma. How accumulation of TG is related to liver disease progression remains obscure. To elucidate the molecular basis of FLD, we used 1H magnetic resonance spectroscopy to measure hepatic TG content in a multiethnic population-based study [Dallas Heart Study (DHS)]. We found significant differences in prevalence of FLD among ethnic groups (Hispanics>Europeans>Africans), suggesting that differences in genetic ancestry may influence the propensity to accumulate liver TG. Using GWAS, we identified variants in two genes that confer susceptibility to FLD: *PNPLA3*, a lipid droplet protein, and TM6SF2, an ER/Golgi protein. Using both *in vitro* and *in vivo* approaches, we identified the pathways that are perturbed by these variants, thus accounting for the accumulation of hepatic TG. One variant (PNPLA3-148M) is common in Hispanics (allele frequency of 49%) and explains ~70% of the differences in ancestry-associated prevalence of FLD. This variant interferes with ubiquitin-mediated degradation of PNPLA3, resulting in accumulation of PNPLA3-148M on lipid droplets and impairment of TG mobilization. The other variant (TM6SF2-167K) impairs bulk lipidation of nascent VLDL particles, thus limiting TG secretion from the liver. Both variants are associated with liver disease progression. The finding that two variant proteins confer susceptibility

to FLD by different mechanisms suggests that chronic steatosis may not be bland. The role of TG in liver disease progression may thus resemble the role of LDL-cholesterol in coronary atherosclerosis.

Minisymposium 1: Bacterial Infection and Symbiosis

M1

Pyroptosis: from innate immunity to cancer.

F. Shao[1];
[1]National Institute of Biological Sciences, Beijing, China

Pyroptosis was long regarded as caspase-1-mediated monocyte death in response to certain bacterial insults. Caspase-1 is activated upon various infectious and immunological challenges through different inflammasomes. The discovery of caspase-11, -4 and -5 function in recognizing intracellular LPS has expanded the spectrum of pyroptosis mediators and also suggested that pyroptosis is not cell type-specific. We have recently identified the pyroptosis executioner protein, Gasdermin D (GSDMD), which is cleaved and activated by caspase-1 as well as caspase-11/4/5. GSDMD represents a large Gasdermin family bearing a novel membrane pore-forming activity capable of inducing pyroptosis. We therefore redefine pyroptosis as Gasdermin-mediated programmed necrosis. Gasdermins are associated with various diseases, but the cellular function and mechanism of activation for other Gasdermins are largely unknown. Very recently, we show that Gasdermin E (GSDME) is cleaved and activated by caspase-3, which determines pyroptotic cell death in response to various chemotherapy drugs. GSDME is silenced in most cancer cells but expressed in normal tissues. Blocking the caspase-3-GSDME axis could inhibit chemotherapy drugs-induced pyroptosis in primary noncancer cells. Consistently, Gsdme-/- mice are protected from chemotherapy drug-induced adverse effects including tissue damages and weight loss. Thus, the Gasdermin family of pore-forming proteins opens a new area of research on pyroptosis and its functions in immunity, disease, and beyond.

M2

Spatial organization of the human tongue dorsum microbiome at the micron scale.

S.A. Wilbert[1], J.L. Mark Welch[2], F.E. Dewhirst [1,3], G.G. Borisy[1,3];
[1]The Forsyth Institute , Cambridge , MA, [2]Marine Biological Laboratory , Woods Hole, MA, [3]Harvard School of Dental Medicine, Boston, MA

Humans have co-evolved with the microbes comprising our microbiome but the significance of this symbiotic relationship for human health is only beginning to be appreciated. The individual microbiomes of the body may be considered the physiological equivalents of multi-cellular organs. As with other body organs, an understanding of their anatomy is essential for understanding their physiology. In previous work using multiplexed, spectral imaging and fluorescence *in situ* hybridization, we discovered a distinctive, multi-genus consortium in human supragingival plaque consisting of a radially arranged nine-taxon structure (Mark Welch et al 2016. PNAS 113 E791-E800). The distinctive structure permitted us to generate specific hypotheses for how the consortium develops, functions and is maintained. We now apply the multiplexed, spectral imaging approach to the microbiome of the human tongue. We designed oligonucleotide probes for taxa both abundant and prevalent on the human tongue dorsum as determined by oligotyping re-analysis of data from the Human Microbiome Project. Multiplexed imaging revealed a highly structured spatial organization ranging in linear dimension from fifty to hundreds of microns. However, the organization differed from supragingival plaque in both structural detail and taxonomic composition. Featured on the tongue were consortia whose members were primarily cocci

and bacilli drawn from the genera *Streptococcus*, *Rothia*, *Neisseria*, *Actinomyces* and *Veillonella*. Fifteen additional genera were detected at lower abundance and prevalence. The consortia radiated outward from a core of epithelial cells, with individual taxa clustering in domains suggestive of clonal expansion. The range expansion/contraction of these domains permits inferences regarding the selective advantage of the taxa during consortium development. Variations in taxon abundance from sample to sample and person to person were used to calculate correlations in relation to cell-cell and cell-host interactions. Finally, species-level analysis revealed distinctive tropisms in the oral microbiome. For example, the *Rothia* genus member on the tongue was determined to be almost exclusively *R. mucilaginosa*. Species level identification in combination with genomic information revealed that several of the tongue taxa possess nitrate reductase, a metabolic capacity not encoded by the human genome. This suggests that the tongue microbiome plays an important symbiotic role in nitric oxide homeostasis which is of significance for systemic control of vasodilation and blood pressure. Our work illustrates how high resolution analysis of micron-scale organization can provide insights into physiological functions and microbiome-host interactions. Supported by NIDCR Grant DE 022586.

M3

Microbe-host mitochondria signaling crosstalk in metabolic health and longevity.

M.C. Wang[1,2];
[1]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, [2]Huffington Center on Aging, Baylor College of Medicine, Houston, TX

Microbes and their hosts share the same environment, and microbial factors play pivotal roles in maintaining the physiological homeostasis of hosts and modulating their susceptibility to diseases. Among all the resident microbes in the host, bacteria are the most abundant components and actively communicate with one another. On the other hand, mitochondria are ancient relatives of bacteria in eukaryotic cells, and are dynamically interconnected through organellar fusion and fission. Given the close relationship between bacteria and eukaryotic mitochondria during evolution, my group is interested in understanding the critical role of their communication in regulating host metabolic health and longevity. We discovered that in response to environmental methionine availability, bacteria could adjust their methyl metabolism to regulate mitochondrial fusion-fission dynamics and consequently lipid metabolism in the host. This interesting environment-microbe-host metabolic axis is mediated by an endocrine crosstalk comprising NR5A nuclear receptor and hedgehog signaling. We also conducted genome-scale screens to decipher how microbial genetic composition impacts host longevity, and identified a series of microbial genetic factors regulating host lifespan and resistance to age-related pathologies. Mechanistic characterization of these microbial factors leads to the discovery of one bacteria-secreted metabolite, colanic acid that fine-tunes the mitochondrial fusion-fission balance and consequently promotes longevity across different host species. Together these studies demonstrate a novel mode of signaling communication between bacteria and mitochondria, and its vital impacts on host metabolic fitness and longevity.

M4

Monte Carlo simulations of *Listeria monocytogenes* cell-cell spread predict a stratified spreading behavior crucial for survival in the intestinal epithelium.

F.E. Ortega[1], E.F. Koslover[2], J.A. Theriot[1,3,4];
[1]Biochemistry, Stanford School of Medicine, Stanford, CA, [2]Physics, University of California San Diego, San Diego, CA, [3]Microbiology and Immunology, Stanford School of Medicine, Stanford, CA, [4]Howard Hughes Medical Institute, Stanford, CA

Bacterial pathogens use diverse mechanisms to colonize their hosts. After invading an intestinal epithelial cell during an oral infection, the pathogenic bacterium *Listeria monocytogenes* can spread to neighboring cells without exposing itself to the bactericidal extracellular milieu. This mechanism, known as cell-cell spread, enables *L. monocytogenes* to breach epithelial and endothelial barriers, and to reach distant organs such as the liver, placenta, and brain. To learn about the dynamics of *L. monocytogenes* cell-cell spread, we established a live microscopy system which allowed us to track fluorescent intracellular *L. monocytogenes* as they spread through polarized epithelial monolayers. Qualitatively, we observed that the dynamics of *L. monocytogenes* cell-cell spread resembled those of a random walk. To test whether a random walk alone could explain bacterial movement during cell-cell spread, we performed a Monte Carlo simulation in which these movements were modeled based on the Normal distribution. Surprisingly, we discovered that the observed speed of spread in our experimental data was higher than that predicted by our simulated data, suggesting that we cannot use a random walk alone to model *L. monocytogenes* cell-cell spread. However, Monte Carlo data generated by simulating stratified spread, a form of spread characterized by stochastic dispersal events over longer distances, agreed well with our experimental data. Importantly, further simulation demonstrated that stratified spread increases the probability that *L. monocytogenes* will mount a successful infection of the epithelium, which depends on the bacterium's ability to spread and replicate efficiently without killing the host cell.

M5

Chlamydia interfere with an interaction between the mannose-6-phosphate receptor and sorting nexins to counteract host restriction.

C. Elwell[1], N. Czudnochowski[1], K. Pha[1], J. Von Dollen[2], J. Johnson[2], R. Nakagawa[1], J. Sherry[3], K. Mirrashidi[1], N. Krogan[2], O. Rosenberg[1], J.N. Engel[1,4];
[1]Medicine , University of California, San Francisco, San Francisco, CA, [2]Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, CA, [3]Biochemistry, University of California, San Francisco, San Francisco, CA, [4]Microbiology/Immunology, University of California, San Francisco, San Francisco, CA

Chlamydia trachomatis is a leading cause of human genital and ocular infections for which there is no vaccine. *Chlamydia* species replicate within a unique membrane-bound compartment--the inclusion--which is essential for its survival within the hostile intracellular environment. *Chlamydia* secretes a family of effector proteins called inclusion membrane proteins (Incs) that localize to the inclusion membrane, where their cytosolic-exposed N- and C-termini are ideally positioned at the host-pathogen interface to mediate interactions between *Chlamydia* and the host cell. As molecular genetic manipulation of *Chlamydia* species has only recently become possible and as the function and host interaction partners of only a few Incs had been determined, we performed a high-throughput affinity purification-mass spectrometry screen to map the *C. trachomatis* Inc-Human interactome. We identified at least one predicted high confidence interaction for 2/3 of the 58 Incs. We have explored

the biologic implications of several of the predicted Inc-host interactions. We uncovered a high confidence interaction between IncE and the SNX-BAR components of the retromer. SNX5 or SNX6 form a heterodimer with SNX1 or SNX2 at the endosome. Together with the cargo recruitment complex, comprised of VPS26/29/35, the SNX heterodimer transports cargo from endosomes to the trans-Golgi network. We demonstrated that the C-terminal 24 amino acids of IncE was necessary and sufficient to bind directly to the SNX5/6 Phox-homology (PX) domain. During *C. trachomatis* infection, SNX1/2/5/6 relocalize from endosomes to the inclusion membrane. Overexpression of IncE is sufficient to disrupt retromer-mediated trafficking in human cells. Depletion of SNX5/6 enhances *Chlamydia* infection, suggesting that retromer functions as a novel pathogen restriction factor. To understand the molecular details of the SNX5-IncE interaction, we solved the crystal structure of the SNX5-PX:IncE complex. IncE binds to a unique and highly conserved hydrophobic groove on SNX5. Mutagenesis of the SNX5-PX:IncE binding surface disrupts a previously unsuspected interaction between SNX5 and the mannose-6-phosphate receptor (CI-MPR). Addition of IncE peptide to *C. trachomatis* lysates inhibits the interaction of CI-MPR with SNX5. *C. trachomatis* infection interferes with the SNX5-CI-MPR interaction, suggesting that IncE and CI-MPR share the same binding surface on SNX5. We are currently following up on additional binding partners of IncE that were identified in the AP-MS screen. Our results provide new insights into retromer assembly and underscore the power of using pathogens to discover disease-related cell biology.

M6

Structure, function and dynamics of the bacterial Type VI secretion systems.

M. Basler[1];
[1]Biozentrum, University of Basel, Basel, Switzerland

Transport of macromolecules across membranes is achieved by various molecular nanomachines. The bacterial Type VI secretion system (T6SS) is assembled from three main parts: membrane complex, baseplate and sheath-tube polymer. Contraction of the sheath quickly pushes the inner rigid tube with associated effectors into both eukaryotic and bacterial cells and thus T6SS plays an important role in bacterial pathogenesis and ecology. The mechanisms behind the generation of power needed to pierce target cell membranes are unknown due to limited information about an inherently unstable pre-firing state of the T6SS.
I will present the structure of T6SS sheath solved by cryo-electron microscopy and the implications for T6SS dynamics and assembly. I will discuss how secreted proteins are efficiently exchanged among by-standing cells within minutes of initial cell-cell contact and can be subsequently reused to form a functional T6SS assembly. I will update on the structure of the T6SS in the pre-firing state and discuss implications for T6SS attachment to the bacterial cell envelope as well as the role of individual proteins in the T6SS assembly.

M7

A Rab32 trafficking pathway that prevents bacterial infections.

M. Baldassarre[1], V. Solano-Collado[1], D. Mancuso[1], S. Spano[1];
[1]Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom

Host defense mechanisms protect complex organisms against the attack of microbes. Intracellular bacterial pathogens such us Salmonella, evolved sophisticated, often redundant, strategies to overcome host defense and cause infection. Despite sharing many strategies to overcome host defense, different Salmonella serovars show different pathogenic behaviors, suggesting the existence of unknown bacterial

killing mechanisms. We previously identified a host trafficking pathway to the bacterial intracellular vacuole that prevents the human-restricted bacterial pathogen Salmonella Typhi from surviving in mouse macrophages and therefore infecting mice. This pathway depends on the host GTPase Rab32 and its guanine nucleotide exchange factor BLOC-3. We showed that in contrast to Salmonella Typhi, the broad-host pathogen Salmonella Typhimurium infect mice by counteracting the Rab32 trafficking pathway through the delivery of two type-III-secretion effectors: GtgE, which is a specific protease cleaving Rab32; and SopD2, which is a Rab GTPase activating protein (GAP). A Salmonella Typhimurium strain deficient for both of these effectors is unable to infect mice, yet it is fully virulent in BLOC-3 deficient mice. Therefore, the Salmonella Typhimurium effectors GtgE and SopD2 act redundantly to neutralize a powerful host defense pathway that can prevent bacterial infections. However, the Rab32-dependent mechanisms directly involved in bacterial killing are still unknown. Our new results indicate that Salmonella is not the only pathogen susceptible to this pathway and suggest that other intracellular pathogens have to counteract this host-defense pathway to be able to survive inside the host cell and to cause infection.

M8

Linking ISG15 to Cellular Stress Responses: Lessons from *Listeria* infection.

L. Radoshevich[1,2], M. Foecke[1], F. Impens[3], K. Knobeloch[4], P. Cossart[1];
[1]Bacteria Cell Interactions, Institut Pasteur, Paris, France, [2]Microbiology , University of Iowa, Iowa City, IA, [3]Proteomics Expertise Center, VIB, University of Ghent, Ghent, Belgium, [4]Institute of Neuropathology, University Clinic Freiburg, Freiburg, Germany

ISG15 is an interferon-stimulated, ubiquitin-like protein, with anti-viral activity, however its role during bacterial infection had not been addressed. We previously found that ISG15 restricts *Listeria monocytogenes* infection both in vitro and in vivo and identified ISGylated proteins that could be responsible for the protective effect. Strikingly, infection or overexpression of ISG15 leads to ISGylation of ER and Golgi proteins, which correlates with increased secretion of cytokines known to counteract infection. More recently, we endeavored to map the *in vivo* ISGylome following *Listeria* infection to mechanistically understand the function of this pathway in host defense. To do so we combined a genetic approach employing a murine model of hyper-ISGylation with quantitative proteomics of immunoenriched endogenous ISG15 modification sites. In addition, our approach mapped the endogenous ubiquitylome following infection with *Listeria in vivo* including the identification of several modified bacterial proteins. Interestingly, we detected ISG15 sites in a number of ER and Golgi proteins, which we had previously shown to be ISG15 targets *in vitro*, as well as proteins known to control cellular stress responses such as ER stress and autophagy. Taken together, this work will open the door to understanding mechanisms of action of an understudied ubiquitin-like protein.

M9

Neisseria gonorrhoeae modifies its infectivity based on the properties of human cervical epithelial cells.

Q. Yu[1], L. WANG[1], D.C. Stein[1], W. Song[1];
[1]Cell Biology and Molecular Genetics, University of Maryland, College Park, MD

Gonorrhea, caused by *Neisseria gonorrhoeae* (GC), is one of the most common sexually transmitted infections. It has recently re-emerged as a public health crisis due to increases in multidrug resistant strains. However, the pathogenesis of this bacterium is not well understood due to a lack of infection models that mimic the human disease and the phase variation of multiple molecules on this bacterium,

including opacity-associated protein (Opa). GC infection in the female reproductive tract starts from the cervix. The mucosal surface of the human cervix consists of three types of epithelial cells: non-polarized multilayer squamous at the ectocervix, polarized monolayer columnar at the endocervix, and transforming epithelial cells in between. This study examined the cellular mechanisms by which GC overcome the mucosal epithelial barrier to establish infection in the human cervix. We developed an ex vivo infection model using human cervical tissues and bacterial strains expressing no Opa or Opa proteins that cannot undergo phase variation. Using three-dimensional immunofluorescence microscopy, we found that GC that express phase variable Opa selectively penetrate into the subepithelium of the transformational zone and the endocervix but not that of the ectocervix. However, GC colonize the mucosal surface at all three regions, but the level of colonization at the transformation zone is higher than those at the ectocervix and endocervix. These findings are consistent with previous clinical observations using patients' biopsies. The expression of phase invariable Opa that binds to the host adhesion molecules CEACAMs enhances GC colonization at the ectocervix and the transformation zone but inhibits GC penetration into the endocervical epithelium. In contrast, Opa expression does not affect GC infectivity in the transformational zone. Surprisingly, epithelial cells in the transformation zone do not express CEACAMs on their surface. GC break the cervical epithelial barrier by disrupting their adherens junction, which leads to GC penetration and epithelial cell shedding. Opa expression restores the epithelial adherens junction in a CEACAM-dependent manner, thereby inhibiting GC penetration and epithelial cell shedding. Our results collectively suggest that GC modify their infectivity based on the availability of the host cell receptors CEACAMs on cervical epithelial cells and the expression of Opa isoforms on GC. Our cervical tissue explant model is a useful tool for studying pathogenesis and developing treatments of sexually transmitted pathogens.
This work has been supported by NIH grants.

M10

Zebrafish modeling defines complex innate immune mechanisms in sepsis and repetitive intestinal injury.

L. Chuang[1], N. Hsu[1], P. Labrias[1], S. Nayar[1], J. Facey [1], K. Gettler[2], N. Villaverde [1], G. Boschetti [3], M.A. Mucci [1], E. Chen[1], M. Giri [1], Y. Sharma[1], M. Merad [3], J. Chu[4], J. Cho [1];
[1]Department of Genetics and Genomic Sciences,, Icahn School of Medicine at Mount Sinai, NEW YORK, NY, [2]Department of Genetics, Yale University, NEW HAVEN, CT, [3]Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, NEW YORK, NY, [4]Department of Pediatrics and Mindich Institute for Child Health, Icahn School of Medicine at Mount Sinai, NEW YORK, NY

Inflammatory bowel disease (IBD) is a complex chronic intestinal human disease composed of genetic, environmental and host immune factors. Establishing a robust and predictive in vivo animal model to study cellular function during acute and chronic inflammation has been challenging. Our previous genome-wide association study (GWAS) showed strong association of increased IBD risk with SNPs in innate immunity and autophagy. Live in vivo imaging of intestinal innate immunity can be uniquely performed on zebrafish because of their transparency and the presence of solely innate immunity over its initial weeks of life. For these reasons, we examined the effects of intestinal injury on time-course and cell-type dependent responses, with a particular focus on prostaglandin E2 (PGE2). Dextran sodium sulfate (DSS) induces chemical intestinal inflammation and has been extensively utilized to study intestinal innate immunity. We compared the effects of single and repeated DSS treatment in zebrafish and reported the mortality, lysosomal function and mucin production. Using previously reported doses, we observed dose-dependent mortality with repeated injury (Oehlers et. al., 2012, 2013). Since neutral red accumulation indicates normally functioning acidic lysosomes, we quantified neutral red intensity in

the midgut with single and repeated DSS treatment for one and two days post DSS. Our results showed that the initial damage to lysosomal function fully recovered in two days after single injury but remains impaired after repeated injury. Mucin production was quantified from alcian blue stained fixed zebrafish. Alcian blue intensity increased in single injury but decreased from 60 to 40% after repeated injury. We observed high mortality with impaired recovery of lysosomal function and mucin production after repeated injury. We then applied PGE2 to the single injury model. By adding 1uM PGE2 for 1, 3, and 6 hours after DSS removal, PGE2 promoted recovery of neutral red intensity, positively correlated to earlier PGE2 treatment. PGE2 treatment dose-dependently increased alcian blue intensity. We further applied fluorescent killed E. coli to the single injury model and detected a high mortality rate similar to repeated injury. 90 minutes after E. coli treatment, fluorescence signals were detected in the dorsal aorta and the posterior cardinal vein of DSS-treated zebrafish, suggesting that high mortality may be due to sepsis. The bacteria-induced mortality was significantly decreased with PGE2 treatment during recovery or co-treatment with DSS. Phagocytosed E. coli co-localized with autophagosome in mFAP4+ intestinal macrophages. Together, we showed that PGE2 prevents sepsis by increasing mucin production and recovering lysosomal function in intestinal macrophages.

Minisymposium 2: Functions and Mechanisms of Cytoskeletal Motors

M11

Mitochondria distribution to filopodia by the actin-based motor Myo19.

B.I. Shneyer[1], M. Usaj[1], N. Wiesel-Motiuk[1], R. Regev[1], A. Henn[1];
[1]Biology, Technion-Israel Institute of Technology, Haifa, Israel

Eukaryotic cells are exposed to many environmental cues and stress conditions which have profound effects on mitochondria dynamics. The role of the actin cytoskeleton in relation to mitochondria function and dynamics is only now beginning to emerge, revealing new functions for actin-based motors. Here, we focus on the recently discovered actin-based motor, Myo19, which is associated with the mitochondria and on its effects on mitochondrial biology. We show that Myo19 localizes with mitochondria to filopodia tips in response to glucose starvation, ROS and EGF. However, how Myo19 localizes to mitochondria, how its enzymology allows the translocation of mitochondria to filopodia, and what is the function of mitochondria at these filopodia is not known. First, we reveal that Myo19 is integrated to the outer mitochondrial membrane (OMM) through a previously unidentified binding motif in its tail domain, ensuring a highly stable interaction between Myo19 and the OMM. Point mutations within the 30 amino acids of this motif inhibit localization of Myo19 to the OMM. Secondly, using time-lapse fluorescent microscopy we show that Myo19 undergoes both anterograde and retrograde movements in filopodia, which are coupled to their extension and retraction, respectively. Thirdly, by studying the enzymology of Myo19 we provide a detailed reaction mechanism of its ATPase cycle. Both the slow ADP isomerization and ADP release prolong the time Myo19 spend in the strong actin binding state and hence contribute to its relatively high duty ratio. However, the predicted duty ratio based on our measured rate and equilibrium constants is lower than required to support motility as a monomer. Thus, we predict that an ensemble of Myo19 motors is required to efficiently propel mitochondria movement on actin filaments. Finally, we provide a model explaining how Myo19 translocation may be regulated by the local ATP/ADP ratio. Interestingly, local mitochondria concentration in neurons is correlated with increased branching of actin-based protrusions of the dendritic spines. Thus, we suggest that Myo19 acts as an ATP sensor, and translocates mitochondria to regions that demand high local levels of ATP for processes such as actin polymerization.

M12

The 3.5Å cryoEM structure of a fast dynein/dynein complex.

L. Urnavicius[1], C.K. Lau[1], M.M. Elshenawy[2], E. Morales[1], A. Yildiz[2], A.P. Carter[1];
[1]MRC LMB, Cambridge, United Kingdom, [2]UC Berkeley, Berkeley, CA

Dynein and its cofactor dynactin form a highly processive microtubule motor in the presence of a coiled coil adaptor, such as BICD2. Different adaptors link dynein/dynactin to distinct cargos. Here we use electron microscopy (EM) and single molecule studies to show that adaptors can recruit a second dynein to each dynactin. Whereas BICD2 is biased towards recruiting a single dynein, the adaptors Hook3 and BICDR-1 predominantly recruit two. Shifting the equilibrium towards a double-dynein complex increases both force and speed by almost two-fold. A 3.5 Å cryo-EM reconstruction of the dynein tail/dynactin/BICDR-1 complex explains how the second dynein is recruited. Our work provides a structural basis for how diverse adaptors recruit different numbers of dyneins and suggests the mechanism by which adaptors can tune the properties of the dynein/dynactin machine.

M13

KIFC1, a mitotic motor protein expressed throughout the life of the neuron, is enriched in the distal region of the axon where it crosslinks microtubules in a manner that opposes axon retraction.

H. Muralidharan[1], X. Sun[1], P.W. Baas[1];
[1]Neurobiology Anatomy, Drexel University College of Medicine, Philadelphia, PA

KIFC1, also called HSET, is a member of the kinesin-14 family of motor proteins that move toward the minus-end of the microtubule (MT). Like many other mitotic motors, KIFC1 can slide MTs against one another. However, it can also align and crosslink MTs in a manner that opposes their sliding. Despite its high expression in both embryonic and adult neurons, KIFC1's functions in the neuron are unknown. In rat hippocampal and sympathetic cultures, we found KIFC1 to be present throughout the neuron, but enriched in the distal region of the axon and at branch points. We investigated potential roles of KIFC1 in organizing axonal MTs either by depleting it using RNA interference or inhibiting its function using the small molecule inhibitor, AZ82. In neurons depleted of KIFC1 or treated with AZ82, neurite number, axon length, axon branch frequency and growth cone size were all diminished relative to controls, and neuronal polarization was delayed. The distal region of the KIFC1-depleted axon was thickened in diameter and the growth cone had a club-like appearance, similar to stalled growth cones in control cultures. Compared to controls, MTs in KIFC1-depleted neurons were more splayed in the shaft of the axon and more curled back on themselves in the growth cone. MTs in the abnormally thickened distal region of the KIFC1-depleted axon were denser than in controls, and displayed flaws in polarity orientation. KIFC1-depleted axons retracted more than controls in response to noc-7 (a nitric oxide donor), but also failed to show the sinusoidal bends typical of control axons wherein MT bundles normally resist the axon's retraction. Consistent with a role for KIFC1 in opposing axon retraction even after the axon has matured, treatment with AZ82 reduced synapse number when applied to older cultures. Expression of a mutant form of KIFC1 that allows it to crosslink but not slide MTs rescued the stalled growth cone phenotype but not the axon length phenotype. We conclude that the major role of KIFC1 in the axon is to crosslink MTs in a manner that resists their capacity to slide, especially in the distal region of the axon where forces such as the retrograde flow of actin filaments may otherwise push MTs backward. We suspect that KIFC1 may also slide short MTs in the axon to promote its growth, in a manner similar to the established role of cytoplasmic dynein. We posit that the most important role of KIFC1 in adult axons is to provide a bulwark against the axon pulling back from its synapse, a

phenomenon central to the pathology of a number of neurodegenerative diseases. (This work was supported by a grant from the NIH to PWB).

M14

Molecular mechanism of dynein's direction-dependent microtubule-binding strength.

L. Rao[1], F. Berger[2], M.P. Nicholas[1], A. Gennerich[1];
[1]Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY, [2]Rockefeller University, New York, NY

Cytoskeletal motor protein motility requires coordination of ATPase and filament-binding cycles. Mechanical tension strongly influences these processes, and likely regulates motor stepping as external forces resist motor movement and intramolecular tension develops between motor domains. In cytoplasmic dynein, an AAA+ ATPase, applied tension affects microtubule (MT)-binding strength anisotropically –backward tension induces stronger binding– while in the absence of tension, reconfiguration of the coiled-coil 'stalk' (which connects the AAA+ and MT-binding domains) is known to alter MT affinity. Using optical tweezers, mutagenesis, and chemical cross-linking, we show that preventing relative motion of the stalk helices or deleting the 'buttress' (which emerges from the AAA+ domain and contacts the stalk) both eliminate tension-based regulation of MT-binding strength. Thus, tension alters dynein's MT-binding strength by inducing sliding of the stalk helices, and the buttress is a key mediator of this process.

M15

Myosin IIA controls red blood cell membrane morphology and mechanical properties.

A.S. Smith[1], R.B. Nowak[1], S. Zhou[2], J. Wan[2], I.C. Ghiran[3], V.M. Fowler[1];
[1]Molecular Medicine, The Scripps Research Institute, La Jolla, CA, [2]Microsystems Engineering, Rochester Institute of Technology, Rochester, NY, [3]Medicine, Beth Israel Deaconess Medical Center, Boston, MA

Nonmuscle myosin II (NMII) is an F-actin-activated Mg++ATPase that assembles into bipolar filaments and binds a variety of F-actin cytoskeletal structures to generate force to mediate diverse cellular processes such as migration, division, and shape changes. In mammalian red blood cells (RBCs) and other differentiated cell types, F-actin forms a network of short, membrane-associated filaments cross-linked by spectrin tetramers. Previous studies have identified NMII in mature human RBCs, but whether and how NMII interacts with F-actin in the RBC spectrin-actin network, or if NMII controls RBC membrane properties and function, remain completely unknown. Here, we show that NMIIA is the NMII isoform present in RBCs and that it associates with the spectrin-actin network in an ATP-dependent manner, suggesting that this interaction involves NMIIA motor domains and the short F-actins in the network. Indeed, results from epifluorescence and TIRF microscopy reveal NMIIA puncta, identified as bipolar filaments by super-resolution microscopy, localized near the RBC membrane. The NMIIA heavy and light chains are both phosphorylated in vivo in RBCs, indicating that NMIIA motor activity and filament assembly are actively regulated. To test the function of NMIIA in RBC membrane morphology and mechanical properties, we treated RBCs with blebbistatin, an inhibitor of NMII motor activity. The results show that treatment with the active enantiomer of blebbistatin resulted in an altered distribution of NMIIA and F-actin at the RBC membrane, suggesting rearrangements in the long-range organization of the spectrin-actin network. In addition, RBCs treated with active blebbistatin exhibit elongated cell shapes and reduced biconcavity, as well as changes in membrane oscillations and increased membrane deformability in microfluidic assays. Together, this data characterizes a novel

function for NMII in regulating cell membrane morphology and mechanical properties through interactions with the spectrin-actin network.

M16

IDA3 associates with IFT in growing cilia to selectively mediate transport and assembly of axonemal I1 dynein.

E.L. Hunter[1], J. Hwang[1], G. Fu[2], L.M. Alford[3], A. Gokhale[1], R. Yamamoto[4], R. Kamiya[5], H. Lin[6], F. Yang[7], D. Nicastro[2], K.F. Lechtreck[8], M. Wirschell[7], S.K. Dutcher[6], W.S. Sale[1];
[1]Department of Cell Biology, Emory University, Atlanta, GA, [2]Department of Cell Biology and Biophysics, UT Southwestern Medical Center, Dallas, TX, [3]Department of Biology, Oglethorpe University , Atlanta , GA, [4]Department of Biological Sciences, Osaka University, Osaka, Japan, [5]Department of Biological Sciences, Chuo University, Tokyo, Japan, [6]Department of Genetics, Washington University School of Medicine, St. Louis, MO, [7]Department of Biochemistry, University of Mississippi Medical Center, Jackson, MS, [8]Department of Cellular Biology, University of Georgia, Athens, GA

Many ciliary substructures such as dynein arms assemble in the cell body before IFT-mediated transport in the ciliary compartment and docking to the axoneme. How these axonemal cargoes interact with IFT remains largely unknown. Here, we analyze the transport of the conserved inner dynein arm I1/f, a 1.5 MDa dynein complex that preassembles in the cytoplasm and is transported by IFT (Viswanadha et al., 2014). The *Chlamydomonas ida3* mutant (Kamiya et al., 1991) preassembles I1 dynein in the cytoplasm but I1 dynein fails to enter the cilium for assembly on the axoneme, resulting in a slow-swimming phenotype. Cryo-ET confirms that I1 dynein is the sole structural defect in *ida3* axonemes. Using whole genome resequencing, we identified a premature stop codon (W22X) in the *ida3* mutant and loss of a 115 kDa coiled-coil protein with an intrinsically disordered region in the C-terminal half. This mutation co-segregates with the loss of I1 assembly on the axoneme. Several independent intragenic revertants restore I1 dynein assembly and subsequent motility defect. Expression of HA or NeonGreen (NG)-tagged IDA3 also rescues the motility and I1 dynein assembly phenotypes in *ida3*. Immuno-blotting demonstrates that IDA3 is restricted to the cell body in cells with full-length cilia, but IDA3 enters the ciliary matrix in growing cilia. Live-cell TIRF microscopy of cells transformed with IDA3::NG confirms the selective entry of IDA3::NG during ciliary assembly and indicates IDA3 entry is regulated in a cilium-autonomous manner (Craft et al., 2016). IDA3::NG moves by anterograde IFT into elongating cilia and largely exits the cilia by diffusion. Immunoprecipitation of IDA3::HA from the matrix fraction of regenerating cilia reveals an association of IDA3 with the I1 dynein intermediate chain IC140. GFP-tagged IC140 also moves by anterograde IFT. We propose that IDA3 interacts with IC140 and acts as an adapter selectively linking the I1 dynein complex to IFT to ensure transport into and within the growing cilium.

M17

Kinesin-2 motors adapted their stepping behaviour for progressive transport on axonemes and microtubules.

W.L. Stepp[1], G. Merck[1], F. Mueller-Planitz[2], Z. Ökten[1,3];
[1]Department für Physik, Technische Universität München, Munich, Germany, [2]Biomedical Center, Ludwigs Maximilians Universität, Munich, Germany, [3]Munich Center for Integrated Protein Science, Munich, Germany

Kinesin-2 is employed in both cytoplasmic and intraflagellar long-range transport. It therefore employs two structurally distinct filament tracks, axonemes in cilia and microtubules in the cell body. We tracked

the heads of single kinesin-2 molecules with Fluorescence Imaging with One Nanometer Accuracy (FIONA) on both axonemes and microtubules. The distribution of step sizes and the traces observed show that the motors adapt their stepping behavior to the respective track. While kinesin-2 takes directional, off-axis steps on microtubules, it resumes a straight path on axonemes. This provides a molecular explanation for the direction-dependent allocation of cargo to one side of the same doublet microtubule that has been observed previously. The intrinsic tendency of kinesin-2 to take sidesteps on the microtubule lattice restricts the motor to the B-tubule. These results offer first mechanistic insights into why heterodimeric kinesin-2 was co-evolved with the ciliary machine to achieve effective two-way traffic on the axoneme *in vivo*.

M18

She1 affects dynein by interactions with the microtubule and the dynein microtubule-binding domain.

K. Ecklund[1], T. Morisaki[1], L.G. Lammers[1], M.G. Marzo[1], T.J. Stasevich[1], S.M. Markus[1];
[1]Biochemistry Molecular Biology, Colorado State University, Fort Collins, CO

Cytoplasmic dynein is an enormous ~1.2 MDa molecular motor that transports myriad cargoes toward the minus ends of microtubule tracks. Rather than existing as bare tracks, microtubules are bound by numerous microtubule- associated proteins (MAPs) that have the capacity to affect various microtubule-based cellular functions, including motor-mediated transport. One such MAP is She1, a potent effector of dynein motility that plays a role in polarizing dynein- mediated spindle movements in budding yeast. Here we characterize the molecular basis by which She1 affects dynein motility, providing the first such insight into which a MAP can directly modulate motor motility. We find that She1 reduces the rate by which dynein hydrolyzes ATP, likely due to the enhanced microtubule-binding affinity and consequent reduced stepping frequency imparted on dynein by She1. Microtubule binding by She1 is required for it to affect dynein motility. Moreover, we find that She1 and dynein directly interact and that this interaction is sensitive to dynein's nucleotide-bound state. We narrowed down the She1 binding region to the microtubule-binding domain (MTBD) of dynein. Accordingly, a dynein motor with a mutated MTBD displays reduced sensitivity to She1 in vitro and in vivo. Taken together, our data support a model in which simultaneous interactions between the microtubule and the dynein MTBD provides She1 with the ability to reduce dynein velocity and prolong its microtubule encounters.

M19

Regulated autoinhibition of kinesin-1 is essential to polarized dendritic transport.

M.T. Kelliher[1], Y. Yue[2], A. Ng[1], D. Kamiyama[3], B. Huang[4], K.J. Verhey[2], J. Wildonger[1];
[1]Biochemistry, University of Wisconsin - Madison, Madison, WI, [2]Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, [3]Cellular Biology, University of Georgia, Athens, GA, [4]Department of Pharmaceutical Chemistry, University of California - San Francisco, San Francisco, CA

Neuronal polarity relies on the selective localization of cargo to axons or dendrites by molecular motors. Kinesin-1 has a critical role in transportation of cargo into axons, but is also known to be active in dendrites. This raises the question of how kinesin-1 activity is regulated to maintain the polarized distribution of cargo within neurons. Our in vivo structure-function analysis of endogenous kinesin-1 in Drosophila reveals a novel role for autoinhibition in polarized dendritic transport. Mutations that disrupt kinesin-1 autoinhibition result in the mislocalization of Golgi outposts (GO) to axons. Our findings show that disruption of autoinhibition is distinct from mutations that inhibit ATP hydrolysis; axonal mislocalization of GO is not found when kinesin-1 enzymatic activity is impaired. Autoinhibition also

functions to control kinesin-1 localization within the neuron. Uninhibited kinesin-1 accumulates in axons and is depleted from dendrites, resulting in dendrite growth defects. Confirming an essential role for kinesin-1 in dendrite development, dendritic morphology is also altered when kinesin-1 levels are depleted through RNAi. Genetic interaction tests reveal that a balance of kinesin-1 inhibition and dynein activity is necessary to localize GO to dendrites and keep them from entering axons. Lowering both kinesin-1 and dynein levels is not sufficient to drive GO into axons. In contrast, relieving kinesin-1 autoinhibition when dynein levels are reduced is sufficient to disrupt the polarized transport of outposts. We propose a mechanism in which dynein is required to carry outposts into dendrites, yet kinesin-1 activity must be precisely regulated by autoinhibition to achieve the selective dendritic localization of outposts. Finally, we have evidence that localization of other dendritic cargo may rely on the tight regulation of kinesin-1 autoinhibition. This suggests that autoinhibition as a mechanism of regulating kinesin-1 activity may be broadly important for compartment-specific localization of cargo in neurons.

M20

CDK5-dependent activation of dynein in the axon initial segment regulates polarized cargo transport in neurons.

E. Klinman[1], M. Tokito[2], E.L. Holzbaur[2];
[1]Neuroscience, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA,
[2]Physiology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

The unique polarization of neurons depends on selective sorting of axonal and somatodendritic cargos to their correct compartments. Axodendritic sorting and filtering occurs within the axon initial segment (AIS). However, the underlying molecular mechanisms responsible for this filter are not well understood. We found that local activation of the neuronal-specific kinase CDK5 is required to maintain AIS integrity, as depletion or inhibition of CDK5 induces disordered microtubule polarity and loss of AIS cytoskeletal structure. Furthermore, CDK5-dependent phosphorylation of the dynein regulator Ndel1 is required for proper re-routing of mislocalized somatodendritic cargo out of the AIS; inhibition of this pathway induces profound mis-sorting defects. Inhibition of CDK5 activity or blocking Ndel1 phosphorylation and subsequent dynein activation further results in atypical axonal localization of Golgi bodies, which nucleate aberrant plus-end-in axonal microtubules. While inhibition of the CDK5-Ndel1-Lis1-dynein pathway alters both axonal microtubule polarity and axodendritic sorting, we show that these defects occur on distinct timescales; brief inhibition of dynein disrupts axonal cargo sorting before loss of microtubule polarity becomes evident. Together, these studies identify CDK5 as a master upstream regulator of trafficking in vertebrate neurons, required for both AIS microtubule organization and polarized dynein-dependent sorting of axodendritic cargos, and support an ongoing and essential role for dynein at the AIS.

Minisymposium 3: Inside the Nucleus: Genome Organization and Gene Expression

M21

RICC-seq: Variable chromatin structure revealed by in situ spatially correlated DNA cleavage mapping.

V.I. Risca[1], S.K. Denny[2], A.F. Straight[3,4], W.J. Greenleaf[1];
[1]Genetics, Stanford University School of Medicine, Stanford, CA, [2]Biophysics, Stanford University, Stanford, CA, [3]Biochemistry, Stanford University School of Medicine, Stanford, CA, [4]Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA

Chromatin structure at the length scale encompassing local nucleosome–nucleosome interactions is thought to play a crucial role in regulating transcription and access to DNA. However, the local three-dimensional structure of chromatin remains poorly understood compared with the structure of single nucleosomes or long-range looping interactions. We report a genome-wide map of chromatin conformation in human cells at the 1–3 nucleosome (50–500 bp) scale, obtained using ionizing radiation-induced spatially correlated cleavage of DNA with sequencing (RICC-seq). RICC-seq can serve as a nuclease-independent orthogonal method for mapping nucleosome positions, complementing methods that sometimes yield contradictory results about nuclease-labile fragile nucleosomes. Importantly, RICC-seq data from low radiation doses that cause sparse DNA damage also reveal DNA–DNA contacts that are spatially proximal via spatially correlated DNA strand breaks. Analysis of RICC-seq signal spanning tri-nucelosome units reveals regional enrichment of DNA fragments characteristic of DNA-DNA contacts between alternating nucleosomes. These contacts are particularly enriched in H3K9me3-marked heterochromatin and depleted in open or transcriptionally active chromatin. Our data support a model of chromatin architecture consisting of fibers with local zig-zag order and variable longitudinal compaction that correlates with changes in histone modifications.

M22

Chromatin dependent glucocorticoid receptor plasticity within the genome.

J.A. Hoffman[1], K.W. Trotter[1], T.K. Archer[1];
[1] Epigenetics and Stem Cell Biology Laboratory, National Institute of Environmental Health Sciences, Research Triangle Park, NC

Upon hormone stimulus, type I nuclear receptors (NRs) bind to their hormone ligand, enter the nucleus, and interact with regulatory elements within chromatin to elicit changes in transcriptional activity. NRs are required throughout development and in adult life, and NR activities are frequently targeted in the treatment of human diseases. Activation of Glucocorticoid Receptor (GR) with the synthetic corticosteroid dexamethasone is commonly used to promote fetal lung development and to combat auto-immune and inflammatory disorders. Elucidating the genetic and transcriptional mechanisms by which GR and other NRs perform their myriad functions is critical for human health and disease treatment. Brahma Related gene 1 (Brg1), the catalytic ATPase of the human SWI/SNF complex, is critical for hormone-induced transcriptional regulation by GR. Previous studies from our lab demonstrated that upon DNA-binding, GR recruits Brg1 to remodel the local chromatin architecture to alter transcriptional output. Our new work significantly expands this model and suggests that Brg1 may act on hormone-regulated genes prior to hormone stimulus and GR binding. Using ChIP-seq, we have generated a genome-wide map of Brg1 and GR chromatin interactions in untreated and dexamethasone-treated cells. We show that Brg1 interacts with many GR binding sites in untreated cells and is present at these sites upon dexamethasone treatment and subsequent GR binding. Using these

and other data sets, we have identified specific classes of GR binding sites that are differentially associated with hormone-dependent transcriptional events. These classes of binding site showed distinct patterns of chromatin accessibility, histone modification, and transcription factor binding. Examination of GR binding in Brg1-deficient cells revealed that disruption and restoration of Brg1 expression selectively alters the chromatin environment at these classes of GR binding sites. Taken together, these data suggest that GR elicits the transcriptional response to hormone via multiple distinct mechanisms that are dependent on specialized chromatin environments. Our classification of these genomic environments provides a more detailed understanding of nuclear receptor function that will allow new approaches in drug development and disease treatment.

M23

Single-Molecule Analysis of Transcription Factors in the Nucleus of Living Cells.

V. Paakinaho[1], D.M. Presman[1], D.A. Ball[1], T.S. Karpova[1], E.E. Swinstead[1], G.L. Hager[1];
[1]NCI, NIH, Bethesda, MD

Population-based assays such as ChIP-seq, DNase-seq and FAIRE are widely used to investigate the interactions of transcription factors (TFs) with chromatin and are often interpreted in terms of sequential and static binding. However, fluorescence microscopy techniques reveal a more dynamic binding behavior of TFs in live cells. Hence, the dynamic status of many TFs in living cells remains unclear. Interestingly, the classical pioneer factor model suggests long term static binding events between pioneer TFs, such as FoxA1, and chromatin. However, little is known on the dynamic action of FoxA1 in living cells. Recently, a new and critical fluorescent microscopy technique, single-molecule tracking (SMT) has emerged. This technique enables the real-time tracking of individual TF molecules, making it possible to measure the distribution of TF binding times.
We have examined the behavior of steroid receptors and cofactors, including FoxA1, at the single-molecule level in living cells. These factors were fused with either Halo or Snap tags, and labeled with the bright and stable fluorophore JF549. Single-molecule tracking of these proteins (using HILO microscopy) reveals a highly dynamic binding behavior of TFs and cofactors. This supports transient rather than stable TF chromatin interactions. Specifically, two distinct binding populations have been observed for all factors tested; fast or slow stops. Fast stops represent genomic scanning of TF, while slow stops, with residence time of 6-14 sec, represent specific binding events at response elements. The vast majority of molecules at any given time are either diffusing or exhibit fast stops, while only a small percentage exhibit slow stops. However, hormone activation of steroid receptors results in an increase in the percentage of molecules with slow stops compared to the unstimulated state. Additionally, the residence time of several cofactors is increased upon steroid receptor activation. Importantly, disrupting the interaction of TF and its cofactors decreases the factor's residence time, and proportions of molecules with slow stops.
These data support the concept that slow stops represent functional binding events at specific response elements. In addition, SMT experiments in live cells revealed a highly dynamic interaction of FoxA1 with chromatin in vivo contrary to the predictions of the pioneer paradigm. Our results indicate that only a small proportion of factors are specifically bound to chromatin at any given time. In conclusion, our single-molecule observations affirm the general model that many TFs are highly dynamic in their chromatin binding activity.

M24

CRISPR-Based DNA Imaging in Living Cells Reveals Cell Cycle-Dependent Chromosome Dynamics.

H. Ma[1], L. Tu[2], A. Naseri[3], Y. Chung[4], D. Grunwald[2], S. Zhang[3], T. Pederson[1];
[1]Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, [2]RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, MA, [3]Department of Computer Science, University of Central Florida, Orlando, MA, [4]Kavli Institute for the Physics and Mathematics of the Universe, University of Tokyo, Tokyo, Japan

In contrast to their well-studied condensation and folding during mitosis, the dynamics of interphase chromosomes are less understood. We developed a multicolor and sensitive system, CRISPR-Sirius, allowing us to track the dynamics of pairs of loci on a single human chromosome in real time throughout interphase. We measured the inter-locus distances from kilobase to megagase scales as compared to the DNA physical map and found cell cycle stage-dependent changes in the inter-locus distance. In addition, we resolved two distinct modes of dynamics: local saltatory movements as well as longer-range domain translational movements. The magnitude of both of these dynamic substantially increased from early to late G1, whereas the domain translational movement was greatly reduced in early S. The local saltatory fluctuations decreased slightly in early S and declined further in late S. These unanticipated movements are indicative of both a compression-relaxation dynamic of the chromosome fiber operating concurrently with changes in the extent of long-range movements of an observed domain. It should now be possible to address the relationship these dynamic phenomena to DNA replication and repair, epigenetic modification, transcription regulation, and stem cell differentiation.

M25

Interphase chromatin is adaptively folded by ongoing transcription and RNA accumulation.

L. Hilbert[1,2,3], Y. Sato[4], H. Kimura[4], F. Julicher[2,3], A. Honigmann[1], V. Zaburdaev[2,3], N. Vastenhouw[1];
[1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany, [2]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany, [3]Center for Systems Biology Dresden, Dresden, Germany, [4]Tokyo Institute of Technology, Tokyo, Japan

In nuclei of eukaryotes, transcribed DNA is unfolded, while transcriptionally inactive DNA is compacted. How this adaptive folding is achieved remains unclear. We have found that transcriptional activity and the accumulation of resulting RNA transcripts control euchromatin organization in interphase nuclei. Using STED super-resolution microscopy and live cell microscopy in zebrafish embryonic cells, we were able to show that transcriptional activity drives the formation of local microenvironments. Microenvironments are formed by the accumulation of RNA, exclusion of not transcribed DNA, and retention of transcribed DNA. Based on known properties of transcription and chromatin, we developed a physical model, which coherently explains our observations based on two main mechanisms: (1) segregation between chromatin and RNA-protein complexes and (2) the tethering of RNA-protein complexes to transcribed DNA. The microscopic mechanisms and effective patterns place this model in a new class of physical systems, active microemulsions. Our findings explain how transcriptional activity and RNA accumulation shape local microenvironments, which serve as basic units of euchromatin organization throughout the cell nucleus. We propose that such microenvironments might function as hubs for genome organization and transcriptional activity.

M26

Chromatin state contributes to nuclear mechanics.

J.F. Johnston[1], S. Mochrie[2,3], M.C. King[1];
[1]Department of Cell Biology, Yale School of Medicine, New Haven, CT, [2]Department of Physics, Yale University, New Haven, CT, [3]Department of Applied Physics, Yale University, New Haven, CT

In mammalian cells, an integrated network of heterochromatin, a polymer mesh of intermediate filaments (lamins), and integral inner nuclear membrane proteins provides a robust nuclear structure that protects the genome. In particular, this network plays a critical role in maintaining nuclear integrity under mechanical strain, yet it is unclear how chromatin, and its condensation state, individually contributes to the overall structure and mechanics of the nucleus. Here we show that deletion of epigenetic modifiers is sufficient to alter nuclear deformability in yeast. We use a novel three-dimensional (3D) image reconstruction software that tracks fluorescentlylabeled nuclear envelope fluctuations over time, as well as a novel force spectroscopy assay that employs optical tweezers to directly measure the viscoelastic properties of isolated nuclei. We find that deletion of the heterochromatin reinforcing factor Swi6 (an HP1 orthologue) results in increased nuclear deformability, while loss of the H3K9 demethylase Epe1 (a KDM2B orthologue) that drives heterochromatin spreading results in decreased nuclear deformability. Our results support the hypothesis that changes in epigenetic modifications, in addition to influencing the transcriptome, have a strong influence on nuclear mechanics. As epigenetic modifications of histones are strongly suggested to contribute to certain disease states, understanding how these chromatin changes act in parallel to influence nuclear integrity and genome stability will be essential to elucidating the full effect of epigenetic alterations on disease pathogenesis.

M27

Lamin A regulates the activity and dynamics of nucleoli.

A.L. Buchwalter[1], M.W. Hetzer[1];
[1]Molecular and Cell Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA

The nuclear lamina is an intermediate filament meshwork that underlies the nuclear envelope, extends through the nuclear volume, and interacts with the genome. Previous analyses have linked lamins to processes including transcription, replication, gene silencing, and cell migration. However, embryonic stem cells lacking all lamins can proliferate and differentiate[1]. This surprising finding underscores how little we understand about the essential functions of lamins. Mutations to lamins cause a wide spectrum of pathologies, including Hutchinson-Gilford progeria syndrome (HGPS). HGPS is a rare, fatal premature aging disorder caused by a mutation to lamin A that generates a truncated protein termed progerin. Using HGPS as a model system to probe lamin biology, we discovered that A-type lamins have a role in regulating the activity of nucleoli. Nucleoli are membraneless organelles within the nucleus that coalesce around actively transcribing ribosomal DNA (rDNA) loci. Nucleoli produce ribosomal RNA, participate in ribosome biogenesis, and receive inputs from cellular signaling pathways. In HGPS-derived cell cultures and in normal cells induced to ectopically express progerin, nucleoli are enlarged. In HGPS, enlarged nucleoli are also more active and produce more ribosomes, which in turn results in globally elevated protein translation. Progerin expression drives the global depletion of heterochromatin marks by an unknown mechanism[2]. We find that loss of repressive DNA methylation on rDNA loci allows their over-activation in HGPS, suggesting that lamin A influences the chromatin state of rDNA. Depletion of lamin A from normal cells also allows nucleoli to expand and produce more ribosomal RNA. Intriguingly, depletion of lamin A increases the nuclear dynamics of nucleoli. We speculate that A-type lamin

networks within the nucleus limit both nucleolar mobility and transcriptional activity of rDNA loci. Since the expression of A-type lamins increases dramatically as cells exit pluripotency and varies widely across differentiated cell types, it is possible that nucleolar activity also changes in tandem with shifts in lamin A expression. These findings generate new insight into the cellular defects associated with HGPS and define a new function for lamin A in regulating the nucleolus.

1. Kim, Y., Zheng, X. & Zheng, Y. Proliferation and differentiation of mouse embryonic stem cells lacking all lamins. Nature Publishing Group 23, 1420–1423 (2013).
2. Shumaker, D. K. & Goldman, R. D. Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. Proceedings of the National Academy of Sciences 1–6 (2006).

M28

Optogenetic control of nuclear body assembly.

H. Zhang[1], C. Aonbangkhen[2], M. Liu[1], R. Dilley[3], R.A. Greenberg[3], D.M. Chenoweth[2], M.A. Lampson[1];
[1]Biology, University of Pennsylvania, Philadelphia, PA, [2]Chemistry, University of Pennsylvania, Philadelphia, PA, [3]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Nuclear bodies are membrane-free sub-organelles found in the nucleus of mammalian cells. Recent studies show that nuclear bodies such as nucleoli assemble by phase transition through liquid-liquid demixing, based on evidence from in vitro reconstitution and in vivo observation. However, our understanding of this process in vivo and its functional consequences is limited due to the lack of tools to experimentally manipulate phase transition in live cells. We developed an optogenetic approach to induce phase transition in live cells by controlling protein-protein interactions using photocaged chemical inducers of protein dimerization. We applied those tools to probe nuclear body assembly and its biological functions in vivo, focusing on a subset of promyelocytic leukemia (PML) nuclear bodies that associates with telomeres in telomerase-negative cancer cells. Those cells employ an alternative lengthening of telomere (ALT) pathway using homologous recombination to maintain telomere length. ALT-associated PML nuclear bodies (APBs) are a hallmark of ALT cancers and are used for diagnosis, but the assembly mechanism and functional role of APBs are not clear. We induced de novo APB assembly with light and provide evidence that APBs assemble by liquid-liquid demixing, which promotes telomere clustering in ALT cells. Our results demonstrate the use of our optogenetic tools for manipulating phase transition to probe biological function in vivo.

M29

Contribution of Nucleus Size and Cell Size to Genome Activation.

H. Chen[1], R. Fuentes[1], M.C. Mullins[1], M.C. Good[1,2];
[1]Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA, [2]Bioengineering, University of Pennsylvania, Philadelphia, PA

Following egg fertilization, embryonic cells rapidly divide without growing and do so although their nuclei are not transcriptional active. At a stereotypic time point following fertilization, the nuclear genomes of these cells, termed blastomeres, are suddenly activated, leading to large-scale zygotic transcription. However a major question is how the cells or their nuclei are able to measure time or developmental progression to correctly trigger genome activation. Intriguingly, the physical dimensions of cells and nuclei drastically reduce during blastomere cleavages. Therefore it has been proposed that cells contain a ‘sizer’ - a means of measuring cell or nucleus volume - to explain the precise onset of nuclear activity. Alternatively, it has been proposed that cells contain a ‘timer’ or clock, and possibly an event ‘counter’ that tracks completed rounds of DNA replication. Importantly, a sizer and counter differ

from a timer because the decision to activate the zygotic genome is made by individual cells or rather than the embryo. Traditional transcriptomics studies, although useful, lack spatial information on genome activation at a single-nucleus level within embryos and thus cannot distinguish between these paradigms. Therefore, we developed an imaging method to measure nascent transcription within individual nuclei, and leveraged embryos from the frog, Xenopus laevis, that exhibit broad cell size variation from one pole to the other. By doing so we are able to investigate factors that regulate zygotic genome activation (ZGA) in isolated blastomeres and intact embryos. Surprisingly, we found that at the canonical time point designating ZGA onset, embryonic nuclei do not uniformly turn on transcription; a result that opposes the timer paradigm. Instead transcriptional output is cell autonomous – the onset of ZGA correlates strongly with cell volume and nucleus:cytoplasm volume ratio, as well as spatial position within the embryo. Using mini-embryos and by increasing cell size variation we are contrasting the contributions of sizer and counter paradigms. Histone titration by DNA has been proposed as a mechanism that controls the timing of ZGA. Histone density on DNA may read-out cell volume, whereas histone concentration within the nucleoplasm may be regulated by nucleus size. Using embryos with reduced levels of core histones we investigated their contribution to ZGA. We found that their levels regulate the timing of genome activation, a DNA damage response that coincides with ZGA, and impact nuclear morphology. At present we are determining which phenotype is sufficient to modulate ZGA onset within cleavage-stage embryos. The results of this study support the presence of a sizer molecular that regulates ZGA.

M30

Nuclear organogenesis requires nuclear surface area regulation through nucleo-cytoplasmic trafficking.

V. Boudreau[1,2], J. Hazel[3], J. Sellinger[1], H.G. Garcia[2,4,5], J.F. Allard[6], J.C. Gatlin[3], P.S. Maddox[1];
[1]Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, [2]Physiology Course, Marine Biological Laboratory, Woods Hole, MA, [3]Molecular Biology, University of Wyoming, Laramie, WY, [4]Molecular Cell Biology, University of California, Berkeley, Berkeley, CA, [5]Physics, University of California, Berkeley, Berkeley, CA, [6]Mathematics, University of California, Irvine, Irvine, CA

Throughout development, nuclei must be assembled following every cell division to establish a functional organelle from compact chromatin. During nuclear organogenesis, chromatin expands to establish a nucleus of a given size, which is dictated by cell size. Determining how nuclear expansion is regulated during nuclear organogenesis is particularly significant in the context of certain cancers in which scaling relationships between cell and nuclear sizes are not maintained.
In Xenopus egg extract droplets of specified volumes, we determine that nuclear surface area does not scale with cytoplasmic volume, whereas nuclear volume does. Looking to explain differential nuclear scaling relationships, we developed a simple mechano-chemical mathematical model. In simulating biological perturbations *in silico*, our model predicted crucial roles for nucleo-cytoplasmic trafficking in regulating nuclear expansion and in regulating the recruitment of a nuclear surface area factor, which we propose is LMNB1. Inhibiting nuclear export increased nuclear expansion rates and reduced the amount of LMNB1 being recruited to assembling nuclei in mammalian cells, supporting our model's predictions. Targeting nuclear export in the Drosophila syncytial embryo, we show that nuclear expansion rates are also increased in this biological context, consistent with our model. Additionally, we demonstrate a role for nuclear export in regulating transcription activation timing and dynamics following nuclear organogenesis, suggesting that regulating nuclear assembly is crucial for downstream nuclear function.

Taken together, we propose a simple model through which nuclear organogenesis is achieved in several organisms and demonstrate a role for nuclear export in regulating nuclear expansion and nuclear surface area. Subsequent work will be aimed at identifying the molecular requirements for nuclear expansion and nuclear lamina assembly.

Minisymposium 4: Multicellular Interactions: Tissue Assembly and Morphogenesis

M31

Fish scales dictate the pattern of adult skin innervation.

J.P. Rasmussen[1], N. Vo[1], A. Sagasti[1];
[1]Molecular, Cellular and Developmental Biology, University of California, Los Angeles, Los Angeles, CA

The skin is profusely innervated by axon endings of touch-sensing neurons. As animals mature from embryonic to adult stages, the skin grows and adds epithelial strata, specialized cells, and dermal appendages, like hairs, feathers and scales. How cutaneous sensory axons adapt to these dramatic changes is poorly understood. By characterizing remodeling of skin innervation in zebrafish, we discovered that sensory axons are delivered to the adult epidermis in strikingly organized nerves patterned by features in bony scales. These sensory axons associate with Schwann cells, blood vessels and osteoblasts. Osteoblasts create paths that independently guide nerves and blood vessels, during both development and regeneration. By preventing scale regeneration and examining mutants lacking scales, we found that scales dictate the pattern of axon distribution in the epidermis, suggesting a new mechanism for achieving comprehensive innervation of the adult skin. Thus, scales coordinate a metamorphosis-like transformation of the skin with sensory axon remodeling. We speculate that regularly spaced dermal appendages in other animal classes may also pattern skin innervation.

M32

Profiling the gene set controlling *C. elegans* embryonic development using an automated method that enables phenotypic comparison in 4D imaging data.

R.A. Green[1,2], R. Khaliullin[1,2], S. Ochoa Mikrut[2], Z. Zhao[1,2], S. Wang[3], J. Hendel[1,2], R. Biggs[4], A. Gerson[1], A.B. Desai[1,2], K. Oegema[1,2];
[1]Ludwig Institute for Cancer Research, La Jolla, CA, [2]Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, [3]National Institutes of Health/NIDCR, Bethesda, MD, [4]Department of Molecular Biosciences, Northwestern University, Evanston, IL

An important challenge is to systematically define the contributions of the genes that control embryogenesis to specify cell fate and position and drive the formation and morphogenesis of multi-cellular tissues. To address this challenge, we developed methods to film embryogenesis in the model metazoan *C. elegans* following individual RNAi-based knockdown of the ~2600 genes specifically required for embryonic development. Knockdowns were performed in two engineered strains expressing fluorescent markers that: (1) mark nuclei in cells in the three germ layers (endoderm, ectoderm and mesoderm) in three different colors, and (2) mark epithelial cell junctions and the surface of a subset of neurons in green and red (morphogenesis strain). Developing an automated method to analyze this 4D data and compare the complex phenotypes resulting from gene knockdowns presents a significant challenge. To tackle this problem, we manually scored ~7000 individual embryo time-lapse datasets for a pilot set of 500 genes to generate a data set that identified the spectrum of embryonic developmental defects and served as a guide for the development of custom automated algorithms. Automated algorithms were developed that: (1) monitor the increase in the number of nuclei in each of

the three germ layers over time and (2) measure the distribution of nuclei in the germ layer strain and the fluorescent signals in the morphogenesis strain over time by measuring their relative center of mass and moment of inertia around orthogonal axes bisecting the embryo. To evaluate the phenotypic similarity between RNAi conditions, the distance between phenotypes is measured in n-dimensional space, where n is the number of measured parameters. To correct for the fact that the distance between genes increases non-uniformly as phenotypes become more severe, we measure the angle between the average phenotypes for the two conditions in n-dimensional space (phenotypic angle of deviation; PAD). This automated method is highly effective in identifying groups of genes that yield similar phenotypes, suggesting that they function together in specific developmental pathways. This work represents the first fully automated high-content screen of an intact developing organism and is the most complex morphological profiling effort to date.

M33

Counter-rotational cell flows drive morphological and cell fate asymmetries in mammalian hair follicles.

M. Cetera[1], L. Leybova[1], B. Joyce[1], D. Devenport[1];
[1]Molecular Biology, Princeton University, Princeton, NJ

Organ morphogenesis is a complex process dependent on the interplay between cell fate specification, epithelial-mesenchymal interactions, and tissue polarity. The emergence of the regularly spaced, globally aligned pattern of hair follicles across the mammalian skin is a striking example of organ morphogenesis that arises through coordinate epidermal-dermal signaling and planar polarized morphogenesis controlled by the planar cell polarity (PCP) pathway. Here, using a combination of live imaging, automating cell tracking, mouse genetics, and laser ablation, we discover a novel program of PCP-dependent cell rearrangements that drive planar polarization and polarized cell fate specification of mammalian hair follicles. Upon specification, hair placode epithelial cells undergo dramatic cell rearrangements that generate a counter-rotational pattern of cell flows. These movements reposition placode cells along the anterior-posterior axis such that posterior cells converge toward the center of the placode and flow anteriorly, while anterior and lateral cells flow outward and posteriorly toward the placode rear. Counter-rotational cell flows are driven by myosin-dependent, spatially polarized cell neighbor exchanges where asymmetric PCP protein localization correlates with junction shrinkage and disassembly. Strikingly, this pattern of cell rearrangements generates not only the morphological polarization of the hair follicle, transforming the placode from vertical to anterior-directed growth, but also directs the planar polarized organization of progenitor fates upon which the future hair follicle is built. Specifically, Shh-expressing hair germ progenitors shift from a central to anterior location while an outer ring of Sox9-expressing stem cell progenitors moves posteriorly toward the placode rear. Cell fate specification also feeds into polarized morphogenesis, as counter-rotational movements are lost in the absence of radially symmetric cell fates. Further, we show that PCP-dependent cell rearrangements displace a crucial stromal signaling center - the dermal condensate - toward the anterior, where it is necessary for the maintenance of polarized hair germ and stem cell progenitor fates. Our results define the cellular mechanisms that generate planar polarity in complex multicellular structures and demonstrate how a simple pattern of local cell rearrangements directs both morphological and cell fate asymmetries.

M34

Planar polarized Rab35 functions as an oscillatory ratchet during cell intercalation.

C. Jewett[1], T. Vanderleest[2], H. Miao[1], Y. Xie[1], D. Loerke[2], J.T. Blankenship[1];
[1]Biological Sciences, University of Denver, Denver, CO, [2]Physics, University of Denver, Denver, CO

The control of cell shape is a fundamental property required for epithelial tissue architecture and function. Here, we examine the relationship between membrane trafficking and actomyosin networks that regulate cell shape. We show that a Rab protein, Rab35, is planar polarized during epithelial tissue remodeling. We use CRISPR-mediated knock-in to examine the dynamics of endogenously-tagged *Drosophila* Rab35 and show that Rab35 compartments are more numerous and dynamic at contractile interfaces of actively intercalating cells. Individual compartmental behaviors have lifetimes of ~140 seconds at AP interfaces, and correlate with periods of rapid interface contraction. When Rab35 function is disrupted, apical cell areas maintain oscillatory behaviors and interfacial contractile periods are observed. However, contractile periods are followed by reversals and interfaces re-lengthen, producing a failure in interface shortening. This “wobble” behavior is consistent with Rab35 functioning as a ratchet ensuring unidirectional movement during interface contraction. The AP patterning system is responsible for engaging ratcheting behaviors, redirecting compartment formation from apical surfaces to AP interfaces and increasing compartment lifetimes. Although tensile actomyosin forces have been conventionally thought to drive interface contraction, initiation of Rab35 compartmental behaviors does not require Myosin II function. However, when Myosin II function is disrupted, Rab35 compartments do not terminate and continue to grow into large elongated, tubular structures. These compartments are contiguous with the cell surface, and are likely hubs of endocytosis. Intriguingly, the absence of paired Rab35 compartments at individual interfaces suggests that asymmetric force generation occurs during interface contraction and provides the first evidence of interfacial monopolarity. Consistent with this, adjacent cells trigger actomyosin area contractions only on the termination of a Rab35 compartment. Finally, we demonstrate that Rab35 is involved in a common contractile cell-shaping mechanism, as cells undergoing apical constriction during mesoderm invagination also form Rab35 compartments at their shrinking surfaces, but with distinct kinetics and an absence of planar polarity. Our results suggest that the coordination of membrane trafficking and cytoskeletal forces converge on Rab35 compartmental behaviors to direct cell shaping events.

M35

Erk-dependent control of epithelial morphogenesis.

H.E. Johnson[1], S.Y. Shvartsman[2,3], J.E. Toettcher[1];
[1]Molecular Biology, Princeton University, Princeton, NJ, [2]Chemical and Biological Engineering, Princeton University, Princeton, NJ, [3]Lewis–Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ

The role of Ras/Erk signaling in cancer, growth, and differentiation has long been appreciated. In contrast, the roles of Erk signaling in cell motility, collective cell migration, and tissue-level morphogenesis are complex and remain poorly defined. Here, we set out to define the Ras/Erk pathway's role in orchestrating collective cell movement during gastrulation in the early Drosophila embryo. Using an optogenetic input to Ras, we found that Erk activity is sufficient to induce cells to adopt a contractile cell fate at nearly any illuminated location within the embryo. This tissue mimics gene expression and physical organization of the posterior midgut (PMG), a tissue normally patterned by the Torso receptor tyrosine kinase. We define the transcriptional network by which Erk programs PMG cell fate, leading to the accumulation of apical myosin and tissue contraction at gastrulation. By

systematically varying the timing and duration of Erk activity, we define the spatiotemporal features of the Erk signal that is required to program these fates. We find that the early embryo responds to the cumulative load of Erk activity delivered over a two hour window in early embryogenesis, revealing a previously unknown long-term memory of signaling that spans multiple nuclear division cycles. Our work mechanistically defines an Erk-dependent cell fate choice and establishes a model system for interrogating how signaling pathway activity can program morphogenesis in vivo.

M36

Role of Cdc42 Pathways in Regulating Group Cooperation and the Transition to Differentiated Multicellularity.

J. Chow[1], H.M. Dionne[1], A. Prabhakar[1], A. Mehrotra[1], J. Somboothum[1], M. Edgerton[2], P.J. Cullen[1];
[1]Biological Sciences, SUNY-Buffalo, Buffalo, NY, [2]Oral Biology, SUNY-Buffalo, Buffalo, NY

Cooperation among individuals provides an evolutionary benefit and is thought to underlie major transitions including the development multicellular organisms. The decision-making processes surrounding social cooperation are poorly understood. Here, we identify decision-making pathways (Cdc42-MAPK) as regulators of group cooperation. By exploring nutrient foraging in unicellular fungal microorganisms including budding yeast, we identify a response where cells assemble into multicellular aggregates. The Rho GTPase Cdc42 and one of its effector MAPK pathways were required for aggregate formation. Aggregates assembled by a mechanism where individuals from different groups interlocked to knit groups together, which resembled intercalation, a zipper-like assembly process in metazoan development. The uncanny resemblance between Cdc42 pathways in regulating group responses in microbes and their roles in tissue development in metazoans led to an idea where decision-making pathways may have been critical for the transition to multicellular life. This idea was supported by a directed selection experiment, which showed the dependency of signaling modules on differentiated multicellular organisms that were generated in the laboratory.

M37

Cytoneme-mediated cell-cell communication creates a morphogenetic gradient of FGF during branching morphogenesis of Drosophila trachea.

L. Du[1], S. Roy[1];
[1]Cell Biology and Molecular Genetics, University of Maryland, College Park, MD

Fibroblast growth factor (FGF) is a conserved paracrine signaling protein that is essential for branching morphogenesis of airway epithelium of Drosophila trachea, a genetic model for vertebrate lung development. Growing Drosophila tracheal branches are assumed to follow the chemotactic extracellular gradient of FGF secreted from dynamic signal sources surrounding the tips of the growing branches. The pattern of FGF gradient and mechanism of formation of this gradient is not known. With an endogenously expressed GFP tagged FGF, which we generated by using CRISPR/Cas based genome editing, this study characterized the patterns of FGF signal and signaling gradient during the development of a larval tracheal branch, air-sac primordium (ASP). This branch buds out to form tubular branch during the L3 larval stage in response to the FGF signal produced in the Drosophila wing imaginal disc. Our results showed that FGF forms an asymmetric gradient, only in the recipient field of cells lining the ASP cells. Similar to a classical morphogen, rather than a chemoattractant, FGF gradient induces threshold dependent activation of differential gene expression in distinct groups of tracheoblast cells of the ASP epithelium. Contact-dependent interactions of the ASP cells with the wing disc FGF-source via specialized actin-based signaling filopodia, cytonemes is necessary to create FGF gradient. Cytonemes

emanated from the ASP cells reach out and contact the disc-source to receive FGF at the point-of contact directly. Quantification of the number of cytonemes projected from the ASP cells located at various distances from the source indicated that the amount of FGF received by the ASP cells is directly proportional to the number of cytonemes that the cell produces to contact the FGF-source. FGF gradient does not form without cytoneme-mediated cell-cell contact. Therefore, understanding the mechanism of complex cellular interactions that create and interpret gradient is of fundamental importance to our understanding of tissue patterning, which will provide a conceptual framework for the development of future tissue engineering.

M38

Cell-cell fusion facilitates aneuploidy tolerance in a developing organ.

N.G. Peterson[1], K.P. Schoenfelder[2], B.M. Stormo[1], R. Lee[2], D.T. Fox[2];
[1]Cell Biology, Duke University, Durham, NC, [2]Pharmacology and Cancer Biology, Duke University, Durham, NC

Aneuploidies are chromosomal imbalances that can be generated by chromosome mis-segregation during mitosis and meiosis. Aneuploidies can cause proteotoxic stress and promote additional genome instability in diverse settings. As aneuploidies frequently occur in cancer, infertility, birth defects, and other disorders, there is much interest in how aneuploid cells survive *in vivo*.
Here, we report a novel role for cell-cell fusion in negating aneuploidy *in vivo*. We previously found the papillar cells of the *Drosophila* hindgut to undergo error-prone mitosis as part of their normal developmental program. These papillar cells are remarkably tolerant of mitotic errors such as multipolar division and DNA bridging. We thus examined how papillar cells tolerate aneuploidies generated by multipolar divisions. Using photoactivatable GFP and multi-color lineage labeling approaches, we discovered that cytoplasmic proteins diffuse between papillar cells. Using live imaging, we found that papillar cells consistently undergo cell-cell fusion to form a syncytium as part of their developmental program. Finally, by transmission electron microscopy we observe a developmentally programmed removal of lateral membranes during syncytium formation.
We then hypothesized that the cytoplasmic sharing permitted by syncytium formation allows cells to survive chromosomal imbalances. We thus conducted a genome-wide screen for aneuploid-specific lethal mutants and identified 36 aneuploid-specific lethal mutants. From these mutants, we then conducted a secondary screen for aneuploidy-defective mutants with disrupted papillar cell fusion. These screens, along with candidate mutant screening, implicated multiple regulators of membrane vesicle acidification, such as the Vacuolar ATPase subunit Vha16 and the small GTPase Rab11, in the mechanism of papillar cell fusion. Papillar cell-cell fusion is distinct from another well-studied *Drosophila* fusion model in myoblasts, as canonical myoblast fusion regulators are not required for papillar cell fusion. Instead, the involvement of vesicle acidification in cell fusion shows mechanistic similarity to multinucleation of mammalian osteoclasts and fusion of several human enveloped viruses with host cells. We thus have a powerful genetic screening platform to identify novel yet conserved cell fusion regulators. Additionally, our work reveals a new cell fusion model in the *Drosophila* hindgut and identifies a novel role for cell fusion in the tolerance of mitotic errors.

M39

A microtissue-building toolbox to study biophysical effects on cell dynamics.

W. Jung[1], K. Elawad[1], D. Maity[1], J. Kim[1], S. Park[1], S.X. Sun[1], S.H. Kang[1], Y. Chen[1];
[1]Mechanical Engineering, Johns Hopkins University, Baltimore, MD

Despite the widely acknowledged notion that biophysical properties of the microenvironment are integral in maintaining tissue homeostasis or disease development, it is still technically challenging to study cell dynamics in 3D and *in vitro*, with well-defined biophysical parameters such as geometry, stiffness, porosity, micro-organization of the extracellular matrix (ECM), and water permeability. We have developed a toolbox to grow microtissues with desired biophysical parameters described above. First, we can combine 3D printing and soft lithography to form epithelium-lined channels with varied radii at sub-100 μm scale. Secondly, we can apply micro-molding techniques and modulate polymer swelling to form capillary-like structures. Third, by using stereolithography to crosslink photo-curable, cell-laden biopolymers such as functionalized gelatin, alginate or collagen, we can fabricate micro-tissues with specific porosity, stiffness and shapes. Fourth, we have devised a self-induced rolling membrane platform, where rectangular thin elastic films are rolled cylindrically by strain mismatching between the two sides of the film, to study the effect of continuum curvature on subcellular organization in the epithelium. Fifth, by externally applying magnetic fields to cell-laden biopolymers containing paramagnetic nanoparticles, we can control the orientation of the polymers, thus the diffusion anisotropy for soluble factors within the microtissues. Using the toolbox, we are able to build microtissues and made following observations: E-cadherin and Vimentin expression in the epithelium varies depending on the curvature; cortical actin assembly is associated with tissue geometry and cell-cell adhesion; the steady-state response of the epithelium to hydrostatic pressure perturbations is independent of ECM elasticity but dependent on its porosity and its extent of crosslinking. In summary, our toolbox is versatile and can be used to construct microtissues with pre-defined biophysical parameters for cell dynamics studies at the multicellular and/or tissue levels.

M40

Cell shortening for epithelial folding via a polarity-coupled, homeostatic microtubule scaffold.

M. Takeda[1], M.M. Sami[1], Y. Wang[1];
[1]Laboratory for Epithelial Morphogenesis, RIKEN Center for Developmental Biology, Kobe, Japan

Epithelial folding is typically driven by changes in myosin contractility that acts on the cortical actin meshwork. It remains unclear, however, how tissue deformation arises when myosin levels are low and uniform, e.g. during dorsal fold formation that occurs in the gastrulating *Drosophila* embryo. Cells that initiate the formation of dorsal folds initially shrink the cell apices and subsequently reduce the apical-basal cell height. Prior to cell shape change, these cells display downregulation of Par-1, the MARK family kinase that specifies the basal-lateral membrane, and yet the mechanism that causes cell shortening is not known. We show that polarity-coupled cell shortening is controlled by a non-centrosomal microtubule network that is anchored at the apical cortex by the CAMSAP minus end binding protein Patronin. Prior to gastrulation, the apical microtubule network appears to scaffold the apical membrane domain, forming spherical dome shapes, via dynein-dependent pushing forces. The microtubule network ensures the homogeneity of apical dome size across the tissue and such size homeostasis requires the microtubule severing enzyme Katanin that counteracts Patronin in a negative feedback circuit. During dorsal fold initiation at the onset of gastrulation, Patronin undergoes basal redistribution as Par-1 becomes downregulated, thereby coupling the cortical scaffold to the basal polarity shifts. Such coupling repurposes the homeostatic microtubule network for the shrinkage of cell

apices that ultimately leads to shortening of the initiating cells, which can otherwise be blocked by overexpression of Patronin that abolishes its differential redistribution downstream of Par-1 downregulation. Our findings thus link modulation of polarity to epithelial folding via a novel, microtubule-based mechanical mechanism.

Minisymposium 5: Organelles in Metabolism and Stress Responses

M41

Lipid Droplet Biogenesis Is Spatially Coordinated at Yeast ER-Lysosome Contact Sites in Response to Nutritional Stress.

H. Hariri[1], S. Rogers[1], R. Ugrankar[1], Y. Liu[1], R. Feathers[1], M. Henne[1];
[1]Cell Biology, UT southwestern medical center, DALLAS, TX

Eukaryotic cells store lipids in cytosolic organelles known as lipid droplets (LDs), which they harvest for energy during prolonged periods of starvation. LDs bud from the endoplasmic reticulum (ER), yet how cells spatially coordinate LD production is poorly understood. Here, we demonstrate that, yeast utilize ER-lysosome/vacuole contact sites (NVJs) to spatially organize lipid metabolism and LD biogenesis. In response to stress, the NVJ physically expands, recruiting and organizing key lipid biosynthesis enzymes such as fatty acyl-CoA synthases and HMG-CoA reductases. Artificially modulating the NVJ size affects LD biogenesis. Using super-resolution microscopy, we also show that NVJ contacts are spatially organized into “core” and “peripheral” sub-regions, with NVJ tether Mdm1 regulating fatty acid metabolism at the NVJ periphery. Altogether, we propose that the NVJ is a metabolic platform that spatially orchestrates the ensemble of enzymes necessary for LD production in response to starvation. Our findings present broad implications for understanding the role for inter-organelle contacts in regulating adaptive cellular metabolism in response to stress.

M42

Measurement of caveolin-1 densities in the cell membrane for quantification of caveolar deformation after exposure to hypotonic membrane tension.

M. Tachikawa[1], N. Morone[2], S. Suetsugu[3];
[1]Theoretical Biology Laboratory, RIKEN, Wako, Japan, [2]MRC Toxicology Unit, University of Leicester, Leicester, United Kingdom, [3]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Japan

Caveolae are abundant flask-shaped invaginations of plasma membranes that buffer membrane tension through their disassembly. Few quantitative studies on the deformation of caveolae have been reported. Each caveola contains approximately 150 caveolin-1 proteins. In this study, we estimated the extent of caveolar deformation by measuring the density of caveolin-1 projected onto a two-dimensional (2D) plane. The caveolin-1 in a flattened caveola is assumed to have approximately one-quarter the density of the caveolin-1 in a flask-shaped caveola. The one-quarter-density caveolin-1 increased after increasing the tension of the plasma membrane through hypo-osmotic treatment. The one-quarter-density caveolin-1 was soluble in detergent and formed a continuous population with the caveolin-1 in the caveolae of cells under isotonic culture. The distinct, dispersed lower-density caveolin-1 was soluble in detergent and increased after the application of tension, suggesting that the hypo-osmotic tension induced the dispersion of caveolin-1 from the caveolae possibly through flattened caveolar intermediates.

M43

Membrane dynamics during cellular wound repair.

N.R. Davenport[1,2], K.J. Sonnemann[3], K.W. Eliceiri[3,4], W.M. Bement[1,3,5];
[1]Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI, [2]Thermo Fisher Scientific, Eugene, OR, [3]Laboratory of Cell and Molecular Biology, University of Wisconsin-Madison, Madison, WI, [4]Laboratory for Optical and Computational Instrumentation, University of Wisconsin-Madison, Madison, WI, [5]Department of Zoology, University of Wisconsin-Madison, Madison, WI

Cells rapidly reseal after damage, but how they do so is unknown. It has been hypothesized that resealing occurs due to formation of a patch derived from rapid fusion of intracellular compartments at the wound site. However, patching has never been directly visualized. Here we study membrane dynamics in wounded *Xenopus laevis* oocytes at high spatiotemporal resolution. Consistent with the patch hypothesis, we find that damage triggers rampant fusion of intracellular compartments, generating a barrier that limits influx of extracellular dextrans. Patch formation is accompanied by compound exocytosis, local accumulation and aggregation of vesicles, and rupture of compartments facing the external environment. Subcellular patterning is evident as annexin A1, dysferlin, diacylglycerol, active Rho, and active Cdc42 are recruited to compartments confined to different regions around the wound. We also find that a ring of elevated intracellular calcium overlaps the region where membrane dynamics are most evident and persists for several minutes. The results provide the first direct visualization of membrane patching during membrane repair, reveal novel features of the repair process, and show that a remarkable degree of spatial patterning accompanies damage-induced membrane dynamics.

M44

Deciphering the function of CLYBL, a missing human gene and a mitochondrial orphan metabolic enzyme.

H. Shen[1], V. Mootha[1];
[1]Department of Molecular Biology, Massachusetts General Hospital/Howard Hughes Medical Institute , Boston, MA

CLYBL is a "missing" human gene that is absent in approximately 2.7% of human chromosomes and whose homozygous loss is associated with reduced circulating vitamin B12 levels. CLYBL encodes an orphan mitochondrial matrix enzyme whose enzymatic activity, pathway assignment, and link to B12 are unknown. Here, we demonstrate that CLYBL loss leads to a cell autonomous defect in the mitochondrial B12 metabolism. By combining enzymology, structural biology and activity-based metabolite profiling we discover that CLYBL operates as a citramalyl-CoA lyase. Cells lacking CLYBL accumulate citramalyl-CoA, an intermediate in metabolism of the C5-dicarboxylates, including itaconate, a recently identified human antimicrobial metabolite and immunomodulator. We find that itaconyl-CoA is a substrate analogue, cofactor-inactivating inhibitor of the mitochondrial B12-dependent methylmalonyl-CoA mutase (MUT). Our work de-orphans the activity of CLYBL, explains why its loss leads to B12 deficiency, and reveals a novel mechanism of B12 poisoning by the CoA ester of itaconate, an immunomodulatory metabolite.

M45

The Unfolded Protein Response Maintains Lipid Homeostasis by Selective Autophagy during Lipid Perturbation-Induced ER Stress.

J. Koh[1], L. Wang[2], C. Beaudoin-Chabot[1], G. Thibault[1,2];
[1]School of Biological Sciences, Nanyang Technological University, Singapore, Singapore, [2]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore

Metabolic disorders such as obesity and nonalcoholic fatty liver disease (NAFLD) are emerging disorders that affect the global population. One facet of the disorders is attributed to the disturbance of membrane lipid homeostasis. Perturbation of endoplasmic reticulum (ER) homeostasis through changes in membrane phospholipid composition results in activation of the unfolded protein response (UPR) and causes dramatic translational and transcriptional changes in cell. To restore cellular homeostasis, the three highly conserved UPR transducers ATF6, IRE1, and PERK mediate cellular processes upon ER stress. The roles of the UPR in proteostatic stress caused by the accumulation of misfolded protein is well understood but lipid perturbation-induced UPR remains elusive. We found that genetically attenuated PC synthesis in *C. elegans* causes lipid droplets accumulation if not for the intervention of the UPR program. Transcriptional profiling of lipid perturbation-induced ER stress animals shows a unique subset of genes modulated in an UPR-dependent manner that are unaffected by proteostatic stress. Among these, we identified IRE1-modulated autophagy genes that trigger liberation of free fatty acids from excess lipid droplets suggesting a stress release mechanism by which free fatty acids are rechanneling to restore lipid homeostasis. Considering the important role of lipid homeostasis and how its impairment contributes to the pathologies in metabolic diseases, our data uncovers the indispensable role of a fully functional UPR program in regulating lipid homeostasis in the face of chronic ER stress.

M46

TFEB regulates lysosomal positioning by modulating TMEM55B expression and JIP4 recruitment to lysosomes.

R.A. Willett[1], J.A. Martina[1], G. Hammond[2], J.P. Zewe[2], R. Puertollano[1];
[1]NHLBI, National Institute of Health, Bethesda, MD, [2]Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA

Lysosomal distribution within cells is directly linked to the role of lysosomes in many cellular functions, including autophagosome degradation, cholesterol homeostasis, antigen presentation, and cell invasion. Moreover, alterations in lysosomal positioning contribute to different human pathologies, such as cancer, neurodegeneration and lysosomal storage diseases. Here we report the identification of a novel mechanism of lysosomal trafficking regulation. We found that the lysosomal transmembrane protein TMEM55B promotes recruitment of JIP4 to the lysosomal surface, thus inducing dynein-dependent transport of lysosomes toward the microtubules minus-end. Accordingly, over-expression of TMEM55B causes lysosomes to collapse into the cell center, whereas depletion of either TMEM55B or JIP4 results in dispersion toward the cell periphery. TMEM55B levels are transcriptionally upregulated following TFEB and TFE3 activation by starvation or cholesterol-induced lysosomal stress. TMEM55B or JIP-4 depletion abolishes starvation-induced retrograde transport of lysosomes and prevents fusion between autophagosomes and lysosomes. JIP4-mediated retrograde transport is also observed upon induction of lysosomal stress by sodium arsenite or curcumin though a mechanism that requires MAPK activation. Overall, our data reveal a novel role of TMEM55B linking lysosomes to microtubule motors and suggest that the TFEB/TMEM55B/JIP4 pathway plays a critical role in cellular adaptation to stress by coordinating control of lysosomal movement in response to variations in nutrient and cholesterol levels.

M47

Mitochondrial subpopulations exhibit differential dynamic responses to support increased energy demand during exocytosis.

N. Porat-Shliom[1], L.N. Malec[1], O. Harding[1], R. Weigert[1];
[1]Laboratory of Cellular and Molecular Biology, National Institute of Health, NCI, Bethesda, MD

Mitochondria are highly dynamic organelles that undergo fission, fusion, and translocation in support of cellular metabolic needs. Although the mechanisms regulating these processes have been extensively studied in cultured cells, little is known about mitochondrial dynamics and their overall distribution in cells within tissues. We applied Intravital Microscopy (IVM) to study the spatio-temporal regulation of mitochondrial dynamics in the salivary glands of anesthetized mice. We found that 60-70% of mitochondria are positioned within 2 microns of the basolateral plasma membrane while 30-40% are scattered in the cytosol. A similar distribution pattern is conserved in other exocrine glands including the pancreas, lacrimal and parotid glands. Interestingly, by using a transgenic mouse expressing a mitochondrial-targeted photoswitchable probe (i.e. Dendra2) we found that under basal conditions: 1) basolateral mitochondria are static, whereas central mitochondria exhibit a microtubule-dependent motility; 2) both populations exhibit a low rate of fusion and fission; and 3) both populations do not mix over the course of 4 hours of observation. Since the differential distribution, dynamic properties, and segregation of these two subpopulations suggest distinct functions, we investigated their behavior under conditions of increased energy demand namely, during stimulated protein or water secretion, which are regulated by the b-adrenergic and the muscarinic receptors, respectively. We found that central mitochondria motility significantly increases during protein exocytosis, but not water secretion. In contrast to non-stimulated conditions, this increase in motility correlated with fusion and elongation of central mitochondria that consequently lead to mixing with the basolateral mitochondria. In support of the increased fusion, b-adrenergic stimulation triggered Protein Kinase A (PKA)-dependent phosphorylation of the fission protein Drp1 on the residue S637, resulting in inhibition of its fission activity. Finally, under these conditions the central mitochondria increased the frequency of interactions with lipid droplets. Current experiments explore the possibility of free fatty acids transfer, as a mean to provide substrates for ATP production. Taken together, our results define for the first time two distinct mitochondria populations in the secretory epithelia that undergo acute dynamic remodeling in response to increase in tissue energy demand.

M48

Regulated recruitment of C9orf72 to lysosomes supports diverse signaling and degradative functions.

J. Amick[1], A. Tharkeshwar[1], C. Amaya[1], S.M. Ferguson[1];
[1]Cell Biology, Yale University, New Haven, CT

Expansion of a hexanucleotide repeat in an intron of the C9orf72 gene causes amyotrophic lateral sclerosis and frontotemporal dementia (ALS-FTD), two related neurodegenerative diseases. These insights from human genetics have stimulated significant interest in understanding C9orf72 functions. However, beyond bioinformatics predictions that have suggested structural similarity to a subset of DENN domain containing proteins that include folliculin (FLCN), the Birt-Hogg-Dubé syndrome tumor suppressor, little was known until recently about the normal cellular functions of the C9orf72 protein. To address this problem, we used genome editing strategies to investigate C9orf72 interactions, subcellular localization and knockout (KO) phenotypes. We found that C9orf72 robustly interacts with SMCR8 and WDR41 (proteins of previously unknown function). Epitope tag insertion into the endogenous human

C9orf72 gene locus, allowed us to establish that C9orf72 dynamically binds to the cytoplasmic surface of lysosomes and that such localization is negatively regulated by amino acid availability. Analysis of C9orf72 KO cell lines revealed diverse phenotypes that are consistent with a function for C9orf72 at lysosomes. These include abnormally swollen lysosomes in the absence of C9orf72, impaired responses of mTORC1 signaling to changes in amino acid availability (a lysosome-dependent process) and defects in lysosome-mediated recycling of both endocytic and autophagic cargoes. Through the use of additional CRISPR-Cas9 knockin and knockout strategies targeted towards SMCR8 and WDR41, we have gained new insights into specific roles for individual components of the C9orf72-SMCR8-WDR41 complex in regulating complex assembly, recruitment to lysosomes and downstream effects on mTORC1 signaling. Collectively, these results identify strong, constitutive, physical interactions between C9orf72, SMCR8 and WDR41 and support an important lysosomal site-of-action for this protein complex. Such insights are relevant for both interpreting the contributions of C9orf72 haploinsufficiency to neurodegenerative disease as well as understanding the fundamental mechanisms that match lysosome function to ongoing changes in cellular demand.

M49

3D ultrastructural analysis of the progressive restructuring of the endoplasmic reticulum by a coronavirus provides insight into its subversion of the ERAD tuning pathway.

E.M. Mihelc[1], S.A. Tinney[1], S.C. Baker[2], J.K. Lanman[1];
[1]Biological Sciences, Purdue University, West Lafayette, IN, [2]Microbiology and Immunology, Loyola University Chicago Stritch School of Medicine, Maywood, IL

Viruses commonly modify organelle membranes and subvert cellular pathways for the various stages of their lifecycles. Coronaviruses extensively utilize the endoplasmic reticulum (ER), inducing drastic architectural changes to the ER through the course of infection. ER membrane is used for both formation of double-membrane vesicles (DMVs) associated with replication, as well as for assembly and envelopment of new virions. Early in infection, the ER is utilized to form DMVs, which have been shown to contain markers for the ER-associated degradation (ERAD) tuning pathway. ERAD tuning vesicles, termed EDEMosomes, bud from the ER and are rapidly trafficked to lysosomes for degradation. Unlike EDEMosomes, DMVs are bounded by two lipid bilayers, are believed to remain connected to the ER via a membranous network, and accumulate within the cell over the course of infection. In this work, we sought to visualize coronavirus-induced restructuring of the ER as it progresses through the infection. Specifically, we investigated how this class of EDEMosome-type vesicles form from the ER, and whether they are able to evade the degradative fate of an EDEMosome. In order to observe morphological changes both at high resolution and over a large cellular volume, we used large-volume EM tomography to image the ER at different timepoints post-infection and to perform detailed 3D analysis of the structures within the volumes. In one volume (dimensions ~8 x 8 x 2 µm), we observed nearly 700 DMVs in the cytoplasmic space. Analysis of the images reveals that DMV inner vesicles form first on or within the ER, then obtain an outer bilayer by budding out of the ER, and eventually separate from the ER. At late timepoints in infection, the ER's role in infection shifts as it accommodates the assembly and budding of new virus particles into its lumen. Concurrent to this architectural restructuring, we observed that the number of DMVs drastically decreases, and DMVs can be visualized inside lysosomal compartments. Chloroquine treatment prevented the decrease in DMVs, indicating that DMVs indeed are degraded in lysosomes and thus follow the ERAD tuning pathway to its end. Live cell fluorescence imaging experiments are underway to further characterize the dynamics of coronavirus interaction with this pathway. In conclusion, 3D ultrastructural analysis of coronavirus-infected cells allowed us to

observe the morpho-functional flux of the ER by an obligate pathogen and to move toward a functional understanding of the involvement of cellular pathways in infection.

M50

The peroxisomal AAA-ATPase Pex1/Pex6 unfolds substrates by processive threading.

B.M. Gardner[1], D.T. Castanzo[1], S. Chowdhury[2], G. Stjepanovic[1,3], M.S. Stefely[1], J.H. Hurley[1,3,4], G.C. Lander[2], A. Martin[1,4,5];
[1]Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, [2]Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA, [3]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA, [4]California Institute for Quantitative Biosciences, University of California, Berkeley, Berkeley, CA, [5]University of California, Berkeley, Howard Hughes Medical Institute, Berkeley, CA

The AAA-ATPases Pex1 and Pex6 are essential for peroxisome stability and peroxisomal matrix-protein import in eukaryotes. While mutations in Pex1 and Pex6 cause the majority of peroxisome biogenesis disorders in humans, their exact function is unclear. Together, Pex1 and Pex6 form a heterohexameric motor that is recruited to the peroxisomal membrane by the tail-anchored protein Pex15. At the peroxisome, Pex1/Pex6 is thought to extract the matrix protein receptor Pex5 for repeated rounds of import. Here we determined that *in vitro* Pex1/Pex6 from *S. cerevisiae* is a protein translocase that unfolds Pex15 in a pore-loop and ATP-hydrolysis dependent manner. Our structural studies of Pex15 in isolation and in complex with Pex1/Pex6 illustrate that Pex15 binds the N-terminal domains of Pex6 and presents its C-terminal disordered region for engagement by the pore loops of the AAA motor, which then processively threads Pex15 through the central pore. *In vitro*, Pex5, the expected substrate of Pex1/Pex6, was not engaged by the motor. Instead, we found that Pex15 binds directly to the cargo receptor Pex5 and thereby links Pex1/Pex6 to other components of the peroxisomal import machinery. Our results thus support a role of Pex1/Pex6 in mechanical unfolding of peroxin proteins and their extraction from the peroxisomal membrane during matrix-protein import, but raise questions as to the true Pex1/Pex6 substrate *in vivo*.

Minisymposium 6: Regulation of Cell Size, Mitosis and Meiosis

M51

Prevalence and Regulation of Cell-Size-Independent Gene Expression.

D. Chandler-Brown[1], K.M. Schmoller[1], J.M. Skotheim[1];
[1]Biology, Stanford University, Stanford, CA

Although cells of a given type span a large range of sizes, most proteins and RNA are maintained at constant, size independent, concentrations. This ensures that biochemical reactions proceed independently of cell size. However, our recent work in budding yeast has shown that a key cell cycle regulator, the G1/S transcriptional inhibitor Whi5, is synthesized independent of cell size. The dilution of Whi5 in larger cells links cell size to G1/S cell cycle progression. This raises two fundamental questions: (1) Are there additional genes whose synthesis is decoupled from cell volume? (2) If most gene expression is proportional to cell size, what molecular mechanism promotes cell-size-independent gene expression? To address these questions, we analyzed flow cytometry data collected using the yeast GFP-fusion library. We identified approximately 200 genes whose expression is not proportional to cell volume. We validated a fraction of these candidates using quantitative live-cell microscopy and showed that protein accumulation over time does not depend directly on cell size. Gene ontology analysis

revealed that non-scaling genes are enriched for genes with roles in DNA-templated processes and membrane transport. This suggests that cells employ differential protein synthesis to coordinate protein requirements with the scaling properties of cellular structures because membranes are expected to scale as volume$^{2/3}$ and DNA content is independent of size. To understand the mechanisms that underlie size-independent gene expression, we used transcriptional reporters of non-scaling genes, including *WHI5*, and determined that cell-size-independent regulation of some genes is due non-scaling transcription rates. Targeted analysis of the WHI5 promoter showed that the region between 1000 bases and 550 bases upstream of the translation start site are required for cell-size-independent gene expression. This suggests there is a molecular element within this region required for non-scaling gene expression. Finally, we identify a partitioning mechanism ensuring proteins are partitioned in dividing cells in amounts that are independent of the asymmetric sizes of the mother and daughter cells. Tight chromatin association ensures that proteins are segregated in equal amounts despite asymmetric division. Consistent with this model, while Whi5 is normally partitioned in equal amounts, a Whi5 protein that lacks the domain required for association with transcription factors is partitioned in proportion to the mother-daughter cell size ratio. Taken together, our work demonstrates a functional role for differential size-dependency of protein synthesis and gives insights into the underlying molecular mechanism(s).

M52

Supergrowth: Effect of osmotic oscillations on the rate of cell growth and the regulation of the proteome.

B. Knapp[1], E.R. Rojas[2], K.C. Huang[2], F. Chang[1];
[1]Cell and Tissue Biology, UCSF, San Francisco, CA, [2]Bioengineering, Stanford, Stanford, CA

Cell growth is a complex process in which the expansion of cellular dimensions are globally coordinated with biosynthesis of cellular components. While much is known about how certain intracellular structures such as chromosomes are replicated, less is known about how the proteome itself is duplicated in the cell cycle. Most cell types, including the fission yeast S. pombe, exhibit exponential growth, in which the growth rate of individual cells scales with cell size, suggesting that the amount of some cellular component(s) dictates the rate of growth. As the majority of proteins are maintained at fixed concentrations during the cell cycle, protein synthesis rates may be coordinated with the rate of cellular expansion. The mechanisms responsible for exponential growth and the coordination of global protein synthesis with growth rate are not well understood. Here, we identify a condition in fission yeast in which the rate of protein synthesis is globally de-coupled from growth, causing cells to grow many times faster than normal (supergrowth). In studying the functions of turgor pressure, we subjected cells to osmotic oscillations and then released them back into normal media. Cells grew slowly under these oscillations, but upon release, grew 2-3-fold faster than control cells for a few generations; we term this unprecedented rapid growth “supergrowth.” Cells maintained normal cell morphology and size control, and these effects were independent of the osmotic response pathway. The concentrations of a large majority of representative proteins were found to increase 10-100% higher than normal during typical oscillations (depending on protein and condition), and then were depleted back to normal concentrations during the supergrowth period. Cell growth and protein synthesis were thus uncoupled in these cells, as during oscillations, proteins were synthesized at normal rates even though cell elongation was stalled. We provide evidence that this abnormal accumulation of proteins may drive supergrowth, as blocking this accumulation with cycloheximide prevented subsequent supergrowth. Our current model is that growth rates are set by an effective amount of “growth materials,” which accumulate to abnormal levels during oscillations and thus provide extra fuel for growth. During

oscillations, cells may lack a negative feedback mechanism for controlling protein homeostasis, as they may not be able to properly sense growth or size. These studies provide one of the first examples of decoupling protein synthesis from cell growth/size control, and begin to give insights into this coordination and the mechanisms that control growth rates and the global regulation of the proteome.

M53

Mechanistic Basis of Spindle Size Control and Scaling.

R. Farhadifar[1,2], G. Fabig[3], M. Rockman[4], M.J. Shelley[1], D.J. Needleman[2];
[1]Center for Computational Biology, Flatiron Institute, New York, NY, [2]Molecular and Cell Biology, Harvard University, Cambridge, MA, [3]Experimental Centre, Technische Universität Dresden, Dresden, Germany, [4]Biology, New York University, New York, NY

The size and morphology of intracellular structures such as the nucleus, Golgi apparatus, and mitotic spindle dramatically vary between different species and different cell types, yet the mechanisms that regulate the size of these structures are poorly understood. The size of many intracellular structures scales with cell size, i.e. larger cells tend to have larger nucleus and spindles. Many models have been proposed to explain such scaling behavior, but it has been very challenging to rigorously test their validity. We have combined the statistical framework of quantitative genetics, with cell biology and biophysics to develop a general methodology to quantitatively test models of size control and scaling. We have applied this approach to study the mechanism of size control and scaling of the mitotic spindle in *C. elegans*.
We used a high-throughput microscopy platform to study the first mitotic division in ~200 genotyped recombinant inbred *C. elegans* lines and observed quantitative variations for all measured aspects of the cell, pronuclei, the spindle, and centrosomes. Analysis of these variations across lines allows us to reject a number of possible models of spindle size regulation and scaling, and to develop a novel model based on the interplay between microtubules and the cortex. Associating the genotype information of the recombinant inbred lines with cell biological variation provides additional tests of our model and allows us to map genetic loci for these processes, which we are validating using CRISPR. We are using laser ablation, other biophysical approaches, and additional genetic perturbations to further explore our proposed model. The combination of quantitative genetics with cell biology and biophysics provides a systematic and rigorous method to study mechanisms that contribute to size regulation of intracellular structure.

M54

Integrated cytoplasmic reorganization during human iPS cell mitosis.

S.M. Rafelski[1], .. Allen Inst. for Cell Science[1];
[1]Allen Institute, for Cell Science, Seattle, WA

The Allen Institute for Cell Science is developing high-replicate, dynamic image data on cell organization and activities using endogenous fluorescently tagged human induced pluripotent stem cells (hiPSCs). To date, we have generated a collection of genome-edited hiPS cell lines (WTC line) with ~15 key GFP-tagged structures (www.allencell.org and other Institute poster abstracts). These include major organelles, like the nucleus, mitochondria, ER, and Golgi, as well as other key organizational structures, such as microtubules, centrioles, actin bundles, and cell-cell junctions. One of our goals is to quantify the relative location and dynamics of major cellular structures and activities as the stem cells traverse the cell cycle. As an internal positional reference, we label the cells with fluorescent dyes that localize to the plasma membrane (CellMask Deep Red) and nucleus (Hoechst DNA staining) as a proxy. We image the

cells in 100s-1000s replicates using 3D live-cell spinning disk microscopy. The DNA dye also allows us to identify 8 stages of the cell cycle, using DNA morphology and texture attributes. The stages are: two stages of prophase, two stages of prometaphase, metaphase, anaphase, telophase/cytokinesis, and interphase. We manually annotated a dataset of 50-100 cells in each mitotic stage. These cells were used as expert input to train a supervised machine learning-based classifier that enabled automated cell cycle staging, performant at >90% accuracy. We used a parallel approach to integrate and analyze intracellular localization of cellular organelles and structures over the 8 stages of the cell cycle, permitting us to identify and correlate localization patterns in time for all of the structures studied. For example, we observed very similar localization for both the ER (sec61beta) and the nuclear envelope (laminB1), contrasting an anti-localization pattern for the mitotic spindle (alpha-tubulin) and mitochondria (tom20p) throughout mitosis. We validate our single timepoint-derived observations with live-cell timelapse imaging, which permits us to directly observe transitions between intracellular structure localization patterns. As part of our workflow, we are performing 3D image processing and a statistical analysis of the variation of organelle position and morphology during mitosis.

M55

Cycling clouds of actin filaments regulate mitochondria size and distribution in mitotic cells.

A.S. Moore[1], J.J. Nirschl[1], C.L. Simpson[1,2], E.L. Holzbaur[1];
[1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA,
[2]Department of Dermatology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

In mammalian cells, mitochondria form interconnected networks that fragment prior to cell division to allow for segregation of mitochondrial mass between daughter cells. Equitable partitioning of mitochondria is critically important to prevent bottleneck effects and the potential expansion of deleterious mitochondrial DNA mutations. To date, the precise mechanism regulating mitochondria inheritance remains controversial. Previous work from our lab identified a dynamic, rotating cloud of actin filaments propagating through interphase mitochondrial networks. We observed that actin clouds move in a continuous manner, cycling onto and off of subpopulations of mitochondria, transiently inducing fission. Here, using HeLa cells and primary epidermal keratinocytes, we find that mitochondrial actin cycling persists through all stages of the cell cycle, but is markedly upregulated at the G2/M transition. Specifically, we observe that mitochondrial actin clouds cycle nearly three times faster in mitotic cells (interphase: 1049±201 sec/cycle; m-phase: 384±87 sec/cycle). Consistent with this observation, we find that mitotic actin clouds are 2.86 times larger, 183% faster, and display a stronger bias for persistent, unidirectional motility as compared to interphase clouds. Enhanced cycling may be dependent on CDK1, as inhibition of the mitotic kinase with either ro3306 (20µM, 1h) or CGP74514a (10µM, 1h) robustly blocks actin cloud formation. We observe that rapidly transiting mitotic actin clouds have profound effects on mitochondrial network organization. In prometaphase, actin clouds promote and maintain mitochondrial fragmentation. In metaphase, cycling actin filaments induce microtubule-independent motility of mitochondria which ensures homogenous distribution of mitochondria in both xy and z planes. Finally, in telophase, we observe selective actin assembly on mitochondria localized to the cleavage furrow. Based on these observations, we hypothesize that mitochondrial actin clouds function as a mechanism to fragment and symmetrically partition mitochondria during cell division.

M56

Exploring the dynamic regulation underlying synchronous sister chromatid separation at anaphase onset.

J. Kamenz[1,2,3], T. Mihaljev[4], T. Boluarte[1,3], S. Legewie[4], S. Hauf[1,3,5];
[1]Department of Biological Sciences, Virginia Tech, Blacksburg, VA, [2]Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA, [3]Biocomplexity Institute, Virginia Tech, Blacksburg, VA, [4]Institute of Molecular Biology (IMB), Mainz, Germany, [5]Center for Soft Matter and Biological Physics, Virginia Tech, Blacksburg, VA

The regulation of major cell cycle transitions typically involves positive feedback, which ensures that the transition occurs quickly and is irreversible. At the onset of anaphase, chromosomes split abruptly and synchronously, which is triggered by the protease separase. Whether this synchrony necessitates positive feedback regulation is unclear. We have systematically analysed the dynamics of sister chromatid separation in fission yeast at the single-cell level. As expected, all chromosomes split during a narrow time window. Separase activity and the degradation kinetics of its inhibitor, securin, are the main determinants of this synchrony. Combining our results with computational modelling, we suggest that synchrony is established in the absence of feedback regulation. Simple assumptions about securin-separase association and securin degradation are sufficient to explain rapid separase release and abrupt chromosome splitting. Hence, sister chromatid splitting, being already irreversible by nature, may be one of the few major cell cycle transitions that can operate without positive feedback.

M57

Microtubule destabilizing activity of selfish centromeres drives non-Mendelian segregation.

T. Akera[1], E. Trimm[1], M.A. Lampson[1];
[1]Department of Biology, University of Pennsylvania, Philadelphia, PA

One of the very few “laws” in biology is Mendel’s Law of Segregation, which states that each allele has an equal chance to transmit to the gametes. However, it is increasingly clear that this law can be violated by selfish genetic elements, which manipulate the production of gametes to increase their own rate of transmission. This genetic cheating in meiosis, meiotic drive, potentially occurs whenever haploid gametes are produced from diploid parents. In female meiosis, selfish elements drive by preferentially segregating to the egg. Our previous results in a mouse model show that selfish centromeres have higher levels of inner and outer kinetochore proteins compared to centromeres of the homologous chromosomes, and that drive of these selfish centromeres depends on asymmetric microtubule (MT) tyrosination within the spindle. These findings raise the question of how selfish centromeres interact with the asymmetric spindle to bias their orientation towards the egg side. Here we show that selfish centromeres preferentially destabilize interactions with MTs on the cortical side of the spindle, thus promoting re-orientation from the cortical side to the egg side. Several lines of evidence support this conclusion. First, we observe re-orientation events initiating with selfish centromeres losing MT attachments. Second, selfish centromeres have higher levels of MT destabilizing factors, including the depolymerizing kinesin-13 MCAK and Aurora B kinase, compared to their homologous partners, suggesting that they drive the re-orientation process. Third, tyrosinated MTs enriched on the cortical side of the spindle are less stable, which gives directionality to the re-orientation. Fourth, selfish centromeres frequently lose cold-stable MT attachments only when facing the cortical side. Together, these findings provide the first mechanistic insights into how selfish elements exploit meiotic spindle asymmetry to bias their transmission.

M58

A compartmentalized, self-extinguishing signaling network mediates crossover control and faithful chromosome segregation in meiosis.

L. Zhang[1,2,3,4], S. Köhler[1,2,3,4], R. Rillo-Bohn[1,2,3,4], A.F. Dernburg[1,2,3,4];
[1]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, [2]Howard Hughes Medical Institute, Chevy Chase, MD, [3]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA, [4]California Institute for Quantitative Biosciences, Berkeley, CA

During meiosis, homologous chromosomes pair, synapse, and undergo crossover recombination. This process leads to the formation of stable interhomolog connections known as chiasmata, which enable homologs to bi-orient and segregate during the first meiotic division. Defects in meiotic chromosome segregation lead to aneuploidy, the primary cause of spontaneous abortion and congenital birth defects in humans. To ensure faithful meiotic chromosome segregation, meiotic crossovers must be tightly regulated. Each chromosome pair typically undergoes at least one crossover event (crossover assurance) but these exchanges are also strictly limited in number and widely spaced along chromosomes (crossover interference). This has implied the existence of chromosome-wide signals that regulate crossovers, but their molecular basis remains mysterious. Recent work from our group suggests that the synaptonemal complex (SC) is a liquid-like compartment that assembles between homologous chromosome axes through phase separation (Rog et al., 2017). The structural proteins of the SC are highly mobile within assembled complexes, and polycomplexes of SC proteins behave as droplets with liquid-like properties in vivo. Additionally, both SCs and polycomplexes are rapidly and reversibly dissolved by aliphatic alcohols such as hexanediols, indicating that their structural integrity depends on weak hydrophobic interactions. These liquid crystalline properties suggested that the SC might act as a conduit for a crossover interference signal. We now characterize a family of four related RING finger proteins in C. elegans. These proteins are recruited to the synaptonemal complex between paired homologs, where they act as two heterodimeric complexes, likely as E3 ubiquitin ligases. Our genetic and cytological analysis reveals that they act with additional components to create a self-extinguishing circuit that controls crossover designation and maturation. Our finding suggests a signal flows through the liquid crystalline channel between the surface of two homologous chromosomes to mediate crossover control and to ensure faithful meiotic chromosome segregation. In addition, we find that these proteins act upstream of other regulators to direct chromosome remodeling in response to crossover formation, which enables stepwise cohesion loss and chromosome segregation. Work in diverse phyla indicates that related mechanisms mediate crossover control across eukaryotes.

M59

Asymmetric centrosomes clustering defines the evolution of newly formed tetraploid cell populations.

N. Baudoin[1], J.M. Nicholson[1], O. Sharakhova[1], K. Soto[1], M. Giam Xue Lin[2], G.I. Rancati[2], D. Cimini[1];
[1]Department of Biological Sciences and Biocomplexity Institute, Virginia Tech, Blacksburg, VA, [2]Agency for Science, Technology, and Research (ASTAR), Institute of Medical Biology, Singapore, Singapore

Tetraploid cells are common features of pre-cancerous lesions and are also seen in tumors of various stages. It is often speculated that a second common and persistent feature of cancers - supernumerary centrosomes – can arise through tetraploidization. This proposal is logical, because several pathways by which tetraploidy can arise intrinsically lead to the generation of cells with doubled centrosome number. However, this link between tetraploidization and supernumerary centrosomes, specifically in

terms of the fate of supernumerary centrosomes following tetraploidization, has never been investigated. Here, we study the early fate of supernumerary centrosomes in two cultured mammalian cell lines (DLD-1 and $p53^{-/-}$ RPE-1 cells) treated with cytochalasin B to induce tetraploidy via cytokinesis failure. Because of the link between supernumerary centrosomes and chromosomal instability (CIN, another common feature of cancer cells), we further investigated the concurrent karyotype evolution that takes place in the early time window (~2 weeks) after cytokinesis failure. We found that cells with supernumerary centrosomes rapidly disappeared from the population, leaving a population of tetraploid cells containing mostly normal centrosome number. Over the course of the same time period, highly aneuploid cells generated from multipolar divisions are eliminated from the population while the near-tetraploid population acquires a significant amount of karyotypic heterogeneity in the form of moderate levels of numerical - but not structural - chromosome aberrations. To investigate the mechanism of supernumerary centrosome reduction, we used a combination of fixed-cell analysis, long-term live-cell imaging, and mathematical modeling. We found that newly formed tetraploid cells that assemble bipolar spindles can do so by randomly clustering their centrosomes symmetrically or asymmetrically. Asymmetric clustering (3:1) generates one G1 daughter cell with a single centrosome. Such a cell is likely to keep dividing and generate viable and stably proliferating progeny. Conversely, its sister cell, as well as daughters resulting from symmetric centrosome clustering (2:2), all inherit supernumerary centrosomes, which make them prone to multipolar cell divisions that generate non-viable daughter cells. Taken together, our data indicate that the emergence and persistence of tetraploid karyotypes can occur independent of the persistence of supernumerary centrosomes. Furthermore, our data suggest that the presence of additional chromosome sets may play a more important role in early tumor evolution than the presence of supernumerary centrosomes themselves, and that supernumerary centrosomes may re-emerge at a later time during tumor evolution.

M60

Serine-dependent sphingolipid synthesis is a metabolic liability of aneuploid cells.

E.M. Torres[1], S. Hwang[1], T.H. Gustafsson[1], C. O'Sullivan[1], C. Klose[2], P. Cavaliere[3], A. Schevchenko[2], R.C. Dickson[4], N. Dephoure[3];
[1]Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, Worcester, MA, [2]Molecular Cell Biology and Genetics, Max Planck Institute , Dresden, Germany, [3]Department of Biochemistry, Weill Cornell Medical College, New York, NY, [4]Department of Molecular and Cellular Biochemistry , University of Kentucky College of Medicine, Lexington, KY

Aneuploidy is a hallmark of cancer. Importantly, the frequency and degree of aneuploidy correlate with tumor aggressiveness and poor prognosis. Therefore, studying the cellular processes affected by aneuploidy can improve our understanding of its role in tumor biology. Our previous studies in aneuploid yeast strains revealed that slow proliferation, increased genomic instability and altered metabolism are characteristics shared by aneuploid cells independent of their chromosomal abnormalities. Subsequent studies showed that the aneuploidy-associated phenotypes first discovered in yeast are also present in mouse and human aneuploid cells. This suggests that the cellular responses to aneuploidy are conserved from yeast to humans.
Identifying the cellular processes affected by aneuploidy can reveal how specific genomic alterations help aneuploid cancer cells survive, adapt and thrive despite harboring an abnormal genome. Due to the negative effects of aneuploidy on cellular physiology and increased genomic instability, selective pressure drives the acquisition of genomic alterations that improve cellular fitness of aneuploid cells. Here, we identified several mutations in genes that regulate sphingolipid synthesis to affect the fitness of aneuploid cells, suggesting that these lipid molecules play an important role in the physiological responses to aneuploidy. Sphingolipids are synthesized from serine and palmitoyl-CoA. Both long-chain

bases (LCBs) and ceramides are sphingolipid intermediates that function as signaling molecules and are rapidly induced upon stress. While ceramides mainly serve to slow cell cycle progression, LCBs activate transcriptional responses and signaling pathways associated with cell wall integrity and survival. Here, we used genetic and biochemical approaches to identify specific sphingolipid molecules that modulate the fitness of aneuploid cells. Transcriptome and proteome analyses of the disomes harboring a mutation that increases LCBs and improves fitness indicate that these molecules regulate membrane protein composition, RNA biosynthesis, and several metabolic pathways that rely on mitochondrial function. Our results provide a better understanding of the physiological role of sphingolipids in controlling the fitness of aneuploid cells. Determining the mechanisms that control the fitness of aneuploid cells can be exploited to target aneuploid cancer cells and to ameliorate the deleterious effects of aneuploidy in Down syndrome or neurodegenerative diseases.

Education Minisymposium: Evidence-Based Education

M61

Developing Future Biologists: creating and assessing a portable short course to engage underrepresented students in developmental biology.

J.M. Pinskey[1], E.A. Dulka[2], S. Barolo[1];
[1]Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, [2]Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI

Many barriers prevent underrepresented students from choosing a career in science. In order for undergraduate students to decide to pursue graduate education, they must first be exposed to a particular subject, develop an interest in that topic, and accumulate related coursework and research experience. Unfortunately, many students from underrepresented groups lack exposure to the field of developmental biology due to limited course offerings and finite resources at smaller institutions. To address this disparity, graduate students at the University of Michigan created a portable short course focusing on developmental biology, titled “Developing Future Biologists” (DFB). This week-long educational initiative provides students with hands-on laboratory sessions, interactive lectures, and professional development workshops, aiming to teach students the core concepts of developmental biology and increase awareness of scientific career options. To evaluate course effectiveness, we developed a pre-post assessment, incorporating key concepts in developmental biology outlined in the BioCore Guide (Brownell et. al., 2011). Here we present results from two separate iterations of the course in Ponce, Puerto Rico (2016) and Ann Arbor, Michigan (2017). We show that student understanding of core developmental biology concepts and perceived experience in developmental biology significantly increased as a result of DFB, despite the abbreviated nature of the course. Pre-post scores improved in all five of our main content areas, including early embryonic development, gene expression, cell signaling, organogenesis, and developmental disease. Furthermore, item analysis of our assessment tool suggested that items on the post-tests were less difficult for students than pre-test items, while simultaneously being better at discriminating between high- and low-performing students in the course. The DFB portable short course model and assessment strategy could easily be adapted to any number of topics and locations in order to connect undergraduate students with opportunities for advanced study and help lower the barriers that exist for underrepresented students in science, technology, engineering, and mathematics.

M62

Steel City Blues: Leveraging a Legacy of Pollution for Research and Reflection in Introductory and Advanced Undergraduate Biology Courses.

K.M. Drace[1], V.K. Gibbs[1], M.L. Styers[1], P.K. Hanson[1];
[1]Biology, Birmingham-Southern College, Birmingham, AL

Place-based education (PBE) is a form of "*in situ*" science pedagogy that helps cultivate a sense of place among students as they interact with their local environment and community. PBE engages students more effectively than traditional science curricula, promotes self-efficacy, and may play a role in identity formation. Despite these benefits, use of PBE has largely been confined to K-12 environmental education in rural settings. The goal of this project is to test the efficacy of PBE in three undergraduate biology courses at an urban liberal arts college. Specifically, heavy metal pollution from local industries is used to frame course-based undergraduate research and critical reflection. In an introductory cell and molecular biology course, students isolate and characterize bacteria from contaminated soils, looking for evidence of co-evolution of resistance to heavy metals and antibiotics. Quantification of interactions between microbes primes students for a subsequent course on evolutionary ecology. In an introductory organismal biology course, students use *Drosophila melanogaster* as a model organism to study the effects of heavy metals on development and behavior. They also develop and test hypotheses regarding the ability of diet to mitigate these effects. In an upper-level course on genetic engineering, students apply concepts from user-centered design to construct novel whole-cell bioreporters for chromate. Whereas the introductory labs will be implemented in Fall 2017, the 2016 pilot of the genetic engineering course suggests that this version of PBE is effective at engaging students. In pre-/post-surveys, 80% of students self-reported gains in "understanding how scientists work on real world problems" and 60% of students self-reported increased "ability to tolerate obstacles in the research process." These gains are particularly notable, given that these students were all seniors, many of whom had prior undergraduate research experience. This cohort (n=10) also reported statistically significant gains in their readiness for more challenging research, their ability to contribute to science, and their understanding of the process of science ($p<0.05$). At the beginning and end of the term, students completed critical reflections addressing civic engagement. Rubric-driven analyses of these reflections suggest a possible increase in students' abilities to articulate the relationship between their research and public interests, as the average score increased from marginal to proficient, and this difference approached statistical significance ($p=0.1$). Consistent with a limited number of reports in the literature, these preliminary data suggest that PBE can be adapted to engage urban undergraduates in STEM.

M63

Assessment of *Mapping the Brain*, a research and neurotechnology based approach for the modern neuroscience classroom.

S.D. Robertson[1], Z.A. Johnson[1], N.R. Sciolino[2], N.W. Plummer[2], P. Jensen[2];
[1]Biotechnology Program, Department of Molecular Biomedical Sciences , North Carolina State University, Raleigh, NC, [2]Neurobiology Laboratory, National Institute of Environmental Health Sciences, Research Triangle Park, NC

Neuroscience research is changing at an incredible pace due to technological innovation and recent national and global initiatives such as the BRAIN initiative. The BRAIN initiative, launched by the White House in 2013, rivals the Human Genome Project in scale and is propelling the field forward through the development of novel neurotechnologies. This transformation of modern neuroscience requires that we reflect on our current approach to neuroscience education, identify best practices, and consider new

strategies in the classroom. Given the wealth of data supporting the value of course-based research experiences for students, we developed and assessed a neurotechnology- and inquiry-based course: *Mapping the Brain*. The goal of the course is to immerse undergraduate and graduate students in research and to explore emerging technologies in neuroscience. In the laboratory portion of the course, students pursue a hypothesis-driven, collaborative National Institutes of Health research project. Using chemogenetic technology (**D**esigner **R**eceptors **E**xclusively **A**ctivated by **D**esigner **D**rugs-DREADDs) and a recombinase-based intersectional genetic strategy, students map norepinephrine neurons and their projections, and explore the effects of activating these neurons in vivo. In lecture, students compare traditional and cutting-edge neuroscience methodology, analyze primary literature, design hypothesis-based experiments, and discuss technological limitations of studying the brain. We evaluated this course over two consecutive years in the Biotechnology Program at North Carolina State University, assessing achievement of student learning outcomes and knowledge of Society for Neuroscience's (SfN) core concepts and essential principals of neuroscience. Using analysis of student assignments and pre/post content- and perception-based course surveys, we found student's attained the course-learning outcomes. Next we assessed whether the course improved student primary literature analysis and neurotechnology assessment. Our analysis revealed the course increased student confidence in their ability to analyze primary research articles and methodologies. Students also report greater focus on data while reading articles post course. In summary, through the integration of authentic research and a neurotechnology focus, *Mapping the Brain* provides a unique model for an introductory neuroscience course. Students finish the course with new technical and intellectual skills along with knowledge of SfN's core concepts and essential principles of neuroscience. We hope that *Mapping the Brain* and similarly designed courses, which actively engage students in the scientific process and cutting edge research, will inspire a new cohort of innovators in the field of neuroscience

M64

Interdisciplinary Collaborations: A Course-based Undergraduate Research Experience (CURE).

R. Roberts[1], J. Koeppe[2], S. Price[1], P. Craig[3];
[1]Biology, Ursinus College, Collegeville, PA, [2]Chemistry, SUNY Oswego, Oswego, NY, [3]Chemistry, Rochester Insistute of Technology, Rochester, NY

Interdisciplinary collaborations are often essential to answer multi-faceted questions, yet they present with many challenges such as understanding the information, methodologies, and norms of another field and effective communication between team members. We must begin to provide young scientists with the skills necessary for success as the call for more interdisciplinary science expands. We have modeled an interdisciplinary collaboration at the undergraduate level through a Course-based Undergraduate Research Experience (CURE). Students in upper-level Biochemistry and Structural Biology courses collaborated to assign functions to proteins "of unknown function" in the Protein Data Bank (PDB). The Structural Biology students used bioinformatics tools including the ProMOL plugin to the PyMOL molecular graphics environment, along with BLAST, Pfam, and Dali to predict protein functions. In parallel, the Biochemistry students expressed and purified the proteins and carried out in vitro testing, informed by the in silico-predicted enzyme function. Students came together in cross-disciplinary teams throughout the project to share progress, explain their discipline-specific methodologies, and discuss next steps. They each presented an oral progress report on the entire project, which required that they not only understand their own work but that of their collaborators. Interdisciplinary teams presented final posters to the campus community and students prepared future directions documents. We had students fill out a pre- and post-assessment for the two years that the CURE has occurred on our campus. The assessment had two foci: the effectiveness of the cross-course collaboration model and assessment of content learning. Students learned the information and

methodologies of both their own discipline and that of their collaborators. Moreover students increased their ability to communicate to and learn from their collaborators. This project is supported by NSF IUSE 1503811 & 1709805.

M65

Integrating Cell Biology Concepts: Comparing Learning Gains And Self-Efficacy In Live And Virtual Undergraduate Lab Experiences.

L. Goudsouzian[1], P. Riola[2], K. Ruggles[2], P. Gupta[2], M.A. Mondoux[3];
[1]Natural Science, DeSales University, Center Valley, PA, [2]Mathematics and Computer Science, DeSales University, Center Valley, PA, [3]Biology, College of the Holy Cross, Worcester, MA

Multiple pedagogical approaches, such as experimental experiences or computer-based activities, have been shown to increase student learning and engagement. We have developed a laboratory module that includes both a traditional "live" experimental component and a student-designed "virtual" computer simulation component. This laboratory employs the mating pathway of the model yeast *Saccharomyces cerevisiae* to demonstrate four fundamental cell biology concepts: cell signaling, cytoskeleton, cell cycle, and cell cycle checkpoints.
In the live laboratory, students add mating pheromone to yeast cultures, then measure the rate of cell division and changes in morphology characteristic of the *S. cerevisiae* mating response. The "virtual" complement to this laboratory was designed using the principles of Design Thinking in collaboration with an undergraduate Computer Science course. The students generated two computer simulations which can support the live laboratory or provide a virtual laboratory experience.
We assessed the live and virtual laboratories at two undergraduate institutions by measuring student learning gains for three learning objectives: applying fundamental cell biology content, developing laboratory skills, and predicting novel experimental outcomes. Students who completed the live lab and the simulation, or the simulation alone, demonstrated gains across all three objectives, with greater gains for students who completed the live lab. Students who performed neither the live nor virtual lab had no significant learning gains. In a student attitudinal survey, students reported positive perceptions of their learning gains, engagement, and self-efficacy after performing the live and virtual labs.
Our data demonstrate that students at both campuses achieved learning gains in key cell biology topics and acquired an improved sense of self-efficacy as a result of performing this laboratory. We believe that the successful implementation of this laboratory at two institutions of varying selectivity indicates that this exercise has a broad appeal for a wide variety of students. The virtual laboratory simulation provides an opportunity for students not enrolled in a laboratory course to achieve some of the learning gains observed with the live laboratory; however, our data underscore the value of a hands-on experimental experience.

M66

Engagement in the publication process improves scientific communication, critique and career skills among graduate students.

E.A. Johnson[1], M.W. Springel[2,3], S.C. Fankhauser[3,4];
[1]Biology, Emory University, Atlanta, GA, [2]Harvard University, Boston, MA, [3]Journal of Emerging Investigators, Boston, MA, [4]Biology, Oxford College of Emory University, Oxford, GA

Reading and critiquing scientific primary literature is an important skill for graduate students, especially in scientific fields, as reviewing literature is critical in progressing science. Results of prior research indicate that graduate students lack in their understanding of effective communication as well as basic

experimental design, but also that graduate students are capable of growth in their experimental design abilities when given proper opportunities. Participating in the primary review process and reading papers with fatal experimental flaws also improves future research efforts yet is not a standard component of graduate student education. The Journal of Emerging Investigators (JEI) provides graduate students with the opportunity to review and edit original research papers submitted by middle and high school student authors. The purpose of this project was to determine whether participation in the primary literature process through JEI effectively aids in developing graduate students' perceived abilities to communicate science effectively, skills in critiquing science, and preparedness for a career in science. A twelve-question survey was developed and distributed using SurveyMonkey to 215 individuals in the JEI reviewer and editor databases. The response rate for this survey was approximately 47%. The survey assessed prior research experience, experience within JEI, motivation for participating in the JEI process, and benefits obtained through participation with JEI in the three domains: communication, critique, and career. Editors, whose role involves the synthesis of feedback from multiple reviewers and interaction with papers in their earliest stages, benefitted more than reviewers in every domain assessed by the survey. Perceived impact on critiquing skills was only rated more highly by reviewers than editors once the graduate students in question had reviewed ten or more papers. Older and more experienced students rated the perceived impact of involvement with JEI on their critiquing skills even more highly than younger and less experienced students, suggesting that even more experienced students can benefit or that these students' experience allowed them to accrue greater benefits through repetition with the JEI process. The results of this research are significant because they suggest that graduate students should participate early in their career in the reading and reviewing of primary literature; furthermore flawed science writing can translate to a practical teaching method that can be applied in graduate education programs to improve experimental design and critique and science communication skills.

M67

Learning in Large Introductory Biology Courses Is Effectively Facilitated by Trained Undergraduate Learning Assistants.

R.P. Donaldson[1];
[1]Biological Sciences, George Washington University, Washington, DC

The purpose of the GWU undergraduate Learning Assistant (LA) program (modeled after the University of Colorado program) is to facilitate learning in active classroom environments. The LA's take a pedagogy class taught by a STEM education specialist and meet weekly with their lead instructor. The GWU LA program is associated with courses in Biology, Chemistry, Physics and Math. Here we report on teams of LA's who participated in the daily teaching of students in Bisc 1111 Introductory Cell & Molecular Biology classes taught 2013-2016. There were 168 students in the Fall 2016 LA class and 170 in a traditional lecture section. Each LA in the Biology classrooms worked with a specified group of 10-18 students, facilitating discussions of clicker questions, worksheet problems and practice quizzes. Each group of 3 students would submit their daily worksheet to their LA for written comments and formative assessment. Students evaluated the effectiveness of the course activities for their learning (the % of students reporting a positive effect in parentheses): assigned readings (93%), weekly online homework (70%), daily in class clicker questions (89%), daily group problem solving (89%), LA facilitation (91%), brief lectures (57%), studying alone (43%), practice quizzes (82%). Most of the students (93%) reported that they would take another course with LA's and 77% felt that the overall course format facilitated their learning. Learning was assessed in short answer exams and by administration of the Introductory Molecular and Cell Biology Assessment concept inventory (Shi et al, 2010, CBE Life Sci. Ed). Students in

the LA supported class performed better on this assessment (74%) vs. students in the traditional lecture (64%). Published reports from other universities show that the LA program increases student retention (Talbot et al, 2015, J. College Sci. Teaching).

M68

Evaluation of an intervention designed to support multicultural collaboration.

A.J. Prunuske[1], J.B. Welch[2], K.M. Nemeth[2];
[1]Microbiology and Immunology, Medical College of Wisconsin, Wausau, WI, [2]Biology, University of Minnesota, Duluth, MN

Research laboratories are multicultural environments that require individuals with different backgrounds, customs, and expectations to work together effectively. There is value in providing students with training to help build cultural humility and their self-efficacy in these situations. We tested the use of a cross-cultural simulation experience, Ecotonos, in an undergraduate biology laboratory course with the goal of making students more aware of the challenges of working across cultures. During the activity, participants are assigned a new, fictitious culture, which they practice in monocultural groups. Participants are then reassorted into multicultural groups of varying composition and in which they perform an evaluation task. We completed a mixed-methods analysis to determine the effectiveness of the experience by coding their responses to reflection questions and by having students complete a cultural intelligence inventory. The activity was effective at raising students' awareness of how cultural factors can impact group dynamics. In addition, the activity increased cultural awareness in the cognitive domain, which relates to having culture specific knowledge that can be used to operate within a culture. Inclusion of these types of experiences will give students not only the scientific knowledge and skills needed to be successful in the lab, but will also prepare them to be more effective members of the laboratory team.

Microsymp 1: Recent Advances in the Molecular Cell Biology of Neurons

E1

Cytoskeletal Regulation of Neurodevelopment in a Human iPSC-derived Autism Model.

T. Rudisill[1], B. Kirk[1], C. Johnson[1], A. Orbita[1], P. Pakala[1], H. Dar[1], S. Davis[1], A.R. Horwitz[2], M.J. McConnell[3], K.A. Litwa[1,2];
[1]Anatomy and Cell Biology, East Carolina University Brody School of Medicine, Greenville, NC, [2]Cell Biology, University of Virginia, Charlottesville, VA, [3]Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA

Autism is a genetically complex neurodevelopmental disorder in which patients exhibit social deficits in verbal and non-verbal forms of communication and display restricted and repetitive behaviors. Emerging evidence suggests that altered neural connectivity, particularly at the level of synaptic connections, contributes to disease pathology. Dynamic rearrangements of the actomyosin cytoskeleton drive neural circuit formation, including neurite extension and the development of actin-enriched spines at excitatory synapses. Actomyosin regulatory pathways are also one of the major molecular mechanisms disrupted by both Autism-associated copy number variants (CNVs) and *de novo* mutations. Yet, it is still unknown how actomyosin regulation shapes developing cortical circuits and the impact of specific actomyosin regulatory pathways on Autism pathology. To understand the cytoskeletal mechanisms that lead to altered neural circuitry in Autism, we develop cortical brain organoids from patient induced pluripotent stem cells (iPSC). Our Autism-derived cortical organoids exhibit increased

excitatory synapse formation, similar to post-mortem patient samples and mouse models of Autism. To address whether myosin-II activity contributes to this observed increased in excitatory synapse area, we acutely treated 3-month old neurotypic cortical organoids with the RhoA kinase (ROCK) inhibitor, Y-27632. We confirmed that Y-27632 treatment reduced ROCK-driven myosin-II activation in the cortical organoids through reduced phosphorylation of the myosin regulatory light chain (RLC) at Ser19. Intriguingly, acute Y-27632 treatment mimicked Autism pathology by significantly increasing excitatory pre-synaptic surface area as labeled by vGlut-1. We hypothesize that a corresponding increase in synaptic Rac1 activity mediates this increase in excitatory synaptic surface area. In support of this hypothesis, we observe increased phosphorylation of the Rac1 downstream target, cofilin, at Ser3 in Y-27632-treated cortical organoids. These results suggest that Rac1 activity dysregulation could be a driving mechanism underlying Autism synaptic pathology, leading us to investigate which Rac1 activity regulators are present during cortical development. For example, in neurotypic cortical organoids, we found that the Rac1 inactivator, ArhGAP23, is enriched at excitatory synapses. Through the use of a human cortical organoid model, we demonstrate that coordinated myosin-II and Rac1 activity underlie excitatory synapse development, and that alterations in the balance between these actomyosin pathways can promote Autism pathology.

E2

Regulation of axon initial segment cytoskeletal architecture and function by βIV-spectrin.

S.L. Jones[1], T.M. Svitkina[1];
[1]Biology, University of Pennsylvania, Philadelphia, PA

The axon initial segment (AIS) of polarized neurons is a specialized structure that, in addition to initiating action potentials, helps maintain neuron polarity by preventing the mixing of axonal and somatodendritc components. This latter function depends, in part, on a submembrane cytoskeletal network comprising ankryinG, actin filaments, and βIV-spectrin. While ankyrinG functions as the master organizer of the AIS, βIV-spectrin is thought to contribute to AIS maintenance. The role of βIV-spectrin in AIS function and structure is poorly understood. AIS structure has been shown by platinum replica electron microscopy (PREM) to contain a dense mixture of fibrils and globules that coat microtubule bundles. We have proposed that this fibrillar-globular coat functions as a selective diffusion barrier that contributes to AIS polarity function. Here we investigate the structural and functional consequences of βIV-spectrin knockdown in hippocampal neurons in vitro using two independent lentiviral shRNAs, compared with scrambled and luciferase shRNA controls. βIV-spectrin depletion caused dramatic increase in AIS lengths (as determined by ankyrinG immunofluorescence) and extensive spreading of AIS membrane proteins (including voltage-gated ion channels and cell adhesion molecules) throughout the axon. These data are consistent with a role for βIV-spectrin in AIS maintenance. We also examined the polarized distribution of several somatodendritic proteins and found that while many of them (e.g., PSD95, transferrin receptor, and MAP2) remained localized to dendrites, the potassium/chloride co-transporter KCC2 re-distributed to axons/AISs of neurons lacking βIV-spectrin. Interestingly, similar results were obtained following treatment with the actin depolymerizing drug latrunculin B. To determine the effects of βIV-spectrin knockdown on AIS structure, we examined AISs by PREM and discovered a fibrillar phenotype characterized by the presence of dense fibrillar bundles that (1) contain fibrils of ~100 nm in length, (2) align parallel to the AIS shaft, (3) contain globular structures at their ends, and (4) appear to repeat periodically along the AS. These fibrillar bundles likely represent ankyrinG molecules since they stain positive by ankyrinG immunogold and other likely candidates (e.g., αII-spectrin, βII-spectrin, βIII-spectrin, septin7, and myosin II) failed to accumulate substantially in the AIS following βIV-spectrin depletion. Efforts are underway to confirm the identity of the fibrillar bundles.

Together, these data indicate that βIV-spectrin is an important component of the AIS cytoskeleton that contributes to maintenance of overall AIS structure and its role in neuron polarity.

E3

The microtubule plus-end-tracking protein TACC3 promotes persistent axon outgrowth and mediates responses to axon guidance signals during development.

B. Erdogan[1], G. Cammarata[1], E. Lee[1], B. Pratt[1], L.A. Lowery[1];
[1]Biology, Boston College, Chestnut Hill, MA

Precise neuronal connection requires proper axon guidance. Microtubules (MTs) of the growth cone are the driving force to navigate the growing ends of axons. Pioneering microtubules and their plus-end tracking proteins (+TIPs) play integrative roles during this navigation. Recently, we introduced the protein TACC3 as a member of the +TIP family regulating microtubule dynamics in Xenopus laevis growth cones and show that manipulation of TACC3 levels affects axon outgrowth by regulating axon outgrowth velocity and the frequency of axon retraction. Additionally, we show that over-expressing TACC3 mitigates nocodazole-induced reduction in MT dynamics parameters suggesting that TACC3 could play a protective role against nocodazole induced MT depolymerization. Moreover, we find that TACC3 and its partner XMAP215, a well-characterized MT polymerase, cooperate to promote axon outgrowth and rescue axon growth defects. Finally, we show that reduction of TACC3 levels causes pathfinding defects in axons of developing spinal cord motor neurons in Xenopus laevis in vivo and increased TACC3 levels interfere with the growth cone response to the axon guidance cue Slit2. Currently we are investigating whether TACC3 could be a potential target of Abelson kinase downstream of the Slit2 guidance cue. Together, our results suggest that by regulating MT behavior, the +TIP TACC3 is involved in axon outgrowth and pathfinding decisions of neurons during embryonic development, and that TACC3 phosphorylation events, which remain to be elucidated, could be important regulators of this involvement.

E4

Pharmaco-genetic toolsets for cell-specific subcellular cGMP and calcium manipulation *in vivo*.

O. Ros[1], K. Loulier[1], S. Ribes[1], S. Couvet[1], Y. Zagar[1], D. Ladarré[2], Z. Lenkei[2], X. Nicol[1];
[1]Vision Institute, Sorbonne Universités, CNRS, Inserm, Paris, France, [2]Brain Plasticity Unit, PSL Research University, ESPCI, CNRS, Paris, France

Second messengers are mid-point relays in signaling cascades governing a wide range of cellular functions. Calcium is central in multiple cellular responses ranging from metabolism and survival to vesicle release and motility. The blockade of calcium signals currently relies on pharmacological strategies to block calcium influx into the cell or chelate extracellular or intracellular calcium. Since calcium is crucial for a wide range of signaling pathways and for the function of a wide range of components of the extracellular matrix, those strategies lack cellular specificity and are plagued with a variety of side effects when applied to patients. Similarly, cGMP is involved in a wide range of signaling pathways and cellular processes including neurotransmission, calcium homeostasis, phototransduction, lipid metabolism and cation channel activity. The diversity of these processes suggests that cGMP signals are tightly controlled in space and time to achieve specific modulation of its downstream pathways. However, manipulating cGMP is mostly achieved using pharmacological approaches either altering the synthesis or degradation of this cyclic nucleotide or manipulating downstream signaling pathways. These techniques lack both cellular and subcellular specificity.

We have developed a pair of genetically-encoded buffers that alter physiological changes in the concentration of cGMP and calcium respectively. These tools enable disrupting signaling cascades with cellular and subcellular resolution. We provide evidence that the cGMP and calcium scavengers, both in soluble form and targeted to the lipid raft or non-lipid raft compartments of the plasma membrane, are able to locally buffer changes in the respective second messenger concentration and to alter downstream cellular processes including axon pathfinding events. When *in utero* electroporated in the developing brain, the cGMP and calcium buffers interfere with the migration of newly generated cortical neurons *in vivo* and highlights a non-cell autonomous impact of second messenger manipulation. These genetically-encoded cGMP and calcium scavengers paves the way to investigate subcellularly-localized signaling *in vivo* , combined with cellular resolution and to directly manipulate these second messengers for therapeutic use.

E5

Mice without c-Abl tyrosine kinase at the CNS show improved learning and memory and increased gene expression of synaptic plasticity.

A. GONZALEZ[1];
[1]Señalización Celular, Pontificia Universidad Católica de Chile, Santiago de Chile, Chile

The memory and learning processes are tightly related with both neuronal plasticity and spine remodeling, the actin cytoskeleton play a key role on these action as well as another important scaffolding proteins localized into the presynaptic terminal such as PSD-95 or SAP-102. The non-receptor tyrosine kinase c-Abl can regulates the postsynaptic scaffolding protein clustering throughout interactions with NMDAR, EphA4 and cdk5 and actin polymerization dynamic into the Central Nervous System (CNS) where is located both pre- and post-synaptic neuron terminals. Also c-Abl regulates transcription factors such as c-Jun or p73 and stabilizes the HDAC2, repressing synaptic genes. Then we hypothesize that c-Abl activity would be involved in memory and learning processes. We evaluated if mice null for c-Abl in CNS have cognitive impairments and if c- Abl has any effect on transcriptome levels of genes related with these processes. We found that absence of c-Abl in CNS specific mouse model improve the memory and learning like shown the different tests in which the c-Abl null animal shown better memory profile than their siblings with c-Abl.Furthermore, our RNAseq study shown diverse highly significant overexpressed pathways related with synaptic plasticity on the transcript of c-Abl null mice with respect to control mice. Our results show that c-Abl play a key role regulating the memory and learning and we propose that this regulation is trough of down-regulation the transcription of several genes related with cytoskeleton dynamic and synaptic plasticity.

E6

Long-term adaptation of G-protein signalling in the brain is facilitated by active G-proteins' feedback control of the amount of RGS proteins.

M.E. Fina[1], S.R. Tummala[2], N. Vardi[3], A. Kashina[1], D.W. Dong[1,3,4];
[1]Biomedical Sciences, University of Pennsylvania, Philadlephia, PA, [2]Department of Bioengineering, University of Pennsylvania, Philadlephia, PA, [3]Department of Neuroscience, University of Pennsylvania, Philadlephia, PA, [4]Institute for Biomedical Informatics, University of Pennsylvania, Philadlephia, PA

Regulation of G-protein signaling is essential for the brain. This regulation is mediated primarily by a class of proteins called Regulators of G-protein Signaling (RGS). With two dozens of RGSs regulating thousands of G-protein signaling cascades, this regulation mechanism is involved in virtually every known physiological process and plays a major role in neural signal processing. Much of what we know

about RGS is derived from the retina. In the first stage of visual processing, RGS9 accelerates the recovery of photo-transduction in both rods and cones. In the second stage, RGS7 and RGS11 accelerate the light-on response of majority of bipolar cells---the Depolarizing Bipolar Cells. For a given receptor activity, decreasing or increasing the amount of RGS proteins leads to more or less active G-proteins, respectively. This allows the signal transduction to change the overall gain without the need to change other components of the cascade. This regulation is believed to be especially important during long-term adaptation such as from daylight to night vision. Much less known is what mediates the change of RGS levels during long-term adaptation. Recent data on dark adaptation in rods and light adaptation in rod bipolar cells indicate that a long-term decrease in a ligand's stimulation of a G-protein signaling cascade should lead to a decrease of RGS protein level in the cascade, and vice versa. Using knockout mouse models, we showed that the decrease of the amount of active G-proteins lead to the decrease of RGS7 and RGS11 protein level in rod bipolar cells. Therefore the RGS protein level and the active G-protein level reach a steady state through their feedback interactions, after a long-term adaptation to a given receptor activity. We developed a computational model of the feedback regulation of G-protein signaling cascades, which provides an over arching principle that reconciles a lot of controversial data in the literature and explains G-protein-dependent regulation in a variety of biological systems.

E7

Determining the localization and function of schizophrenia-linked protein tSNARE1b in the endolysosomal system of developing neurons.

M. Plooster[1], G. Rossi[2], M.S. Farrell[3], P.F. Sullivan[3,4], S.L. Gupton[2,5,6], P. Brennwald[2];
[1]Cell Biology and Physiology Curriculum, University of North Carolina, Chapel Hill, NC, [2]Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC, [3]Department of Genetics, University of North Carolina, Chapel Hill, NC, [4]Department of Psychiatry, University of North Carolina, Chapel Hill, NC, [5]Neuroscience Center, University of North Carolina, Chapel Hill, NC, [6]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC

Schizophrenia is a severe neuropsychiatric disorder characterized by delusions and hallucinations, which lacks effective, targeted therapies, likely due in part to its polygenic etiology. Recently, the largest genome wide association study on schizophrenia to date identified 108 loci associated with the occurrence of schizophrenia. The fifth most significant hit mapped to a locus containing the gene TSNARE1, which encodes the previously unstudied protein t-SNARE domain containing 1 (tSNARE1). tSNARE1 contains a N-terminal c-Myb DNA binding domain and a C-terminal Qa SNARE domain that shares closest homology to Syntaxin 12 (Stx12), an endosomal SNARE protein. Rare variant mutations identified from patients with either schizophrenia or autism spectrum disorder suggest that the SNARE domain is critical to tSNARE1 function. Unlike canonical Qa SNARE proteins, the primary neuronal isoform of tSNARE1, tSNARE1b, lacks a transmembrane domain as well as any other predicted site for membrane attachment, which is thought to be necessary for membrane fusion. Therefore, our central hypothesis is that tSNARE1b acts as an inhibitory SNARE (i-SNARE) of specific membrane trafficking events. This hypothesis is supported by biochemical pull-down assays with recombinant proteins and embryonic brain lysates, which demonstrate that GST-tSNARE1 can replace Stx12 and assemble with the endosomal SNARE proteins Vti1a, Stx6, and VAMP4 into SNARE complexes. Because tSNARE1 shares its closest homology with Stx12, we hypothesized that tSNARE1 functions within the endosomal pathway. High resolution, live-cell confocal microscopy of tSNARE1b-GFP and a battery of spectrally distinct organelle markers in embryonic murine cortical neurons determined to which endocytic compartments tSNARE1b localizes. Colocalization of tSNARE1b and each marker was quantified using a semi-automated, quantitative image-analysis pipeline that robustly identifies colocalization based on two

different measurements. Preliminary evidence suggests that tSNARE1 colocalizes the strongest with late endosome marker Rab7 and lysosome marker LAMP1, suggesting tSNARE1b may regulate trafficking between these organelles. Ongoing studies are exploring how tSNARE1b functions at the membrane trafficking between late endosomes, lysosomes, and autophagosomes with three-color, live-cell imaging.

Microsymp 2: Cytoskeletal Molecular Dynamics

E8

Reconstitution of aster movement and cell division plane positioning in Xenopus egg extract.

J.F. Pelletier[1,2,3], C.M. Field[1,2], N. Fakhri[3], J.S. Oakey[2,4], J.C. Gatlin[2,5], T.J. Mitchison[1,2];
[1]Department of Systems Biology, Harvard Medical School, Boston, MA, [2]Marine Biological Laboratory, Woods Hole, MA, [3]Department of Physics, Massachusetts Institute of Technology, Cambridge, MA, [4]Department of Chemical Engineering, University of Wyoming, Laramie, WY, [5]Department of Molecular Biology, University of Wyoming, Laramie, WY

During early embryogenesis in *Xenopus laevis*, each cell division plane bisects the boundary between a pair of large microtubule asters that propagate from the poles of the mitotic spindle. The asters are a network of short, dynamic microtubules oriented radially outward from the microtubule organizing center (MTOC). Before cleavage, asters grow and move so that the cell divides approximately at its midplane and perpendicular to its long axis. Aster movement is thought to depend on length-dependent forces from cytoplasmic dynein opposed by hydrodynamic drag; however, it remains unclear how these forces propagate through the network of short, and short-lived, microtubules, resulting in aster movement. We reconstituted aster movement in *Xenopus* egg extract, and imaged the growth and interaction of asters under slit-like confinement. Aster boundaries stopped growing when they interacted with neighboring asters. The boundaries between asters formed dynamic, tessellated polygonal patches that resembled 2D foams. MTOCs moved relative to aster centroids and boundaries, and this movement was partially inhibited by the CC1 fragment of dynactin, which inhibits dynein-dependent forces. We measured the dynamic geometries of MTOCs and aster boundaries at different MTOC densities, and will use numerical and analytical modeling to investigate whether known forces can explain the results. Within the egg, microtubule asters must navigate abundant obstacles including lipid droplets and yolk platelets, which are removed during preparation of the extract. To simulate them, we added agarose spheres to the extract, and asters were able to grow around these obstacles. The global organization of microtubules remained radial, and the boundaries between asters remained approximately straight and equidistant between pairs of MTOCs. We also imaged the response of asters to controlled flows. We conclude that the short, dynamic nature of microtubules in large asters facilitates aster growth and movement through a cytoplasm containing abundant obstacles.

E9

Controlling cytoskeletal organization and cellular dynamics by localized optical modulation of microtubule dynamics.

J. van Haren[1], A. Ettinger[2], R. Charafeddine[1], H. Wang[3], K.M. Hahn[3], T. Wittmann[1];
[1]Department of Cell and Tissue Biology, UCSF, San Francisco, CA, [2]Institute of Epigenetics and Stem Cells, Helmholtz Center Munich, Munich, Germany, [3]Department of Pharmacology, UNC Chapel Hill, Chapel Hill, NC

Throughout the cell cycle and during differentiation, the highly dynamic microtubule (MT) cytoskeleton self-organizes to form polarized intracellular tracks that enable directional transport, facilitate chromosome segregation and help to establish cell polarity. MT remodeling through stochastic switching between phases of growth and shortening is critical for all MT functions in cells, but it is unclear how MT dynamics are spatially and temporally coordinated. Amongst the regulators of MT growth dynamics and interactions are a heterogeneous family of >30 plus end tracking proteins (+TIPs) that associate with growing MT ends. End Binding proteins (EBs) associate autonomously with MT ends and are considered core +TIPs, as they recruit most other +TIPs to the MT end. Interestingly, while all growing MT plus ends are decorated with EBs, MT functions vary in different parts of the cell, suggesting that the activity of the +TIP complex is spatially controlled. Because no tools existed to manipulate subpopulations of MTs with high spatiotemporal resolution, we sought to develop a novel optogenetics approach to spatially control the activity of the EB recruited +TIP complex and investigate the functions of different MT populations during cell migration and mitosis. We developed a photo-inactivated (pi) EB1 molecule by utilizing a phototropin LOV2 domain-based protein interaction pair that rapidly dissociates upon blue light exposure. The resulting pi-EB1 replaced endogenous EB function in EB1/ EB3 double CRISPR/Cas9 knockout cells and was rapidly inactivated by blue light in a highly reversible manner. Inactivation of pi-EB1 efficiently disrupted the +TIP network at growing MT ends, induced depolymerization of a population of cell edge associated MTs and reduced persistent growth of MTs in the cell body, without affecting the recruitment of the MT polymerase ch-TOG. Patterned illumination allowed inactivation of pi-EB1 with micrometer precision, and enabled spatial control of MT polymerization in migrating and dividing cells. While the MT network is polarized toward the leading edge of migrating cells, it has remained unclear if this is a cause or consequence of directional cell movement. Our results show that local pi-EB1 inactivation results in an immediate aversive turning response, indicating that EB1-mediated +TIP interactions are essential to maintain migration direction. pi-EB1 also allowed us to investigate how subsets of dynamic MTs contribute to various aspects of mitotic spindle organization and positioning, and found that the EB recruited +TIP complex is required for spindle size homeostasis.

E10

Rac1 promotes septin-mediated guidance of CAMSAP-associated microtubules to focal adhesions.

D.G. Merenich[1], S. Donovan[1], A. Jacobs[1], K.A. Myers[1];
[1]Biological Sciences, University of the Sciences in Philadelphia, Philadelphia, PA

During angiogenesis, endothelial cells (ECs) utilize signaling molecules and microtubule (MT) associated proteins (MAPs) to control the dynamic and coordinated remodeling of the actin and MT cytoskeleton to become polarized. Rac1, a signaling molecule of the Rho GTPase family, promotes cell protrusion and enhances MT plus-end assembly into protrusions, thereby driving EC polarization. As ECs polarize, actin filaments assemble and become linked to focal adhesion (FA) complexes that mature at the positions where the cell interfaces with the extracellular matrix. Recent investigations have identified that a

subset of elongating MTs undergo “capture” at FAs, and that FA-captured MTs promote directed cell migration, yet how MTs are designated for FA capture is not known. CAMSAPs are MAPs that associate with MT minus-ends, where they inhibit MT disassembly and promote plus-end MT growth. Septins are MAPs that promote actin stress fiber-mediated maturation of FAs and that spatially guide MT plus-end dynamics. Collectively, these data point to a potential mechanism used by ECs to delineate MT functions via association with CAMSAPs and septins. Here, we tested the hypothesis that FA capture of MTs is controlled by signaling from Rac1 to promote septin-mediated guidance of CAMSAP-associated MTs for FA capture. Live-cell fluorescence imaging of ECs revealed that MTs captured at FAs are predominantly nucleated at the Golgi-apparatus rather than the centrosome. Expression of a constitutively active Rac1 promoted increased CAMSAP stretch length on Golgi-derived MT minus-ends, while a dominant negative Rac1 had the opposite effect. Analysis of MT growth events revealed that CAMSAP-associated MTs consistently grew into septin stretches that localized adjacent to FAs, resulting in FA capture of MTs in almost all cases. Pharmacologic inhibition of myosin-II, to relieve contractile forces on FA-associated actin filaments, resulted in a significant reduction of both FA-associated septin and FA capture of MTs, and this effect was dependent on Rac1 activity. Expression of either CAMSAP or septin, or simultaneous expression of both proteins, increased EC migration in a wound-healing assay. These results suggest that Golgi-derived, CAMSAP-associated MTs represent a distinct population of MTs that are guided by peripheral septin for FA capture. Moreover, these data support a model in which a balance of Rac1 and myosin-II activity promotes CAMSAP-association with Golgi-derived MTs and increases septin localization to peripheral FAs to promote persistent and directional EC migration. Future investigations will mechanistically determine how CAMSAP-associated MTs are distinguished by septin, and how septin-mediated FA capture of MTs contributes to enhanced EC migration.

E11

C. elegans microtubules are highly dynamic and have non-canonical lattices.

S. Chaaban[1], C. Hsu[1], S. Redemann[2], T. Müller-Reichert[2], J.M. Kollman[3], H.K. Bui[4], G.J. Brouhard[1];
[1]Biology, McGill University, Montréal, QC, [2]Technische Universität Dresden, Dresden, Germany, [3]Biochemistry, University of Washington, Seattle, WA, [4]Anatomy and Cell Biology, McGill University, Montréal, QC

Microtubules of the model organism *C. elegans* differ from the textbook eukaryotic microtubule both in their structure and dynamics. For example, microtubules in somatic cells do not contain the canonical 13 protofilament (pf) lattice seen in most eukaryotes; rather, most cells have 11 pfs. Furthermore, microtubules in various *C. elegans* tissues grow extremely fast (up to 60 um/min) - almost an order of magnitude higher than other model organisms. To determine the origin of these phenomena, we purified tubulin from whole *C. elegans* lysates. Interestingly, we observed a shift to smaller pf numbers for spontaneously nucleated microtubules relative to bovine microtubules. Furthermore, *C. elegans* microtubules grew much faster *in vitro*, with an apparent on-rate constant ~3-fold higher than for bovine tubulin, and underwent more frequent catastrophes, indicating that *C. elegans* tubulin is the most dynamic to date. To determine how *C. elegans* achieves these differences, we solved the structure of *C. elegans* microtubules at 4.8 A resolution. We found that *C. elegans* microtubules have retained the helical parameters of other eukaryotes, suggesting 11 pf microtubules found *in vivo* are super-twisted. We confirmed this result with negative stain electron tomography of the C. elegans embryo. When we mapped sequence conservation onto our structure, we found that the lateral bond interfaces has many divergent residues. Monte-carlo simulations of microtubule growth revealed that a modest increase in lateral bond energies is sufficient to recapitulate the growth rates observed *in vitro*. Together, these data provide insight into tubulin evolution by characterizing divergent dynamic and structural properties in a higher eukaryote.

E12

Cytoskeletal dynamics during wound reepithelialization *in vivo*.

A.S. Kennard[1], J.A. Theriot[1,2,3];
[1]Biophysics Program, Stanford University, Stanford, CA, [2]Howard Hughes Medical Institute, Stanford University, Stanford, CA, [3]Biochemistry, Stanford University, Stanford, CA

A critical step in wound healing is reepithelialization, or migration of epithelial cells into a wounded region. Several recent studies *in vivo* have revealed the tissue-scale patterns of growth and migration during epidermal reepithelialization, but a detailed understanding of the cytoskeletal dynamics in individual cells is lacking. We have observed the migration of the basal epidermal cells of embryonic zebrafish during reepithelialization at high spatial and temporal resolution, using spinning disk confocal microscopy. These basal epidermal cells are also known as keratocytes, and while isolated keratocytes have been studied extensively in culture for their rapid and persistent migration, much less is known about their physiological function *in vivo*.
We have found that keratocytes *in vivo* initiate rapid and dramatic migration en masse within minutes of tissue wounding. This study has revealed several novel insights: **First**, keratocytes adopt an elongated "squid-like" morphology as they migrate, which differs dramatically from their characteristic shape in culture; this suggests that the physical environment of the tissue significantly impacts keratocyte migration. **Second**, during reepithelization keratocytes separate from each other, unlike in collective migration in cultured monolayers, where cells often remain attached even as they migrate. **Third**, cell stopping at the end of reepithelialization is accompanied by a burst of myosin localization at the back of the lamellipodium. Together, these results suggest that collective migratory responses differ markedly *in vivo* and *ex vivo*, particularly in the nature of the physical contact between cells. Furthermore, these results provide insight into the dynamics of cell collectives when they stop migrating, which has been difficult to observe in tissue culture models of reepithelialization. Future work promises to clarify molecular details about the dynamics of adhesions and myosin localization during reepithelialization, shedding light on this classic example of cell migration in an *in vivo* context.

E13

The cykinetic ring is composed of local contractile units that propagate contractility along the ring circumference using time delayed negative feedback.

M.E. Werner[1], A. Sattler[1], D. Cortes[1], A. Patra[1], F. Jug[2], A.S. Maddox[1];
[1]Biology, University of North Carolina Chapel Hill , Chapel Hill, NC, [2]Center for Systems Biology, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Both positive and negative feedback regulation of the cortical actomyosin cytoskeleton have been shown to drive cell shape changes. The cytokinetic ring is an actomyosin based structure that organizes at the cell equator to provide the mechanical force for the physical separation of the two daughter cells during mitosis or meiosis. The molecular regulation of contractile ring assembly is fairly well understood, but the nature of the cytoskeletal rearrangements and dynamics that promote cytokinetic ring contraction largely remain to be elucidated. More specifically, while it has been proposed that RhoA activity is regulated by time delayed negative feedback in other contexts, it is unknown if this phenomenon occurs during cytokinesis. We combined 4 dimensional high spatial- and temporal resolution imaging with custom image analysis to detect and measure the dynamics of cytokinetic ring organization and kinetics. In contrast to previous data with relatively low temporal resolution that suggested that cytokinesis proceeds at a constant speed, we find that the cytokinetic ring alternates between phases of acceleration and deceleration To determine the length scale of this phenomenon we

subdivided the ring into 72 equal segments and measured the oscillatory behavior of each segment over time. Different parts of the contractile ring contract at different speeds at any given time and each contractile unit undergoes multiple oscillatory cycles of acceleration and deceleration during cytokinesis. Furthermore, the segment of the ring that exhibits the highest ingression speed travels along the ring circumference as the furrow ingresses. To define the molecular mechanisms regulating the oscillation and propagation of local contractions, we quantified ring dynamics following depletion of well-characterized regulatory and structural ring components. Both the amplitude and circumferential travel of local contractions are attenuated by partial loss of RhoA or myosin activity. In contrast, we observe enhancement when actin polymerization is perturbed. Ongoing work aims to define the length scale of circumferential contiguity, and to correlate protein enrichment with contractility, to identify the positive and negative feedback loop regulators that drive cytokinetic cytoskeletal remodeling. We propose that the cytokinetic ring is composed of contractile units that propagate contractility around the circumference of the cytokinetic ring in the form of a travelling wave of maximal contractility. This propagation of contractility is promoted by both biochemical and mechanical negative feedback that drives actomyosin reorganization to ensure sustained cytokinetic ring closure. Thus negative feedback appears to be a conserved behavior of actomyosin cortical structures.

E14

BMW is an exceptionally potent actin assembly factor from a human parasite.

M. Winterhoff[1], S. Brühmann[1], M. Kollmar[2], R. Schnabel[3], U. Curth[1], J. Faix[1];
[1]Institut of Biophysical Chemistry, Hannover Medical School, Hannover, Germany, [2]Department of NMR-based Structural Biology, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany, [3]Institute for Genetics, Technical University Braunschweig, Braunschweig, Germany

Assembly, organization and turnover of filamentous actin structures are essential for cell function. The initial and rate-limiting step in actin filament formation is the assembly of actin nuclei onto which additional monomers are subsequently added by filament elongators. As yet three types of actin nucleators have been established: the Arp2/3 complex, formins and tandem-monomer-binding nucleators. Here were characterized a novel and powerful tandem nucleator from a parasitic nematode causing lymphatic filariosis in man. *Brugia malayi* WH2-domain containing protein (BMW) harbors four WH2 domains preceded by proline-rich stretches at its N-terminus suggesting that it could recruit four actin monomers to form a polymerization nucleus. In vitro BMW nucleates actin polymerization at low nanomolar concentrations in a dose-dependent manner and does not require profilin for activity. A short N-terminal fragment encompassing the WH2 domains is sufficient for nucleation, but the nucleation activity is markedly enhanced in longer constructs suggesting synergy with other functional domains for full activity. In line with this notion, but in contrast to other tandem nucleators, BMW contains multiple F-actin binding sites which are required to induce the formation of massive bundles with mixed polarity in vitro as assessed by TIRF imaging. The bundles increase by growth on both ends and by fusion with neighboring bundles. Consistently, ectopic expression of BMW in commonly used cell types also leads to formation of massive bundles composed of hundreds of filaments and is associated with drastic depletion of the cellular G-actin pool arguing that most actin is consumed by BMW. Multi-color TIRF imaging at low BMW concentrations further revealed that the protein primarily nucleates actin at filament barbed ends and is subsequently either released to the filament sides or back into solution. Surprisingly, however, BMW can also associate with the pointed ends of the filaments. Finally, we explored the requirement of the WH2 domains for nucleation and bundling by analyses of deletion mutants in vitro and in vivo. Unexpectedly, nucleation and bundle assembly is markedly enhanced in constructs lacking the first two WH2 domains and is still seen with constructs lacking the first three WH2 domains in biochemical assays and transfected cells. Since BMW is a monomer as assessed by analytical

ultracentrifugation, we conclude that BMW must ulitize a novel nucleation mechanism, which will be discussed.

Microsymp 3: Cell Biology of the Nucleus

E15

LITE imaging: a high numerical aperture, low photobleaching fluorescence imaging technology.

T.C. Fadero[1], T.M. Gerbich[1], K. Rana[2], A. Suzuki[1], M. DiSalvo[3,4], K. Schaefer[1], J. Heppert[1], T.C. Boothby[2], B. Goldstein[1], M. Peifer[1], N.L. Allbritton[3,4], A.S. Gladfelter[1], A.S. Maddox[1], P.S. Maddox[1];
[1]Biology, UNC-Chapel Hill, Chapel Hill, NC, [2]Chemistry, UNC-Chapel Hill, Chapel Hill, NC, [3]Biomedical Engineering, UNC-Chapel Hill, Chapel Hill, NC, [4]Biomedical Engineering, North Carolina State University, Raleigh, NC

The dynamics of cellular processes demand visualization with fluorescence microscopy at high spatial and temporal resolution with; however, conventional fluorescence microscopy techniques are very light-intensive and introduce unwanted photobleaching, phototoxicity, and out-of-focus fluorophore excitation. Light sheet fluorescence microscopy decreases these issues by selectively illuminating the focal plane of a detection objective using an orthogonal excitation objective.1 However, existing light sheet microscopes are physically limited in the numerical aperture (NA) of the detection objective, which decreases both the microscope's efficiency and the native image resolution.1-4 We present a novel light sheet illumination method: Lateral Interference Tilted Excitation (LITE), in which a sheet of light is generated at the focal plane of the imaging objective without a sterically limiting illumination objective. This allows the use of practically any detection objective, including oil immersion, with no upper NA limit. Thus, LITE achieves the low photodamage of light sheet imaging while exploiting the benefits of high spatial resolution, high efficiency, coverslip-based objectives. We demonstrate the efficacy of LITE in imaging animal, fungal, and plant model organisms over many hours at high spatiotemporal resolution. Specifically, we are now able to generate multigenerational nuclear pedigrees in *Ashbya gossypii* over the course of more than 7 hours of continuous imaging, allowing us to study nuclear asynchrony at high spatiotemporal resolution over long developmental times.

E16

Myofibril contraction and cross-linking drive nuclear movement to the periphery of skeletal muscle.

W. Roman[1,2], J.P. Martins[2], F.A. Carvalho[2], R. Voituriez[3,4], J.V. Abella[5], N.C. Santos[2], B. Cadot[1], M. Way[5], E.R. Gomes[2];
[1]Sorbonne Universités, UPMC Univ Paris 06, , INSERM UMRS974, CNRS FRE3617, Center for Research in Myology, Paris, France, [2]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal, [3]Laboratoire de Physique Théorique de la Matière Condensée, CNRS UMR 7600; Université Pierre et Marie Curie, Paris, France, [4]Laboratoire Jean Perrin; CNRS FRE 3231, Université Pierre et Marie Curie, Paris, France, [5]Cellular Signalling and Cytoskeletal Function, The Francis Crick Institute, London, United Kingdom

Nuclear movements are important for multiple cellular functions and are driven by polarized forces generated by motor proteins and cytoskeleton. During skeletal myofiber formation or regeneration, nuclei move from the center to the periphery of the myofiber for proper muscle function. Centrally located nuclei are also found in different muscle disorders. Using theoretical and experimental approaches, we demonstrate that nuclear movement to the periphery of myofibers is mediated by

centripetal forces around the nucleus. These forces arise from myofibril contraction and cross-linking that “zip” around the nucleus in combination with tight regulation of nuclear stiffness by lamin A/C. In addition, an Arp2/3 complex containing Arpc5L together with γ-actin is required to organize desmin to cross-link myofibrils for nuclear movement. Our work reveals that centripetal forces exerted by myofibrils squeeze the nucleus to the periphery of myofibers.

E17

Regulating interactions between SUN and KASH proteins to mediate nuclear migration and anchorage.

N. Cain[1], Z. Jahed[2], H. Hao[1], M. Mofrad[2], G. Luxton[3], D.A. Starr[1];
[1]Molecular and Cellular Biology, University of California, Davis, Davis, CA, [2]Bioengineering and Mechanical Emgineering, University of California, Berkeley, Berkeley, CA, [3]Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN

The LINC complex, consisting of KASH proteins in the outer nuclear membrane and SUN proteins in the inner nuclear membrane mediates nuclear positioning to specific locations in a cell. Defects in LINC components result in failure to position nuclei during fertilization, cell migration, cell polarization, neuronal development, and muscle development. The cores of LINC complexes are the interfaces between KASH and SUN domains in the perinuclear space. However, the regulation of the SUN/KASH interaction is poorly understood. Crystal structures predicted that KASH peptides form three interfaces with SUN proteins. Our goal here is to test the functional consequences of disrupting these three interfaces. Significantly, we show that a conserved disulfide bond between SUN and KASH proteins plays an important role in the developmental switch between nuclear migration and anchorage. We use a three-pronged approach combining *in vivo C. elegans* developmental genetics, a functional assay for nuclear migration in polarizing tissue culture cells, and molecular dynamic simulations to better understand the molecular mechanisms for how SUN and KASH domains interact. The C-termini of KASH proteins are pointed into a SUN protomer forming the first SUN/KASH interface. We showed that extension of the KASH domain by a single alanine residue completely blocked the nuclear migration function of *C. elegans* UNC-83. At the second interface, KASH peptides then interact in a cleft between two SUN protomers. We showed that mutation of tyrosine at -7 to an alanine blocks UNC-83 function. Molecular modeling showed that the alanine is too short to reach a conserved region in of the SUN domain necessary for hydrogen-bond formation. Finally, before leaving for the outer nuclear membrane, KASH peptides stretch along the surface of a second SUN protomer and form a di-sulfide bond. Interestingly, the *C. elegans* nuclear migration KASH protein UNC-83 does not contain this third interaction domain, while the anchorage KASH ANC-1 and its mammalian ortholog Nesprin-2G does. Mutations of the conserved cysteines in SUN or KASH disrupted ANC-1 dependent nuclear anchorage in *C. elegans* and Nesprin-2G/SUN2 dependent nuclear movements in polarizing NIH3T3 fibroblasts. However, the SUN cysteine mutation did not disrupt UNC-83 function during nuclear migration. Finally, molecular dynamic simulations showed that the disulfide bond is necessary to transfer maximal forces from the cytoskeleton to the nucleoskeleton. Thus, the intermolecular disulfide bond is critically important for LINC complex-mediated mechanotransmission and the developmental switch from nuclear migration to nuclear anchorage.

E18

The LINC complex contributes to epithelial cell homeostasis.

V. Narayanan[1], P.T. Arsenovic[1], C. Mayer[1], G. Luxton[2], D.E. Conway[1];
[1]Biomedical Engineering, Virginia Commonwealth University, Richmond, VA, [2]Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN

Mechanical forces, both at cell-cell junctions and cell-matrix adhesions, have been shown to be important for regulating homeostatic processes of the epithelium, such as proliferation, collective cell migration, and 3D organization. More recently the nuclear LINC (linker of the nucleoskeleton and cytoskeleton) complex has emerged as another critical structural and mechanosensitive region of the cell. However, the majority of studies of the LINC complex have occurred in fibroblasts. We hypothesized that the LINC complex, both structurally and mechanically, was necessary for epithelial function. Using a previously developed nesprin-2G force biosensor, we confirmed that the LINC complex is subject to mechanical tension in 2D MDCK cell monolayers. We also observed that biaxial stretch increased the mechanical force applied across the LINC complex, indicating that externally applied forces affect LINC complex force. Additionally, we measured higher nesprin-2G force in 3D epithelial acini (grown in Matrigel) as compared to 2D monolayers. To disrupt the LINC complex, we developed an MDCK II cell line expressing inducible dominant negative (DN) KASH, a peptide which disrupts the endogenous nesprin-SUN interactions and blocks nuclear-cytoskeleton interactions. Cells expressing DN KASH exhibited slower migration speeds in response in a scratch wound cell migration assay, consistent with prior reports in fibroblasts. Expression of DN KASH in MDCK 3D acini cultures resulted in a rapid filling of the central lumen with cells, suggesting that the LINC complex is necessary for acini equilibrium. This filling of the lumen occurs without measurable increases in cellular proliferation, suggesting either a defect in apoptosis, migration, or cellular polarity. Taken together our results indicate that the LINC complex is a critical structure in the epithelium.

E19

Nanoscale Nuclear Envelope Dynamics and Spatial Organization of the Muscular Dystrophy Protein Emerin.

A. Fernandez[1], M. Bautista[2], T. Chung[1], F. Pinaud[1,2,3];
[1]Biological Sciences, University of Southern California, Los Angeles, CA, [2]Chemistry, University of Southern California, Los Angeles, CA, [3]Physics and Astronomy, University of Southern California, Los Angeles, CA

Emery-Dreifuss Muscular Dystrophy (EDMD) is a laminopathy caused by mutations of emerin, an inner nuclear envelope protein that participates to nucleo-cytoskeleton mechanotransductions, maintenance of nuclear shape and nucleus stiffening against applied tensions. The mechanosensing functions of emerin and the reasons why its mutations cause altered nuclear mechanics and muscle disease remain unclear. To address the molecular pathogenesis of EDMD at the nanoscale we used single molecule and three-dimensional superresolution optical microscopy in human cells. At the nuclear envelope, the diffusional mobility and the spatial organization of wild type (WT) emerin and a variety of clinically relevant emerin mutants were quantified by single particle tracking photo-activated localization microscopy (sptPALM) and direct stochastic optical reconstruction microscopy (dSTORM) imaging in rescued emerin-null cells. We identified different subpopulations of WT emerin associated with the endoplasmic reticulum, the outer or the inner nuclear envelope and show that it forms diffraction-limited nanoclusters at the nuclear membrane. We further show that the diffusion of emerin and its nanoscale clustering are directly impacted by mutations that cause EDMD and that a complex interplay

between lamin A binding, actin binding and emerin/emerin interactions dictate the dynamics and the nanoscale spatial distribution of emerin at the nuclear envelope. To study how specific mechanical strains at the nuclear membrane influence the normal and pathogenic nanoscale organizations of emerin, we also developed a simple cell micropatterning strategy that provides control of the nucleus architecture and allows steady-state changes in the mechanical landscape of the nuclear envelope while permitting nanometer accuracy single molecule microscopy. Super-resolution imaging of emerin in nuclei subjected to varying mechanically strains reveals that the mechanotransducing functions of emerin are coupled to its clustering state and its nanoscale distributions within the inner nuclear envelope. Together, sptPALM, dSTORM superresolution imaging, emerin mutants and cell micropatterning reveal subtle biophysical properties of nuclear emerin that would otherwise be undetected by traditional diffraction-limited microscopy techniques and help elucidate key molecular mechanisms of EDMD.

E20

High-throughput FISH-based screening approaches to identify regulators of 3D genome organization.

E. Joyce[1,2], L.F. Rosin[1], S. Nguyen[1];
[1]University of Pennsylvania, Philadelphia, PA, [2]Penn Epigenetics Institute, Philadelphia, PA

Eukaryotic genomes encode genetic information in their linear sequence, but appropriate expression of their genes requires chromosomes to fold into complex and spatially distinct three-dimensional structures. However, despite the remarkable conservation of these organizational features and their impact on gene function, we have a very limited understanding of how chromosomes are spatially partitioned, functionally packaged, and relatively positioned in the nucleus. To address this problem, our previous work involved the development of two technologies that use fluorescent in situ hybridization (FISH) to interrogate chromosome positioning at single-cell resolution. The first is a technology for high-throughput FISH (Hi-FISH), and the other, called Oligopaints, is a new type of probe that reduces the cost and increases the resolution of FISH. Using a novel combination of these tools, we are conducting imaging-based screens for novel architectural proteins in Drosophila cells. This work has provided new insights into how chromosomes find and influence each other in the nucleus. In particular, we have isolated the highly conserved condensin II complex as a central organizing factor that can drive chromosome territory formation and epigenome compartmentalization through large-scale chromatin folding during interphase. These results have highlighted a mechanistic relationship between intra- and interchromosomal interactions in general, which we are further exploring through the characterization of additional architectural proteins.

E21

Mutations in nucleoporin NUP88 cause lethal neuromuscular disorder.

E. Bonnin[1,2], P. Cabochette[2], M. Hezwani[1], B. Vanhollebeke[2], B. Fahrenkrog[1];
[1]IBMM, Biology of the Cell Nucleus, Universite Libre de Bruxelles, Charleroi, Belgium, [2]IBMM, Laboratory of Neurovascular Signaling, Universite Libre de Bruxelles, Charleroi, Belgium

Fetal movement is a prerequisite for normal fetal development and growth. Intrauterine movement restrictions cause a broad spectrum of disorders in which the unifying feature is a reduction or lack of fetal movement, giving rise to the term fetal akinesia deformation sequence (FADS). FADS corresponds to a clinically and genetically heterogeneous condition, characterized by multiple joint contractures, facial abnormalities, and lung hypoplasia as a result from decreased in utero movement of the babies.

Affected individuals are often prematurely and stillborn, and those born alive typically die within minutes or hours after birth. The genetic causes for this fatal disorder are ill defined, but FADS often results from mutations in genes affecting the muscle nicotinic acetylcholine receptor (AChR). Here we report mutations in the nucleoporin *NUP88* as a novel cause of lethal FADS. FADS-related mutations in *NUP88* lead to a loss-of-function phenotype. Genomic disruption of *nup88* in zebrafish results in developmental defects that resemble those seen in affected human cases, including facial abnormalities and reduced movement. We provide evidence that loss-of-function mutations in NUP88 impair NFkB activity, which in consequence affects gene expression of a key player in AChR formation. We hereby present the first lethal developmental human disorder due to mutations in a *bona fide* nuclear pore protein.

Microsymp 4: Cancer Cell Biology

E22

Metastasis by tumor epithelial clusters requires E-cadherin expression.

V. Padmanaban[1], A.J. Ewald[1];
[1]Departments of Cell Biology and Oncology, Center for Cell Dynamics, Johns Hopkins University School of Medicine, Baltimore, MD

Introduction : Metastasis is the major driver for cancer related deaths. The genetic/ epigenetic loss of E-cadherin (E-cad) is linked to increased invasion of cancer cell lines and therefore, has been inferred to promote metastasis. Consistent with this concept, invasive lobular breast cancer cells lack E-cad expression and commonly metastasize. However, contrary to this concept, more than 80% of breast cancers are invasive ductal and these tumors retain their E-cad expression in both the primary tumor and distant metastases. We recently demonstrated that cancer cell clusters expressing E-cad contribute to >95% of metastases arising from these tumors. The current study aims to dissect the direct functional contribution of E-cad towards metastasis.
Results : To address this question, we used MMTV-PyMT mice, a frequently used metastatic model of invasive ductal carcinoma. We bred these mice to also include additional transgenes that allow for a Cre-inducible, GFP reported deletion of E-cad. Primary tumor organoids isolated from these mice were infected with Adeno-Cre recombinase to delete E-cad. We observe that loss of E-cad expression results in increased invasion and dissemination in 3D organotypic culture. Disseminated cells typically lack E-cad, but retain expression of other epithelial markers such as cytokeratins. To test the requirement of E-cad expression for metastasis, we transplanted E-cad deficient tumor organoids into immunocompromised host mice.Tumors in these mice grow slower but have a more invasive front compared to tumors with intact E-cad expression. Interestingly, however, cancer cells that have lost E-cad do not contribute to any observable metastases. From *in-vivo* tail vein assays, we concluded that E-cad null cancer cells are defective at seeding distant organs. To then test if E-cad expression promotes survival of cancer cells, we performed colony formation assays using flow sorted cancer cells. E-cad null cancer cells show a 7-fold decrease in colony forming potential. We are currently investigating molecular signaling alterations downstream of E-cad deletion that confer a decreased metastatic potential.
Conclusions: Consistent with previous reports, loss of E-cad increases tumor invasion and dissemination. However, our results reveal that E-cad loss in cancer cells can inhibit metastatic colonization. Interestingly, we observe an uncoupling of the relative efficiency of local invasion/ dissemination and distant metastatic outcome. **We propose an alternate route of collective epithelial metastasis in which cancer cells need to maintain gene expression consistent with their epithelial origin to efficiently metastasize.**

E23

Leader cells are defined by DNA hypermethylation and aberrant gene expression during collective lung cancer invasion.

E.R. Summerbell[1,2], J. Bell[3,4], J. Konen[1,2], J. Kowalski[5], P.M. Vertino[4], A.I. Marcus[2];
[1]Graduate Program in Cancer Biology, Emory University, Atlanta, GA, [2]Department of Hematology and Medical Oncology, Emory University, Atlanta, GA, [3]Graduate Program in Genetics and Molecular Biology, Emory University, Atlanta, GA, [4]Department of Radiation Oncology, Emory University, Atlanta, GA, [5]Department of Biostatistics and Bioinformatics, Emory University, Atlanta, GA

Collective cancer cell invasion, wherein packs of cancer cells invade together, is present in patient samples of most solid tumor types and contributes to cancer metastasis. Within these collective invasion packs, highly-invasive leader cells pioneer migration and invasion while highly-proliferative follower cells travel behind them. Our lab previously developed an image-guided genomics technique to isolate and culture individual leader and follower cells, termed Spatiotemporal Genomic and Cellular Analysis (SaGA). Since epigenetic mechanisms, such as DNA methylation, regulate phenotypic plasticity and cell differentiation in many cellular contexts, we hypothesize that leader and follower cell phenotypes can emerge through epigenetic reprogramming of lung cancer cells. We performed DNA methylome profiling using a DNA methylation array wherein leader cells showed significant global DNA hypermethylation compared to both follower cells and the H1299 lung adenocarcinoma parental population. Furthermore, integrating DNA methylome analysis with RNAseq analysis identified gene expression patterns unique to leader cells that correlate with these changes in DNA methylation. Differentially methylated CpG islands overlapping promoters correlated with significant gene silencing or overexpression of 57 genes in leader cells, including multiple putative tumor suppressors and oncogenes. In addition, leader cells showed differential methylation at FANTOM5 enhancers correlated with many gene ontology pathways critical for collective cancer invasion, including VEGF signaling, which we have previously shown to be critical for collective pack formation. In addition, inhibition of DNA methylation using 5-aza-2'-deoxycytidine (DAC) significantly abrogated collective invasion of 3-D spheroids of H1299 parental cells and even more significantly in spheroids of purified leader cells. DAC treatment also rescued expression of genes in leader cells that had little to no gene expression and high promoter DNA methylation compared to parental and follower cells. Our data suggest a mechanism wherein global DNA hypermethylation can drive the leader cell phenotype and wherein DNA methylation regulates gene expression critical for leader cell behavior. Subsequent validation of differentially methylated target genes may determine functionally-relevant genes driving leader cell-dependent collective cancer invasion.

E24

Loss of MTSS1 results in increased metastatic potential in pancreatic cancer.

A.E. Zeleniak[1,2], W. Huang[2,3], M.K. Brinkman[2,3], R. Hill[2,3];
[1]Integrated Biomedical Sciences, University of Notre Dame, South Bend, IN, [2]Harper Cancer Research Institute, South Bend , IN, [3]Biological Sciences, University of Notre Dame, South Bend, IN

Pancreatic ductal adenocarcinoma (PDAC) has a 5-year survival rate of 7%. This dismal outlook is largely due to the inability to diagnose the disease before metastasis occurs. 53% of patients afflicted with pancreatic cancer are diagnosed at the metastatic stage. Thus, there is a critical need to better understand what causes early tumor cell dissemination and metastatic progression in this disease. One of the hallmarks of early stages of PDAC is inflammation. It is well known that patients with chronic

pancreatitis have a much higher chance to develop PDAC. While many studies have focused on elucidating the mechanisms by which this inflammation drives PDAC progression, few have focused on the role inflammation plays in metastasis. Using a novel inflammation-driven mouse model of PDAC, we were able to uncover a subset of genes that were not only regulated by increased inflammation, but also correlated with poor PDAC patient prognosis. Here, we functionally test the role of one of the genes found in this subset that is involved in metastatic progression, namely, metastasis suppressor protein 1 (MTSS1). We show that loss of MTSS1 leads to increased invasion and migration in PDAC cell lines. Moreover, PDAC cells treated with cancer-associated fibroblast-conditioned media also have increased metastatic potential, which is augmented by loss of MTSS1. Additionally, overexpression of MTSS1 in PDAC cell lines leads to a loss of migratory potential *in vitro* and an increase in overall survival *in vivo.* Furthermore, we present a novel regulatory mechanism for the stabilization of MTSS1 via the tumor suppressor protein, phosphatase and tensin homolog (PTEN). Our data show that PTEN loss in PDAC cells results in both increased metastatic potential *in vitro,* and in a decrease in MTSS1 expression. Furthermore, we show that ectopic MTSS1 expression rescues this effect. Additionally, we demonstrate that PTEN forms a complex with MTSS1 in order to stabilize it from proteasomal degradation. Finally, we show that the inflammatory tumor microenvironment, which makes up over 90% of PDAC tumor bulk, is capable of downregulating PTEN expression, potentially uncovering a novel extrinsic, upstream mechanism of MTSS1 regulation. Collectively, these data offer new insight into not only the role and regulation of MTSS1in suppressing tumor cell invasion and migration, but also a different glimpse as to what molecular mechanisms could be leading to early cell dissemination in PDAC.

E25

Identification of drivers of Chromosome Instability in Breast Tumors.

K. Pfister[1], J.L. Pipka[1], C. Chiang[2], R. Clark[1], M.J. Guertin[1], I. Hall[2], T. Stukenberg[1];
[1]Biochemistry and Molecular Genetics, University of Virginia, School of Medicine, Charlottesville, VA,
[2]McDonnell Genome Instititute, Washington University, St. Louis, St Louis , MO

Numerical Chromosomal instability (CIN) is a form of genomic instability characterized by an increased rate of chromosome missegregation. It is unclear how cancer cells acquire the CIN phenotype. We have developed a simple computational method that measured the degree of aneuploidy or structural rearrangements of large chromosome regions of >500 human breast tumors from TCGA data to identify the drivers of CIN. RNA analysis demonstrates that aneuploid tumors overexpressed regulators of mitosis, including proteins in the centromere signaling network and key kinetochore proteins. Aneuploid tumors also overexpress regulators of the DREAM complex that regulates the transcription of mitotic genes. Overexpression of the DREAM regulators E2F1, MYBL2 and FOXM1 was sufficient to increase the rate of lagging anaphase chromosomes in a nontransformed vertebrate epithelial tissue validating the results of our computational analysis of human tumors and connecting our aneuploidy measurements to CIN. Highly aneuploid human breast tumors were also highly enriched in TP53 mutations. There was strong co-association of DREAM regulators with TP53 mutations in human breast tumors suggesting that tumors require both events to gain evolutionary fitness. Our data suggest a two-step model for the generation of CIN in breast tumors. First, the overexpression of DREAM complex regulators overexpresses key mitotic regulators to generate anaphase lagging chromatids at higher rates. Second, loss of p53 function allows cells that have missegregated chromosomes to escape the resulting induction of senescence or cell death allowing cells with aneuploidy to undergo selection within the tumor.

E26

Prdm14-containing Protein Complexes Regulate Chromatin in Stem Cells, Development and Cancer.

N. Nady[1], A. Gupta[2], N. Arora[1], T. Swigut[1], A. Koide[2], S. Koide[2], J. Wysocka[1];
[1]Department of Chemical and Systems Biology, Stanford University, Stanford, CA, [2]Department of Biochemistry and Molecular Pharmacology, New York University, New York, NY

Prdm14 is a sequence-specific transcriptional regulator of embryonic stem cell (ESC) pluripotency and primordial germ cell (PGC) formation. In addition to understanding the molecular pathways governing the normal development of PGCs, it is pivotal to know what goes awry in the disease setting. Remarkably, PRDM14 is repressed in normal adult somatic tissues and its aberrant overexpression is associated with tumor initiation in a wide variety of cancers, with the highest levels observed in germ cell tumors. Prdm14 exerts its function, at least in part, through repressing genes associated with epigenetic modification and cell differentiation. Here, we show that this repressive function is mediated through an ETO-family co-repressor Mtgr1 (Cbfa2t2), which tightly binds to the pre-SET/SET domains of Prdm14 and co-occupies its genomic targets in mouse ESCs. We generated two monobodies, synthetic binding proteins, targeting the Prdm14 SET domain and demonstrate their utility, respectively, in facilitating crystallization and structure determination of the Prdm14-Mtgr1 complex, or as genetically encoded inhibitor of the Prdm14-Mtgr1 interaction. Structure-guided point mutants and the monobody abrogated the Prdm14-Mtgr1 association and disrupted Prdm14's function in mESC gene expression and PGC formation in vitro as well as germ cell cancer lines. Altogether, our work uncovers the molecular mechanism underlying Prdm14-mediated repression and provides renewable reagents for studying and controlling Prdm14 functions in cancer setting paving the way for the development of new PRDM14 therapeutics.

E27

Analysis of the Nature of Paclitaxel Resistance in APC Knockdown Breast Cancer Cells.

B.J. Berkeley[1,2,3,4], A.H. Arnason[2,3], J.R. Prosperi[1,2,3];
[1]Biochemistry and Molecular Biology, Indiana University School of Medicine, South Bend, IN, [2]Biological Sciences, University of Notre Dame, South Bend, IN, [3]Harper Cancer Research Institute, South Bend, IN, [4]Smurfit Institute of Genetics , Trinity College Dublin, Dublin, Republic of Ireland

Adenomatous Polyposis Coli (APC) is a multi-domain tumor suppressor protein that binds to proteins including β-catenin, axin, and microtubules (MTs). APC is lost in many epithelial cancers and up to 70% of sporadic breast cancers, with a tendency towards triple negative breast cancers (TNBCs). In a mouse breast cancer model of APC loss, MMTV-PyMT;ApcMin/+, our laboratory previously demonstrated that APC loss resulted in metaplastic-like tumors, a subtype of TNBCs that often develops resistance to chemotherapy. Using the human breast cancer cell line, MDA-MB-157, we created APC knockdown cells (APCKD) using lentiviral mediated shRNA knockdown of APC, which demonstrated resistance to the Taxane family chemotherapeutic agent, Paclitaxel. This APCKD model has an increased proportion of tumor initiating cells (TICs), a subpopulation of highly tumorigenic cells found in most cancers that are resistant to traditional chemotherapeutic agents. Given that Taxanes and APC both alter MT dynamics, we sought to understand the molecular mechanisms of APC-mediated resistance. Based on RNA-seq data, we hypothesized that genes involved in the G2/M transition or those co-identified in PTX treated APCKD cells and a TIC signature list would be responsible for PTX resistance. We used three approaches to examine the molecular architecture and cell cycle phase pattern of Paclitaxel resistant APCKD cells: Western blots, RT-qPCR and flow cytometry. Four genes were identified as being selectively regulated by

PTX in the APCKD cells and overlapping with the TIC signature (PLEKHG2, GPR37, MLF1, and IGSF3). RT-qPCR confirmed the RNA-seq data, and future studies will investigate the effect of manipulating expression of these genes. Western blot analysis of the checkpoint proteins CDK1 (Thr14, Thr161, Tyr15) and Cyclin B1 showed no significant difference in expression between the parent MDA-MB-157 and APCKD cells after PTX treatment. Finally, cell cycle analysis demonstrated that PTX treatment of APCKD cells resulting in G2/M arrest similar to the parent cells. Combined, this suggests that the PTX resistance of APCKD cells is not mediated by the G2/M checkpoint but by an alternative mechanism. We propose that APCKD cells evade apoptosis induced by cell cycle arrest in G2/M by utilizing mutant proteins, such as kinases or phosphatases, which interact with checkpoint proteins directly. As the functionality of checkpoint proteins hinges on their phosphorylation profile, an examination of this in APCKD cells and parent cells may explore this as a potential PTX resistance mechanism. By understanding the molecular nature of PTX resistance in APCKD cells, a prospective therapeutic target may be identified to work towards a targeted treatment for some TNBC types.

E28

HPV oncoproteins cause specific types of chromosomal instability in head and neck cancer.

L.C. Funk[1,2,3], D.L. Lee[3], P.F. Lambert[3,4], R.J. Kimple[4,5], B.A. Weaver[1,2,3];
[1]Cell and Regenerative Biology, University of Wisconsin-Madison, Madison, WI, [2]Molecular and Cellular Pharmacology, University of Wisconsin-Madison, Madison, WI, [3]Oncology, University of Wisconsin-Madison, Madison, WI, [4]Carbone Cancer Center, University of Wisconsin-Madison, Madison, WI, [5]Human Oncology, University of Wisconsin-Madison, Madison, WI

Aneuploidy, a karyotype that differs from a multiple of the haploid, is a hallmark of cancer. Aneuploidy can be caused by an ongoing rate of chromosome missegregation during mitosis, known as whole chromosomal instability (CIN). Work from our lab and others has shown that while low rates of CIN promote tumorigenesis, high levels of CIN can be tumor suppressive. Human papillomavirus (HPV), specifically HPV subtype 16, is a growing cause of head and neck cancer worldwide. In cervical cancer, HPV is associated with CIN, which has been attributed largely to the expression of its E6 and E7 oncoproteins. HPV+ head and neck cancer patients show improved responses to radiation therapy than HPV- head and neck patients, potentially due to an increased basal rate of CIN. Here, we tested the ability of HPV E6, E7, or E6+E7 expression to induce CIN in normal oral keratinocytes (NOKs). Expression of E6 alone robustly induced misaligned chromosomes and multipolar spindles with supernumerary centrosomes, while E7 expression alone produced more moderate increases. Combined expression of E6 and E7 exacerbated the phenotypes observed in NOKs expressing E6 alone. Interestingly, HPV+ patient-derived xenograft (PDX) models of head and neck squamous cell carcinoma showed less evidence of mitotic defects than NOKs expressing E6 alone. A subset of HPV+ PDX tumors showed an increase in misaligned chromosomes when compared to HPV- tumors, but no increase in lagging chromosomes was observed. Together, these data suggest HPV infection causes an incompletely penetrant increase in specific types of CIN in head and neck cancer.

Microsymp 5: Cell Death, Cell Volume and Cytokinesis

E29

Stem Cell Proliferation is Induced By Engulfment of Apoptotic Bodies from Adjacent Dying Cells During Epithelial Tissue Maintenance.

C.K. Brock[1], S.T. Wallin[1], A. Mandal[1], E.A. Sumner[1], G.T. Eisenhoffer[1];
[1]Department of Genetics, The University of Texas MD Anderson Cancer Center, Houston, TX

Epithelial tissues require the removal and replacement of damaged or defective cells to sustain a functional barrier. Apoptotic cells provide instructive cues that can influence surrounding cells to proliferate. Yet, how dying cells transmit these signals to their healthy neighbors to control individual cellular behaviors and population dynamics during tissue homeostasis remains poorly understood. Here we show that dying stem cells facilitate communication with adjacent stem cells by caspase-dependent production of WNT8a-containing apoptotic bodies to drive cellular turnover in a living epithelial tissue. Basal stem cells engulf the extracellular apoptotic bodies, activate WNT signaling, and are stimulated to undergo division to maintain tissue-wide cell numbers. Inhibition of either cell death or WNT signaling eliminated the apoptosis-induced cell division, while overexpression of WNT8a signaling combined with increased cell death led to an expansion of the stem cell population. We conclude that ingestion of apoptotic bodies represents a novel regulatory mechanism linking death and division to maintain overall stem cell numbers, and thus, is key for epithelial tissue homeostasis.

E30

Two small GTPases function antagonistically in corpse removal of a developmental non-apoptotic dying cell.

L.M. Kutscher[1], W. Keil[1], S. Shaham[1];
[1]Laboratory of Developmental Genetics, The Rockefeller University, New York, NY

Programmed cell death and subsequent cell clearance are necessary in the development and maintenance of organisms and tissues. Defects in cell clearance can result in inappropriate inflammatory responses and autoimmune disorders. Much is known about apoptotic corpse removal; however, our understanding of mechanisms driving the clearance of cells dying by non-apoptotic means is rudimentary. Linker cell death in *C. elegans* is an excellent model of non-apoptotic cell death. The linker cell is a male-specific cell that is born in the second larval stage, leads the elongation of the developing gonad, and then dies in a programmed manner independently of known apoptosis, autophagy, or necrosis genes. Importantly, linker cell corpses are robustly engulfed in mutants lacking the capacity to engulf apoptotic bodies, suggesting utilization of a novel engulfment program. To elucidate the mechanism of linker cell corpse removal, we carried out a forward genetic screen to identify mutants that are defective in this process. F2 mutant males were propagated by artificial insemination, as surviving linker cells or corpses may block spermatid exit and promote male sterility. From this screen, we isolated two loss-of-function mutants in the RAB-35 guanine nucleotide exchange factor (GEF), *rme-4*, and a gain-of-function mutant in the small GTPase, *arf-6*. Using a variety of genetic and cell biological methods, we have uncovered the relevant GEFs, and GTPase activating proteins (GAPs) for RAB-35 and ARF-6 in this process, and have determined that these two proteins and their regulators have antagonistic functions; RAB-35 promotes corpse clearance, and ARF-6 inhibits it. Using a microfluidic imaging-device we developed, we imaged the dynamics of these proteins over a 20h period, capturing linker cell migration, engulfment, and degradation. We found that YFP-RAB-35 is localized to the extending pseudopod membrane, and remains at the membrane for the duration of phagosome

maturation. While ARF-6-YFP is also localized to the extending pseudopods, it changes localization from the plasma membrane to intracellular vesicles after engulfment is complete. These results, together with our genetic and protein interaction studies, suggest that ARF-6 and RAB-35 localize to the plasma membrane during corpse recognition and engulfment, but RAB-35 then inactivates ARF-6, likely through its GAP CNT-1, to allow phagolysosome maturation to proceed. Rab35 and Arf6 have been previously implicated together in the recognition and removal of foreign particles in mammals, but not in the degradation of programmed cell death corpses. *rab-35* and *arf-6* may work together to preferentially degrade corpses that die by non-apoptotic mechanisms.

E31

Cell Tension and Mechanical Regulation of Cell Volume.

N.P. Gonzalez[1], J. Tao[2], N.D. Rochman[1], K. . Guan[3], D. Wirtz[1], S.X. Sun[1,2];
[1]Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, [2]Mechanical Engineering, Johns Hopkins University, Baltimore, MD, [3]Pharmacology, UC San Diego, La Jolla, CA

Animal cells use an unknown mechanism to control their growth and physical size. Here, using the fluorescence exclusion method, we quantitatively measure cell volume for adherent cells on substrates of varying stiffness in stationary culture. We discover that the cell volume has a complex dependence on substrate stiffness, but is correlated with the size of the cell adhesion to the substrate. From a mechanical force balance condition that determines the geometry of the cell surface, we find that the observed cell volume variation can be quantitatively explained by the distribution of active myosin throughout the cell cortex. To connect cell mechanical tension with cell size, we quantified the nuclear localization of YAP, a transcription factor involved in cell growth and proliferation. We find that the level of nuclear YAP is positively related to the average cell volume. Moreover, the level of nuclear YAP is also connected to cell tension, as measured by the amount of phosphorylated myosin. Cells with greater apical tension corresponds to higher levels of nuclear YAP and cell volume.
To further quantify the role of YAP and the Hippo pathway in regulating cell growth, we monitored growth of live cells over many hours to measure their growth and division rates. Results show that YAP knockout cells exhibit overall slower growth rates and a greater tendency to transition into quiescent state (G0) than the WT cells. In contrast, LATS knockout cells show a faster growth rate and reduced transition into the G0 state. YAP knockout cells are also smaller in volume while LATS knockout cells are larger in volume.
Taken together, these results point to a size-sensing mechanism based on cell mechanical tension: the cell tension increases as the cell grows, and increasing tension biochemically feeds back to the Hippo pathway to regulate growth and proliferation control.

E32

A cdc2 homolog is required for completion of oral development during regeneration and cell division in the giant ciliate *Stentor coeruleus*.

S.B. Reiff[1], W.F. Marshall[1];
[1]Biochemistry Biophysics, UCSF, San Francisco, CA

The giant unicellular ciliate *Stentor coeruleus* has the ability to fully regenerate after being cut into pieces, in a way that perfectly preserves the original polarity and structure. This regeneration ability is particularly remarkable because *Stentor* has a complex cortical architecture, with longitudinal ciliary rows running between an oral apparatus at the anterior end and a holdfast at the posterior end. The molecular details behind this incredible phenomenon have remained largely unstudied, and we wish to

understand how the regeneration process is coordinated at the molecular level. To identify candidates for RNAi knockdown we analyzed the kinome of *Stentor*. *Stentor* was found to encode over 2000 kinases, making up 6% of the total protein coding genes. Many of these consist of expansions in mitotic kinase families such as CDKs, PLKs, Auroras, NDRs, and NEKs. As some of the details of the regeneration process parallel the events of cell division, we wish to understand whether the cell co-opts cell division signaling pathways to initiate and control regeneration. To begin to investigate the role of the cell cycle in regeneration, we performed RNAi knockdown of CDK1/cdc2 homologs in *Stentor*. For some of the cdc2 genes, knockdown resulted in slowed division with daughter cells of strikingly unequal size. For other homologs, however, knockdown results in cells unable to complete division. In these cases, the cells are still able to initiate oral development but ultimately exhibit aberrant oral morphology, both prior to division and during regeneration. We conclude that the oral regeneration process employs CDK signaling pathways. In future work we aim to understand the full extent to which *Stentor* uses cell cycle signaling pathways to coordinate and control regeneration.

E33

A novel correction mechanism regulates nuclear position and ensures proper DNA segregation during late cytokinesis.

A. Pacquelet[1], M. Jousseaume[1], G. Michaux[1];
[1]Institut de Génétique et de Développement de Rennes, CNRS, Université Rennes 1, Rennes, France

Ensuring correct DNA segregation is an essential feature of cell division which relies on the proper assembly of the mitotic spindle and its coordination with the cytokinetic machinery. Here we present a novel mechanism which corrects DNA segregation defects due to cytokinetic furrow mispositioning.
We recently showed that tight regulation of myosin is required to coordinate furrow and spindle positions during the first division of *C. elegans* embryos: abnormal accumulation of myosin at the anterior cortex induces a strong displacement of the furrow towards the anterior, thereby uncoupling cytokinetic furrow and spindle positions and leading to DNA segregation defects (Pacquelet et al;, J Cell Biol, 210,1085). However, we unexpectedly found that these DNA segregation defects can be corrected at the end of cytokinesis. This correction occurs when the mitotic spindle midzone is being disassembled and after nuclear envelop reformation. It relies on the concomitant displacement of the furrow and of the anterior nucleus towards the posterior and anterior poles, respectively. Genetic experiments demonstrated that the displacement of the anterior nucleus requires the interaction of the nucleus with microtubules as well as myosin activity. We also found that this displacement coincides with an anteriorly directed flow of cytoplasmic particles and with the curvature of the ingressing cytokinetic furrow, likely revealing the existence of a higher tension in the posterior region of the embryo compared to the anterior region. Importantly, both cytoplasmic flow and membrane curvature require myosin activity, suggesting that myosin contributes to nuclear displacement by regulating intracellular tensions and cytoplasmic flow.
Altogether, our work reveals the existence of a so far undescribed correction mechanism which ensures that DNA segregation defects due to the mispositioning of the cytokinetic furrow are corrected during late cytokinesis. This correction involves the regulation of nuclear position by the concomitant action of microtubules and myosin and is critical to ensure the robustness of cell division.

E34

FLIRT: Fast local infrared thermoptogenetics for spatiotemporal control of ts protein function during cytokinesis.

S. Hirsch[1], S. Sundaramoorthy[2], Y. Zhuravlev[1], T. Davies[2], J.C. Waters[3], M.M. Shirasu-Hiza[1], J. Dumont[4], J.C. Canman[2];
[1]Genetics and Development, Columbia University Medical Center, New York, NY, [2]Pathology and Cell Biology, Columbia University Medical Center, New York, NY, [3]Cell Biology, Harvard Medical School, Boston, MA, [4]Cell Division and Reproduction, Institut Jacques Monod, Paris, France

We developed FLIRT (Fast Local InfraRed Thermoptogenetics) to manipulate fast-acting temperature-sensitive (ts) protein function at precise times and locations during complex cellular processes. Molecular control of complex and transient cellular behavior such as cell motility or cell division involves a high degree of spatiotemporal regulation, which is difficult to study using traditional genetic approaches. Fast-acting (≤20 sec) ts mutants permit studies of the temporal regulation of protein function by simply shifting to the restrictive temperature to conditionally inactivate protein function during the complex cellular behavior of interest. To harness the power of fast-acting ts mutants for spatiotemporal studies, FLIRT works by focusing an infrared laser at distinct sub-cellular structure(s) or on specific cells to locally heat and inactivate ts proteins at precisely defined moments during a complex cellular behavior while simultaneously monitoring the kinetic effects on that process in vivo. Here we use FLIRT to probe the spatiotemporal regulation of the core cytokinesis machinery in worms; however, FLIRT is applicable to any cellular or developmental process in any model system accessible by light microscopy. Cytokinesis is the physical division of one cell into two that occurs at the end of the cell cycle. We use FLIRT in *C. elegans* to study the spatiotemporal regulation of cytokinesis using our growing collection of fast-acting and reversible ts cytokinesis-defective mutants. Cytokinesis is driven by constriction of a contractile ring composed of diaphanous formin-nucleated f-actin and the motor myosin-II. We found that FLIRT irradiation of half of the contractile ring in *formin(ts)* or *myosin-II(ts)* mutant embryos halts ring constriction on the irradiated side of the cell, but not on the non-irradiated side. This suggests the contractile ring is made up of individual contractile units that can function independent of a full contractile ring. FLIRT irradiation does not inhibit cytokinesis in control embryos lacking ts mutations, or in *formin(ts)* and *myosin-II(ts)* embryos irradiated outside of the division plane (in the polar cell cortex). Further, FLIRT is reversible and cytokinesis can complete when the IR laser is turned off mid-way through contractile ring constriction in *myosin-II(ts)* mutants. These results highlight the high degree of rapid and reversible spatial control that can be achieved with FLIRT. We are currently using FLIRT to test the contributions of subcellular regions of actomyosin contractility in cytokinesis and expanding our analysis to other fast-acting ts cytokinesis-defective mutants.

E35

Precise tuning of cortical contractility regulates mechanical equilibrium during cell division.

N. Taneja[1], M.R. Bersi[2], A.M. Fenix[1], J.C. Snider[2], J.A. Cooper[1], R. Ohi[1], V. Gama[1], W.D. Merryman[2], D.T. Burnette[1];
[1]Cell and Developmental Biology, Vanderbilt University, Nashville, TN, [2]Biomedical Engineering, Vanderbilt University, Nashville, TN

The animal cell cortex comprises a thin network of actin filaments underneath the plasma membrane. Contractile force generation by myosin II (MII) in the cortex drives shape changes during cell division, cell migration and tissue morphogenesis. During the cytokinetic (C)- phase of cell division, large contractile forces and cortical remodeling are required at the equatorial cortical network to drive furrow

ingression. Outward pressures created by furrow ingression must be balanced by MII contractility at the polar cortical network. The roles for the two MII isoforms, MIIA and MIIB, in the establishment of a mechanical equilibrium between these two cortical networks are not understood. We found MIIA depletion resulted in slower cleavage furrow ingression, and a loss of both MIIA and MIIB filaments in the furrow, with no changes at the polar cortex. MIIB depletion, in turn, resulted in intense blebbing at the polar cortex, with no changes in furrow ingression. We predicted the cortex of MIIB depleted cells should be softer; however, AFM measurements revealed no changes in stiffness. This suggested molecular scale behaviors may be driving cortical mechanics, which may not be reflected in macroscopic measurements such as cell stiffness. We therefore sought to develop a robust coarse-grained mathematical description of cortical behavior, incorporating experimentally measured kinetic parameters. Measurement of localization patterns and lifetimes of MII isoforms during C-phase revealed MIIA had faster turnover and more transient kinetics compared to MIIB. Modeling the cortex based on active gel theory surprisingly revealed no significant differences in outward pressure and cortical tension in the two knockdown conditions. Instead, our model predicted differences in the duty ratios of MII isoforms and total contractility must account for the observed differences in cortical stability in the two knockdown conditions. We verified these predictions using both MII chimeras and biophysical assays. We further found Rho kinase and Myosin Light Chain Kinase regulated the recruitment and turnover of MII isoforms, respectively. Our working model posits that in the unperturbed state, competition between MIIA and MIIB regulates cortical turnover, while competition between MLCK and ROCK regulates the turnover of MII itself. Depletion of either isoform leads to compensation at the polar cortex, driving the cell to extremes of contractility, with MIIA and MIIB depletion leading to hypo- and hyper-contractility, respectively, leading to cytokinetic failure. Therefore, our data support a model where an intermediate level of contractility leads to efficient cytokinesis, allowing MIIA driven ingression at the equator balanced by MIIB driven tension generation at the poles.

Microsymp 6: Cellular Regulation of the Cytoskeleton

E36

Non-centrosomal microtubules and not the centrosome control endothelial cell polarity and sprouting angiogenesis.

M. Martin[1], A. Veloso[2], J. Wu[1], F. Dequiedt[2], E. Katrukha[1], A. Akhmanova[1];
[1]Cell Biology, Utrecht University, Utrecht, Netherlands, [2]GIGA-Molecular Biology in Diseases , University of Liège, Liege, Belgium

Microtubules regulate cell polarity during diverse processes such as directional migration. In dividing mammalian cells, microtubules are traditionally believed to be organized into a radial, centrosomally-anchored network where the relative positioning of the centrosome and the nucleus has been long considered to play a pivotal role in setting up asymmetry. Here, by using sprouting angiogenesis as a paradigm of a polarised physiological process, we deciphered the contribution of centrosomal and non-centrosomal microtubules to controlling cell asymmetry. In strong contradiction with the crucial role attributed to the centrosome in setting up polarity, we showed that the loss of centrosomes had no effect on the ability of endothelial cells to polarize and move in 2D and 3D environments. In contrast, by silencing the microtubule minus-end-stabilizing protein CAMSAP2, we uncovered a key function for non-centrosomal microtubules in establishing endothelial cell polarity during the process of sprouting angiogenesis. Non-centrosomal microtubules controlled Golgi positioning and trafficking, 2D directional migration and formation of large persistent protrusions in 3D. Moreover, by interfering with CAMSAP2 recruitment to the Golgi apparatus, we showed that although Golgi-tethered microtubules are

important for regulating polarity, other non-centrosomal microtubules could also contribute to polarized cell sprouting in 3D. Importantly CAMSAP2 was also required for persistent endothelial cell sprouting during *in vivo* zebrafish vessel development. In the absence of CAMSAP2, cell polarization in 3D could be partly rescued by centrosome depletion, indicating that the centrosome acts as an inhibitor of cell polarity. We propose that CAMSAP2-protected non-centrosomal microtubules are needed for breaking the symmetry imposed by the radial centrosome-anchored microtubule array to enable microtubule enrichment in a single cell protrusion.

E37

Non-random γ-TuNA-dependent spatial patterning of microtubule nucleation at the Golgi.

A.A. Sanders[1], K. Chang[1], X. Zhu[1], R.J. Thoppil[1], W.R. Holmes[2], I. Kaverina[1];
[1]Cell and Developmental Biology, Vanderbilt University, Nashville, TN, [2]Physics and Astronomy, Vanderbilt University, Nashville, TN

Non-centrosomal microtubule (MT) nucleation at the Golgi generates MT network asymmetry in motile vertebrate cells. Investigating Golgi-derived MT (GDMT) distribution, we find that MT asymmetry arises from non-random nucleation sites at the Golgi (hotspots). Using computational simulations, we propose two plausible mechanistic models of GDMT nucleation leading to this phenotype. In the “Cooperativity” model, formation of a single GDMT promotes further nucleation at the same site. In the “Heterogeneous Golgi” model, MT nucleation is dramatically upregulated at discrete and sparse locations within the Golgi. While computationally both models are equally probable, GDMT nucleation leans toward simultaneous rather than sequential, supporting Heterogeneous --Golgi model. Investigating the molecular mechanism underlying hotspot formation, we have found that hotspots are significantly smaller than a Golgi subdomain positive for scaffolding protein AKAP450, which is thought to recruit GDMT nucleation factors. We have further probed potential roles of known GDMT-promoting molecules, including γ-TuRC-mediated nucleation activator (γ-TuNA) domain-containing proteins and MT stabilizers CLASPs. While both γ-TuNA inhibition and lack of CLASPs resulted in drastically decreased GDMT nucleation, computational modeling revealed only γ-TuNA inhibition suppressed hotspot formation. We conclude that clustered GDMT nucleation is a result of γ-TuNA-dependent local activation of γ-TuRC at the Golgi.

E38

The mitotic spindle is chiral due to torques generated by motor proteins.

J. Simunic[1], B. Polak[1], M. Novak[2], Z. Boban[2], B. Kuzmić[1], N. Pavin[2], I.M. Tolić[1];
[1]Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia, [2]Department of Physics , Faculty of Science, University of Zagreb, Zagreb, Croatia

Mitosis relies on forces generated in the spindle, a micro-machine composed of microtubules and associated proteins. Forces are required for the congression of chromosomes to the metaphase plate and separation of chromatids in anaphase. However, torques may also exist in the spindle, yet they have not been investigated. Here we show that the spindle is chiral. Chirality is evident from the finding that microtubule bundles follow a left-handed helical path, which cannot be explained by forces but rather by torques acting in the bundles. STED super-resolution microscopy, as well as confocal microscopy, of human spindles shows that the bundles have complex curved shapes. The average helicity of the bundles with respect to the spindle axis is 1.5 degrees/µm. Inactivation of kinesin-5 (Eg5/Kif11) abolished the chirality of the spindle, suggesting that this motor generates the helical shape of microtubule bundles. To explain the observed shapes, we introduce a theoretical model for the balance

of forces and torques acting in the spindle, and show that torque is required to generate the helical shapes. We conclude that torques generated by motor proteins, in addition to forces, exist in the spindle and determine its architecture.

E39

Inhibitable kinesin motors to study intracellular trafficking.

M.F. Engelke[1], B. Waas[1], B.L. Allen[1], K.J. Verhey[1];
[1]Cell Developmental Biology, University of Michigan Medical School, Ann Arbor, MI

The kinesin-2 motor KIF3A/KIF3B/KAP is the essential driver of intraflagellar trafficking (IFT) in mammalian cells and interference with its function results in the absence of cilia. In contrast, KIF17 appears to be an accessory IFT motor, as knockout of KIF17 alone has little effect in mice, but results in synthetic effects when combined with other knockouts. The lack of fast and specifically acting kinesin inhibition methods currently precludes the direct analysis of kinesin-2 function in cilia. Here we adapt a chemical-genetic kinesin inhibition approach that we originally developed for kinesin-1 (Engelke et al., 2016, Nat. Commun.) and demonstrate that this approach yields functional kinesin-2 motors that can be abruptly inhibited by small, cell-permeable molecules. To do this we pursued two strategies: I) We inserted the six amino acid tetracysteine tag into surface loops of the motor domain such that binding of biarsenic dyes conformationally distorts and thereby inhibits motility. II) We fused DmrB dimerization domains to the motor heads such that addition of B/B homodimerizer cross-links the motor domains and inhibits processive motor stepping. Using cellular assays, we show that the engineered kinesin-2 motors are able to transport artificial cargo similarly to the wild type motor, but cargo transport is efficiently inhibited by the addition of inhibitor. Future studies will enable us to deploy the inhibitable kinesin-2 motors and for the first time abruptly inhibit KIF3A/KIF3B/KAP and monitor its function in the genesis, maintenance, and resorption of primary cilia. For KIF17 this approach will allow us to determine the direct involvement of KIF17 in IFT, before compensatory mechanisms can attenuate the resulting phenotype.

E40

Two isoforms of myosin-II cooperate to organize the fission yeast cytokinetic ring for maximal tension production.

S. Wang[1], H.F. Chin[2], S. Thiyagarajan[1], E. Karatekin[3], T.D. Pollard[4], B. O'Shaughnessy[2];
[1]Department of Physics, Columbia University, New York, NY, [2]Department of Chemical Engineering, Columbia University, New York, NY, [3]Department of Cellular and Molecular Physiology, Yale University, New Haven, CT, [4]Department of Molecular Cellular and Developmental Biology, Yale University, New Haven, CT

Cytokinesis of animal, fungal and amoeboid cells is driven by an actomyosin contractile ring that generates tension. How ring components organize to generate tension, and how the organization is maintained are unanswered questions. Here we show that the two isoforms of myosin-II in the fission yeast ring not only exert force, but have equally important roles as crosslinking agents that bundle actin filaments and organize the ring, cooperating to boost ring tension while avoiding fracture.
Recent FPALM super-resolution fluorescence microscopy measurements of the fission yeast ring showed that protein complexes (“nodes”) from which the ring is assembled persist into constricting rings (Laplante et al., 2016). Nodes, anchored to the plasma membrane, contain several components including myosin-II Myo2. A second myosin-II isoform, Myp2, may be unanchored: fluorescence images

show a Myp2 ring of smaller radius than the Myo2 ring (Laplante et al., 2015), and Myp2 vanishes from the ring following Latrunculin A-mediated disassembly (Takaine et al., 2015).
Here we measured ring tension in fission yeast protoplasts. We found ~650 pN tension in wild type cells, ~65% the normal tension in myp2 deletion mutants and ~40% normal tension in myo2-E1 mutant cells with negligible ATPase activity and reduced actin binding.
To understand the relation between organization and tension, we developed a molecularly explicit simulation of the fission yeast ring with the above organization. Our simulations revealed a clear division of labor between the 2 myosin-II isoforms, which maintains organization and maximal tension. (1) Myo2 anchors the ring to the plasma membrane, and transmits ring tension to the membrane. (2) Myo2, extending ~100 nm away from the membrane, bundles half (~25) of the actin filaments in the cross-section due to filament packing constraints, as only ~25 filaments are within reach. (3) To increase tension requires that the ring be thickened, as tensions in the ~25 membrane-proximal filaments are close to fracture. (4) Unanchored Myp2 indeed enables thickening, by bundling an additional ~25 filaments and doubling tension. Anchoring of these filaments to the membrane is indirect, via filaments shared with the anchored Myo2.
In simulated myo2-E1 rings ~20% of the actin filaments peeled away from the ring and formed Myp2-dressed bridges, as observed experimentally in myo2-E1 cells. The organization in simulated Δmyp2 rings was highly disrupted, with ~ 50% of the actin filaments unbundled.
In summary, beyond their widely recognized job to pull actin and generate tension, myosin-II isoforms are vital crosslinking organizational elements of the ring. Two isoforms in the ring cooperate to organize the ring for maximal actomyosin interaction and tension.

E41

A Novel Role for Nonmuscle Myosin II Monomers in Regulation of Focal Adhesion Dynamics.

M. Shutova[1], T.M. Svitkina[1];
[1]Biology, University of Pennsylvania, Philadelphia, PA

The role for nonmuscle myosin II (NMII) in positive regulation of actin stress fiber contraction, cell cortex tension and focal adhesion (FA) maturation is well established. In order to perform these functions, NMII must be activated by myosin light chain (MLC) phosphorylation and, according to the classic model, be polymerized into bipolar filaments. However, bipolar filament assembly and disassembly is regulated separately from NMII activation through MLC. A combination of these regulatory mechanisms, in principle, can produce motor-active but unpolymerized NMII molecules (monomers), which can represent a physiologically relevant functional NMII species in cells. Using methods of molecular cloning, biochemistry, and light and electron microscopy, we provide evidence that NMII monomers can regulate FA dynamics in cells. We show that endogenous MLC-activated NMII monomers exist in the cell and often associate with FAs through their tail domain. To explore their function, we created monomeric mutants of NMIIA and NMIIB isoforms by deleting both Assembly Competence Domains (ACD1 and ACD2) from the respective heavy chains. The monomeric NMIIA and NMIIB mutants neither formed bipolar filaments nor incorporated into stress fibers; however, TIRF microscopy revealed their increased accumulation in active cell protrusions and at the front and sides of the mature FAs. When expressed in cells, monomeric NMIIB mutant produced a strong phenotype on cell adhesion. Specifically, cells overexpressing monomeric NMIIB exhibited more dynamic protrusions, smaller FAs, thinner stress fibers, decreased spreading area, and eventually detached. Quantitative analysis of FA dynamics showed that overexpression of monomeric NMIIB increased rates of both FA assembly and disassembly, but with greater enhancement of the disassembly rate. Time-lapse imaging revealed a correlation between the accumulation of monomeric NMIIB at the front of a FA and subsequent FA disassembly. Monomeric NMIIB mutant lacking the actin-binding motor domain also localized to the vicinity of FAs, whereas the

isolated short C-terminal nonhelical tailpiece did not. We propose a model in which MLC-activated monomeric NMII is targeted to FAs through its tail domain and enhances FA dynamics. The mechanisms of FA regulation by monomeric NMII and NMII tail interaction partners are currently under investigation.

E42

B cell mechanosensing: is it a myth?

S. Shaheen[1], Z. Wan[1], Z. Li[1], W. LIU[1];
[1]School of Life Sciences, Institute for Immunology,Tsinghua University, Beijing, China

Mechanosensing capability of lymphocytes plays an important role in shaping the initiation of an immune response. Although it is evident that B lymphocytes use such capabilities to discriminate the stiffness features of the substrate presenting antigens, but the underlying molecular mechanism remains unexplored. Here, through a combination of molecule imaging, genetic ablation and pharmacological approaches, we demonstrated that B cells with genetic ablation of each of the early BCR signaling molecules, Lyn, Syk, PLCγ2, Btk, BLNK or PKCβ, lost the capability to discriminate substrate stiffness during initiation of B cell activation, while exogenous addition of each molecule rescued the defects, suggesting B cell discrimination of substrate stiffness is dependent on the BCR signaling. In marked contrast, BCR signaling-independent BCR accumulation in the initiation of B cell activation is insensitive to substrate stiffness. Mechanistically, we showed that PMA induced activation of PKCβ can bypass the requirements on Btk and PLCγ2 for the substrate stiffness discrimination ability of B cells. Importantly, we excluded the contribution of PKCβ mediated NF-κB activation to the discrimination capabilities of B cells, instead we provided evidence for a model that PKCβ-dependent activation of focal adhesion kinase (FAK) is required in these events by the FAK-mediated potentiation of B cell spreading and adhesion responses. FAK inactivation or deficiency impaired B cell discrimination against substrate stiffness.As supporting evidence for this model, we showed that the presence of adhesion molecules, ICAM-1 or VCAM-1, greatly enhanced B cell's capability to discriminate substrate stiffness. In contrast, integrin inactivation, drastically impaired the capability of B cell to discriminate substrate stiffness. Lastly, rheumatoid arthritis (RA) patient B cells strikingly exhibited disordered capability to discriminate substrate stiffness in contrast with B cells from healthy controls. All these data shed light on the precise molecular mechanism of how B cells discriminate substrate stiffness through a BCR signaling and PKCβ mediated FAK activation dependent manner in the process of B cell activation, improving our understanding of the sophisticated mechanosensing capability of B cells.

Monday, December 4

EMBO Gold Medal Ceremony and Lecture

A3

Systematic Cell Biology of Organelles.

M. Schuldiner[1];
[1]Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

One of the hallmarks of eukaryotic cells is the presence of membrane-bound organelles that create optimized environments best suited for promoting the various chemical reactions required to sustain life. The presence of organelles has enabled a diversification of functions that is extremely beneficial to the cell, however, it also creates two severe problems: First, thousands of organelle proteins require specific mechanisms to target to their final destination and second, communication between membrane bound entities must occur to enable coordination of cellular function. In my lab we are interested in characterizing solutions to both of these cellular tasks: Organelle protein targeting and organelle communication. We use the yeast Saccharomyces cerevisiae as a discovery platform as although nearly 20 years have passed since the publication of its entire genome sequence, over 30% of the proteins that reside in its organelles have never been studied and more than half of them do not have a known biochemical function. Most of these proteins are conserved to humans and some have been implicated in diseases. One of the great challenges of the post-genomic era is, therefore, to use novel methodologies to fill in these gaps in our knowledge and to uncover the functions of unstudied proteins. My talk will focus on our journey to uncover novel functions for yeast organellar proteins mostly in pathways of targeting and contact sites.

Bruce Alberts Award for Excellence in Science Education

A4

What Does Teaching Sound Like? Introducing DART – Decibel Analysis for Research in Teaching – A New Tool for Systematically Analyzing Teaching Practices across Instructors and Institutions.

K.D. Tanner[1];
[1]Biology, San Francisco State University, San Francisco, CA

What proportion of STEM (science, technology, engineering, and math) instructors in higher education regularly employ teaching strategies beyond lecture? What is the probability that an undergraduate STEM student would have the opportunity to speak, write, or discuss their ideas about science with peers in every class session for every course they take? Current college STEM teaching continues to be primarily lecture-based, even though active learning pedagogies have been repeatedly demonstrated to produce superior learning gains with large effect sizes compared to lecture-based pedagogies. Shifting large numbers of college STEM faculty to include any active learning in their teaching may retain and more effectively educate far more students than having a few instructors completely transform their teaching. Yet, the extent to which STEM faculty are changing their teaching methods is unclear. Here, we describe the development and application of the machine learning-derived algorithm Decibel Analysis for Research in Teaching (DART), which can analyze thousands of hours of STEM course audio recordings quickly, with minimal costs, and without need for human observers (PNAS, 2017). DART analyzes the volume and variance of classroom sound recordings to predict the quantity of time spent on Single Voice (e.g., lecture with question and answer), Multiple Voice (e.g., pair discussion), and No Voice (e.g., clicker question thinking) activities. Applying DART to 1486 recordings of class sessions from

67 courses, a total of 1720 hours of audio, revealed varied patterns of lecture (Single Voice) and non-lecture activity (Multiple Voice and No Voice) use. Courses taught by different instructors varied considerably, with some instructors having high variability between individual class sessions, emphasizing the need for a tool that can analyze each session of an entire course. While DART cannot assess the quality of active learning, our results demonstrate that DART is valuable for high-throughput, cost-effective, and comprehensive analyses of the extent of non-lecture pedagogies employed in courses. As such, the DART tool could enable individual instructors, departments, institutions, and science education stakeholders worldwide to systematically and regularly inventory the presence of evidence-based, active learning pedagogies with ~90% accuracy across thousands of courses in diverse settings with minimal effort. We envision DART as a tool for immediate feedback for individual instructors, as well as a system with which departments and institutions can regularly capture, assess, compare, and demonstrate their added educational value by showing the extent to which their instructors employ effective instructional practices for students.

Symp 3: Cell Biology of Neurons

S6

Somatic mutation and genomic diversity in the human brain.

C.A. Walsh[1];
[1]Division of Genetics and Genomics, Boston Children's Hospita/Harvard Medical School/HHMI, Boston, MA

The role of ‘somatic’ mutations—those arising during prenatal development--in human disease is not well understood, nor do we understand the potential role of genomic variation as a source of normal neuronal diversity. Analysis of blood DNA with high coverage panel sequencing suggests that >25% of undiagnosed patients with brain malformations show causative mosaic mutations in known genes. Hemimegalencephaly (HME) and Focal Cortical Dysplasia (FCD) represent epileptic brain malformations caused by mosaic mutations resulting in mTOR pathway activation, but the mutation is typically present in only a minority of cells (1-30%) within the brain lesion and undetectable in blood DNA. These data show that mosaic mutations causing disease can occur either before, or after, separation of neural tissue from non-neural tissue. Similarly, analysis of whole exome sequence of patients with autism spectrum disorders (ASD) with a new calling algorithm shows that 5-10% have mosaic mutations detectable in blood. Analysis of postmortem ASD brains shows that about 10% of likely causative mutations are mosaic in brain, sometimes unevenly distributed between brain regions. Such somatic mutations have the potential to create a mosaic brain that could underlie other neuropsychiatric diseases, though this remains untested. In parallel experiments analysis of single human neurons, using single cell whole genome amplification and sequencing, reveals that somatic LINE element insertions can be found in up to half of the neurons in normal cerebral cortex; large CNV are also frequent; and hundreds to thousands of SNV occur in each single cerebral cortical neuron. Clonal somatic SNV represent a permanent lineage map of the human brain of quite high density, revealing unique patterns of clonal structure in human brain. On the other hand, nonclonal somatic mutations appear to be driven by transcriptional damage, and occur at even higher rates in rare genetic syndromes associated with precocious neuronal degeneration. Supported by the NIMH, NINDS, and HHMI.

S7

Sorting out polarized transport in neurons.

L.C. Kapitein[1];
[1]Department of Biology, Utrecht University, Utrecht, Netherlands

Proper positioning of organelles by cytoskeleton-based motor proteins underlies cellular events such as signaling, polarization, and growth. For example, the selective transport of different cargoes into axons and dendrites underlies the polarized organization of the neuron, whereas the regulated intra-dendritic transport of receptor-carrying endosomes is important for synaptic maintenance and modulation. To explore how different motor proteins contribute to neuronal transport and to study the site-specific roles of different organelles, we have established optical control of intracellular transport by using light-sensitive heterodimerization to recruit specific cytoskeletal motor proteins (kinesin, dynein or myosin) to selected cargoes. In addition, to unravel how the specialized organization of the neuronal cytoskeleton guides different motor proteins to either axons or dendrites, we have developed novel approaches for optical nanoscopy. One of these, called motor-PAINT, uses nanometric tracking of motor proteins to super-resolve cytoskeletal fibers and determine their polarity. This has revealed a key architectural principle of the neuronal microtubule cytoskeleton that explains how different motor proteins can selectively transport cargoes to either axons or dendrites.

S8

Ligands, receptors and signaling mechanisms for sensory dendrite morphogenesis.

K. Shen[1];
[1]Biology, Stanford University, Stanford, CA

Ligand receptor interactions instruct axon guidance during development. How dendrites are guided to specific targets is less understood. The C. elegans PVD sensory neuron innervates muscle-skin interface with its elaborate dendritic branches. Here, we found that DMA-2, the ortholog of leukocyte cell-derived chemotaxin-2 (LECT2), is secreted from the muscles and required for muscle innervation by PVD. Ectopic expression of DMA-2 from seam cells is sufficient to redirect the PVD dendrites onto seam cells. DMA-2 functions in a multi-protein receptor-ligand complex that also contains two¬ transmembrane ligands on the skin, SAX-7/L1CAM and MNR-1, and the neuronal transmembrane receptor DMA-1. SAX-7 exists in stripes and generates the regular spacing of dendrites while MNR-1 ensures that the dendrites attach to the skin cells. DMA-2 specifies dendritic innervation to muscles. The activation of DMA-1 strictly requires all three ligands, which establishes a combinatorial code to precisely target and pattern dendritic arbors. Intracellularly, we show that high level of filamentous actin is found in branching dendrites during development. Actin regulators including a RacGEF, TIAM-1 and WAVE regulatory complex (WRC) are required for dendrite morphogenesis. Interestingly, TIAM-1 directly binds to the dendrite branching receptor DMA-1, while the WRC binds to a claudin like transmembrane protein HPO-30. DMA-1 and HPO-30 form a coreceptor complex to scaffold RacGEF and WRC to branching dendrites. The synergy of the two actin assembly modulesis required to build the elaborate dendrite arbors. We conclude that the DMA-1-HPO-30 receptor complex induce dendritic branching by scaffolding actin regulators.

Symp 4: Cell Interactions

S9

Capturing principles of tissue dynamics and function by live imaging.

V. Greco[1];
[1]Genetics, Yale University, New Haven, CT

The goal of my research program is to define how tissues maintain themselves throughout the course of our lives in the face of continuous cellular turnover, frequent injuries and spontaneous mutations. Despite the highly dynamic nature of these processes, the mammalian regeneration field has been limited by static approaches that are unable to track the same cells over time to determine which cells and what behaviors are critical for adult tissue function. To overcome this challenge, my lab has developed a method to visualize and manipulate stem cells and their niche in the skin epithelium of an intact, uninjured mouse. Our real time imaging of skin epithelium in live mice has allowed us to demonstrate that location dictates stem cell fate, that stem cells are dispensable for tissue growth while the niche is required, and that tissue correction preserves homeostasis. Based on these insights, we aim to understand first, what factors dictate stem cell behavior? As tissues perform their function, changing demands can create constant variations. Our data shows that epithelial stem cells have highly flexible behaviors and are influenced by the niche. These observations change the way we think about regenerative biology and define the need to decode the many inputs stem cells receive from the cells that surround them. Thus a major goal of my laboratory is to define the cell types, and the modes of molecular communication, that control stem cell behaviors during regeneration. Second, how does a tissue cope with the presence of mutations? Recent work has revealed that phenotypically normal human tissues often harbor mutant cells, some of which carry oncogenic mutations. We have found that skin epithelial tissue maintains its homeostatic equilibrium in the presence of such mutant cells. An important focus of our work is therefore to determine the combination of factors that allow a tissue to integrate, tolerate and/or eliminate cells carrying different mutations while maintaining a homeostatic steady state. Third, what leads to the emergence of cancer from homeostatic tissue growth? Although tissues carrying a single mutation are not malignant, the development of a small wound or a second cooperating mutation can “break” the system and lead to cancer. Our work has identified critical epithelial required to repair a wound as well as return to a homeostatic equilibrium. We plan to use these insights to understand how wounding in combination with a single mutation can lead to cancer, and to contrast this situation with more traditional two-hit models of cancer initiation. This third goal aims to capture the critical cellular behaviors and molecular cross-talk occurring within and between mutant and wild-type populations that drive cancer emergence.

S10

Polarised secretion and frustrated ciliogenesis: the cell biology of serial killer cells.

G.M. Griffiths[1];
[1]Cambridge Institute for Medical Research, Cambridge University, Cambridge, United Kingdom

Treatment against cancer has been revolutionized by recent immunotherapies that focus on finding ways to enable small killer cells of the immune system to recognize and destroy cancerous cells. These cytotoxic T lymphocytes (CTLs) are remarkably effective serial killers that can move rapidly from one target to another, killing each in turn. To do so they use an exquisitely polarized secretion of modified lysosomes containing cytolytic proteins that are released at the immunological synapse formed by the killer with its target. Secretion is focused at a precise point at the membrane of the synapse by the

centrosome that polarizes all the way from the rear of the cell as CTLs move right up to the plasma membrane where it docks with it's distal appendages contacting the membrane directly, exactly as during ciliogenesis. However, in CTLs ciliogenesis is frustrated and docking is transitory, rarely lasting more than 10 minutes, and no transition zone structures form.
Recent studies on the cell biology of secretion from these remarkable cells have focused on the parallels with ciliogenesis, the mechanisms that control membrane specialization across the synapse allowing centrosome docking and secretion. Using insights provided from studies of CTLs from immunodeficient patients and genetic screens that identify novel mutations affecting secretion from CTLs together with high-resolution imaging my lab has focused on the mechanisms that allow CTLs to fine-tune the ability of CTLs to modulate centrosome docking and secretion. The most recent advances will be described in the context of our own and others advances in this field that provide our current understanding of the cell biology of the mechanisms that control polarized secretion from these serial killers.

Minisymposium 7: Actin Dynamics and Function

M69

Direct observation of actin structural deformation in response to mechanical force.

P.S. Gurel[1], Y. Takagi[2], J.E. Bird[3], J.R. Sellers[2], G.M. Alushin[1];
[1]The Rockefeller University, New York, NY, [2]Cellular Biology and Physiology Center, National Heart, Lung, and Blood Institute, Bethesda, MD, [3]National Institute of Deafness and Other Communication Diseases, Bethesda, MD

The ability of a cell to respond to the mechanical properties of its environment ("mechanosense") influences core cellular processes including division, migration, differentiation, and survival. Misregulation of mechanical signal transduction ("mechanotransduction") has been implicated in malignant transformation, tumorogenesis, and metastasis, highlighting this process as a key component of cancer progression, yet the underlying molecular mechanisms remain largely unknown. Cellular mechanics are governed by an intricately coordinated contractile network consisting of the actin cytoskeleton, myosin motor proteins, and actin binding partners (ABPs). Actin filaments are flexible polymers that can adopt multiple conformational states, and recent evidence suggests that forces can regulate interactions between actin and ABPs. Here, we explore how mechanical stimuli alter the actin filament structural landscape, potentially serving as an upstream event in mechanotransduction by regulating ABP binding. We have developed a novel reconstitution system to place actin filaments under mechanical load suitable for structural studies with cryo-electron microscopy (cryo-EM). We immobilize active myosinV (barbed-end directed motor) or myosinVI (pointed-end directed motor) onto the film of holey-carbon cryo-EM grids, and then tether pointed-end biotinylated actin filaments onto grids such that filaments are attached to the surface through one end and free to engage with the myosin throughout the rest of the filament. Using time-lapse fluorescence microscopy, we observe morphological changes in individual actin filaments consistent with myosin V generating compression along the filament axis, while myosin VI generates tension, consistent with the directionality of the motors. Cryo-EM images reveal a novel, persistent actin structural state found in the presence of force generation that is characterized by oscillating areas of high curvature within the filament, which we term "squiggles". Intriguingly, both compressive and tensile forces produce these "squiggle" structures, which cryo-electron tomography reveals to be 3-dimensional spirals. We hypothesize that both compression and tension introduce defects in longitudinal contacts along the filament, and that preferential propogation of defects along one strand induces squiggle formation. Resolving the structure

of squiggles at high-resolution will provide insight into the molecular mechanisms of mechanically regulating actin-ABP interactions and advance the development of targeted therapeutics against specific actin conformational states.

M70

Twinfilin promotes Capping Protein association with actin filament barbed ends by attenuating the inhibitory effects of CARMIL.

D. Hilton[1], A. Johnston[1], A. Simone[1], P. McConnell[2], J.A. Cooper[2], B.L. Goode[1];
[1]Molecular Cellular Biology, Brandeis University, Waltham, MA, [2]Biochemistry Molec Biophysic, Washington University in St. Louis, St. Louis, MO

Nucleation and elongation of actin filaments represent the two critical control points in the cellular regulation of actin network assembly. Until recently, our view of actin elongation regulation was that the growing barbed ends of filaments were either rapidly bound by capping protein, terminating growth, or 'captured' by processive elongators such as formins and Ena/VASP, resulting in accelerated growth. However, work in recent years has clearly demonstrated that cells have multiple mechanisms for spatially and temporally controlling capping and elongation. CARMIL and other CPI motif-containing proteins can rapidly displace capping protein from barbed ends, and other factors can directly attenuate or enhance formin-mediated elongation. Additionally, formins and capping protein can lock horns at the barbed end, catalyzing each other's displacement. Here, we build on this emerging view of complex molecular interplay controlling barbed end growth by addressing the functional significance of Twinfilin binding interactions with capping protein. We find that Twinfilin directly competes with CARMIL for binding capping protein and attenuates CARMIL-mediated displacement of capping protein, enabling capping protein to remain on barbed ends. Consistent with these biochemical effects, Twinfilin silencing in B16F10 melanoma cells leads to an increase in actin assembly and in filopodia density, phenocopying the loss of capping protein. Together, these results show that Twinfilin promotes capping protein association with barbed ends by attenuating CARMIL effects, thus tuning actin network assembly at the leading edge.

M71

Arp2/3 complex- and formin-mediated actin networks tune actin-binding protein sorting in fission yeast.

K.E. Homa[1], C. Suarez[1], D.R. Kovar[1,2];
[1]Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL, [2]Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL

F-actin networks are diverse and specialized to carry out specific cellular processes, such as polarization, endocytosis, and cytokinesis. Each F-actin network is tightly regulated by a unique set of actin-binding proteins (ABPs) that initiates and maintains the organization and dynamics of actin filaments. However, the general mechanistic principles by which specific ABPs sort to particular F-actin networks remain largely unclear. By combining *in vivo* fluorescence microscopy and *in vitro* reconstitution, we discovered that two actin assembly factors, Arp2/3 complex and formin Cdc12, tune the binding of ABPs fimbrin and tropomyosin to specific F-actin networks in fission yeast. Disruption of F-actin networks by small molecule inhibitors or genetic manipulation revealed that fimbrin is preferentially recruited to Arp2/3-complex mediated actin patches, while tropomyosin is preferentially targeted to the contractile ring by Cdc12-mediated filaments. To investigate the role of Arp2/3 complex and Cdc12 in this sorting, we used four-color *in vitro* TIRF microscopy to reconstitute ABP sorting with purified proteins. We discovered

that either fimbrin or tropomyosin alone binds similarly to Arp2/3 complex- and Cdc12-mediated actin filaments. Conversely, sorting of these ABPs to their preferred actin filaments occurs when Arp2/3 complex- and Cdc12-mediated filaments are assembled together in the presence of both fimbrin and tropomyosin. Under these conditions, fimbrin accumulates ~2-fold more at Arp2/3 complex branch points, while tropomyosin association is enhanced 3.5-fold on filaments assembled by Cdc12. This result suggests that competition between fimbrin and tropomyosin is necessary for their sorting to different F-actin networks. In summary, these findings reveal for the first time that the F-actin assembly factors Arp2/3 complex and formin Cdc12 facilitate the recruitment of specific ABPs, thereby tuning ABP sorting and subsequently establishing the identity of a given F-actin network.

M72

CYRIPS (Fam49) proteins are local inhibitors of Rac1 and Scar/WAVE induced lamellipodia.

L. Fort[1], J. Batista[1], P. Thomason[1], H. Spence[1], J. Greaves[2], K. Martin[1], K. Anderson[1,3], P. Brown[1], S. Lilla[1], M. Nielson[1], P. Tafelmeyer[4], S. Zanivan[1], S. Ismail[1], N. Tomkinson[5], L.H. Chamberlain[2], R.H. Insall[1], L.M. Machesky[1];
[1]Beatson Institute for Cancer research, Glasgow, United Kingdom, [2]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom, [3]Crick Institute, London, United Kingdom, [4]Hybrigenics Services, Paris, France, [5]WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, United Kingdom

The driving forces for cell migration depend on a complex interaction between environmental cues and internally generated feedback loops.The Scar/WAVE complex provides a main control point for actin-based protrusion generation; spatiotemporal activation of this complex via activated Rac1 recruits and activates the Arp2/3 complex, triggering branched actin polymerisation. This is reinforced through a positive feedback loop, but it is unclear how the cell restricts the eventual size of a protrusion through buffering of the positive signals to cause splitting or retraction.
We have identified a highly conserved regulator of Scar/WAVE complex, CYRIPS (**Cy**fip related **R**ac **i**nteracting **p**seudopod **s**plitter) from a proteomics screen. CYRIPS shows sequence similarity to CYFIP1/2 in a sequence domain of unknown function DUF1394 and myristoylation at an N-terminal conserved sequence. We demonstrate specific binding of CYRIPS DUF1394 to activated Rac1 in a region of shared homology with CYFIP proteins, establishing DUF1394 as a new Rac1 binding domain. CYRIPS-depleted cells have broad, Scar/WAVE-enriched lamellipodia and enhanced Rac1 signalling. CYRIPS-B knockout *D. discoideum* and mammalian cells also frequently assume a single leading pseudopod suggesting a role for CYRIPS as a local inhibitor of the positive signals that trigger actin-based motility. Conversely, CYRIPS over-expressers show fenestrated dendritic protrusions with reduced Scar/WAVE accumulation. We have identified the primary Rac1 interaction site and created mutants to demonstrate the importance of Rac1 binding *in vivo*.
In a 3D-cyst model, Rac1 signal is fundamental for establishment and maintenance of apical-basolateral polarity. CYRIPS-knockdown cells affect lumen formation and Scar/WAVE complex localization and these phenotypes can be partially reverted by chemical inhibition of Rac1.
We conclude that CYRIPS is a new evolutionarily conserved protein that competes with the Scar/WAVE complex for active Rac via its DUF1394 domain.

M73

Ubiquitin-dependent regulation of filopodia during axon guidance and branching.

N. Boyer[1], C. Monkiewicz[2], S. Menon[2], S.L. Gupton[2,3];
[1]Neurobiology Curriculum, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC, [2]Cell Biology and Physiology, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC, [3]Neuroscience Center, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC

During development, neurons extend axons across long distances to find postsynaptic partners and create neuronal networks. Upon reaching target regions, axons branch to increase network complexity. At the distal end of an extending axon, a dynamic filopodia-rich growth cone senses extracellular guidance cues and facilitates directional extension and branching of the axon. This requires tight spatial and temporal modulation of the F-actin cytoskeleton, which is reorganized rapidly in response to extracellular guidance cues. We found that lower concentrations of the extracellular guidance cue netrin-1 increases growth cone filopodial stability and density and induces attractive axon turning and axon branching, whereas higher netrin concentrations collapse and repulse the growth cone. The mechanisms by which netrin and its receptor DCC alter cytoskeletal machinery in these distinct responses however have not been fully elucidated. Here we show that TRIM67, a brain-enriched member of the tripartite motif family of E3 ubiquitin ligases, interacts with DCC and is required for both attractive and repulsive responses to netrin. Using murine embryonic cortical neurons, we find that growth cones of *Trim67*$^{-/-}$ axons fail to increase in size or number of filopodia in response to a low concentration of netrin, and fail to collapse in response to a high concentration of netrin. However, *Trim67*$^{-/-}$ axons respond appropriately to other guidance cues. We find that TRIM67 regulates filopodia stability and localizes to the tips of filopodia, where it colocalizes and cotransports with the barbed end actin polymerase VASP. We show that TRIM67 and VASP interact and that TRIM67 is required to increase the dynamics of VASP at filopodial tips in response to netrin-1. We previously found that a closely related E3 ligase TRIM9 reduces VASP dynamics and filopodial stability via ubiquitination of VASP. Surprisingly TRIM67 negatively regulates this VASP ubiquitination, potentially via ubiquitination of TRIM9. Together with investigations of *Trim9*$^{-/-}$*:Trim67*$^{-/-}$ neurons, our results suggest that TRIM67 and TRIM9 coordinate to promote cytoskeletal reorganization in response to netrin-1 downstream of DCC. This regulation is likely critical to appropriate neuronal morphogenesis: Netrin-dependent axon branching and axon turning is absent in *Trim67*$^{-/-}$ neurons in vitro, and the netrin-dependent cortical corpus callosum projection is thinner in *Trim67*$^{-/-}$ mice in vivo.

M74

Mechanistic principles underlying regulation of the actin cytoskeleton by phosphoinositides.

Y. Senju[1], M. Kalimeri[2], E. Koskela[1], P. Somerharju[3], H. Zhao[1], I. Vattulainen[2,4], P. Lappalainen[1];
[1]Institute of Biotechnology, University of Helsinki, Helsinki, Finland, [2]Department of Physics, Tampere University of Technology, Tampere, Finland, [3]Faculty of Medicine, University of Helsinki, Helsinki, Finland, [4]Department of Physics, University of Helsinki, Helsinki, Finland

The actin cytoskeleton provides forces for vital cellular processes involving membrane dynamics. Membrane phosphoinositides regulate many actin-binding proteins, including cofilin, profilin, Dia2, N-WASP, ezrin and moesin, but the underlying mechanisms have remained elusive. By applying a combination of biochemical assays, novel photobleaching/activation approaches, and atomistic molecular dynamics simulations, we revealed that these proteins interact with membranes through multivalent electrostatic interactions, and hence function as phosphoinositide density sensors at the

membrane. However, their membrane-binding kinetics differ drastically. Cofilin and profilin display transient, low-affinity interactions with phosphoinositide-rich membranes, whereas F-actin assembly factors Dia2 and N-WASP reside on phosphoinositide-rich membranes for longer periods to perform their functions. Ezrin and moesin, which link cytoskeleton to plasma membrane, bind membranes with very high affinities and slow dissociation dynamics, and do not require high "stimulus-responsive" phosphoinositide density for membrane-binding. Thus, membrane-interaction mechanisms of actin-binding proteins evolved to precisely fulfill their specific functions in cytoskeletal dynamics.

M75

An Amish founder mutation disrupts a PI(3)P-WHAMM-Arp2/3 complex driven autophagosome remodeling pathway.

A. Mathiowetz[1], E. Baple[2], A. Russo[1], A. Coulter[1], E. Carrano[1], J. Brown[1], R. Jinks[3], A. Crosby[2], K.G. Campellone[1];
[1]University of Connecticut, Storrs, CT, [2]University of Exeter Medical School, Exeter, United Kingdom, [3]Franklin and Marshall College, Lancaster, PA

Actin nucleation factors function to organize, shape, and move membrane-bound organelles, yet they remain poorly defined in relation to disease. Galloway-Mowat syndrome (GMS) is an inherited disorder characterized by microcephaly and nephrosis resulting from mutations in the WDR73 gene. This core clinical phenotype appears frequently in the Amish, where virtually all affected individuals harbor homozygous founder mutations in WDR73 as well as the closely linked WHAMM gene, which encodes a nucleation factor. Here we show that patient cells with both mutations exhibit cytoskeletal irregularities and severe defects in autophagy. Re-introduction of wild type WHAMM restored autophagosome biogenesis to patient cells, while inactivation of WHAMM in healthy cell lines inhibited lipidation of the autophagosomal protein LC3 and clearance of ubiquitinated protein aggregates. Normal WHAMM function involved binding to the phospholipid PI(3)P and promoting actin nucleation at nascent autophagosomes. These results reveal a cytoskeletal pathway controlling autophagosome remodeling and illustrate several molecular processes that are perturbed in Amish GMS patients.

M76

Complement mediated phagocytosis involves mechanical coupling of β2 integrins to the actin cytoskeleton by a myosin-independent molecular clutch.

V. Jaumouillé[1], T. Liu[2], E. Betzig[2], C.M. Waterman[1];
[1]National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, [2]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA

αMβ2 and αXβ2 integrins (also called complement receptor 3 and 4) are highly expressed in macrophages, neutrophils and dendritic cells, and are thought to be the main phagocytic receptors for many pathogens, and participate in the clearance of dead cells and tumor cells. In other contexts, integrin functions are intimately linked to the actin cytoskeleton dynamics. For instance, at focal adhesions, coupling of the force provided by the actin retrograde flow to engaged integrins through a molecular clutch generates mechanical tension that is thought to switch integrins to a high affinity conformation and to generate traction on the substrate. Although it is established that phagocytosis requires actin, its dynamics and specific contribution to β2 integrin functions remain largely unknown. Thus, we sought to test the hypothesis that transmission of mechanical tension to β2 integrins by molecular coupling to the actin cytoskeleton is essential for particle binding, outside-in signaling, and engulfment. Using live cell imaging, we observed that engagement of complement-opsonized particles

led to a dramatic reorganization of the actin cytoskeleton, characterized by the formation of large protrusions that wrapped around the particle, contrasting with the particle sinking mechanism previously proposed. Quantitative live cell imaging showed that the Arp2/3 complex, which was required for particle internalization, was recruited to the leading edge of the protrusion and drove phagosome formation. However, slow actin retrograde flow within the phagocytic cup suggested a strong coupling between engaged β2 integrins and the actin network. β2 integrins appeared to form small focal complex-like adhesions at the phagocytic cup, characterized by the recruitment of mature adhesion markers, such as Vinculin, α-Actinin, Zyxin, as well as tyrosine phosphorylation of Paxillin, FAK and Syk. We found that Src family kinases, Syk and FAK/Pyk2 activities were important for particle engulfment. Tyrosine kinases were required for the recruitment of Vinculin, independently of Myosin II, which provided higher transmission of mechanical tension to integrins. Our observations indicate that professional phagocytes co-opt the focal adhesion-lamellipodium machinery, and use signaling to rapidly achieve strong coupling of integrins with the cytoskeleton in order to protrude faster and engulf large particulate material.

M77

A geodesic septin lattice is required for actomyosin contractility on micron-scale curved membranes in vivo.

S. Ebrahim[1], A. Shitara[1], H. Shroff[2], B. Kachar[3], R. Weigert[1];
[1]NCI, NIH, Bethesda, MD, [2]NIBIB, NIH, Bethesda, MD, [3]NIDCD, NIH, Bethesda, MD

The ability of cell membranes to remodel into various curved configurations is fundamental for processes from cytokinesis to intracellular trafficking. Generation of membrane curvature by proteins is an important biological concept that is well studied in nanometer-scale curved membranes such as endocytic vesicles, where curvature-generating proteins include clathrin, and BAR-domain proteins. However, little is known about the proteins or mechanisms driving the formation and dynamics of larger, micron-scale membrane curvature within the cell such as around the cytokinetic furrow, secretory vesicles, or the ciliary base.

To address this question under physiological conditions we used intravital subcellular microscopy (ISMIC) in live mice to study regulated exocytosis in specialized secretory cells of the salivary gland. During this process large (~1.5 μm) vesicles, called secretory granules (SGs), containing proteinaceous cargo fuse with narrow, tube-like canaliculi at the apical plasma membrane (APM) after GPCR stimulation. A fusion pore then forms to allow flow of SG contents into the canaliculi, and integration of the SG membrane into the APM. The canalicular diameter (~0.3 μm) is almost an order of magnitude smaller than the SG diameter, making membrane integration energetically unfavorable. Thus, we asked how the SG membrane is forced into the APM forming the narrow canaliculi, and found that F-actin and non-muscle myosin (NMII) localize to the fused SG membrane and provide the forces needed for integration. We then asked, how these proteins are recruited to and organized on the curved membrane. Using knockin mice expressing GFP-NMIIA and superresolution microscopy, we describe for the first time that F-actin and NMII organize around SGs as a striking geodesic lattice with triskelia-like tripolar vertices, reminiscent of the clathrin cage around endocytic vesicles. NMIIA and actin within the lattice are partially offset, consistent with the lattice being contractile. We also show that septins, which recognize membrane-scale curvature, also form lattices around SGs, that are in register with NMIIA. Disruption of F-actin assembly causes expansion of SGs, but surprisingly does not impair recruitment of NMII or septins. Pharmacological inhibition of septin, however, results in a significant decrease in activated NMII and myosin light chain kinase (MLCK) on fused SGs.

Based on our data, we propose that the septin-lattice 1) acts as a scaffold to recruit the actomyosin network to the surface of SGs, and 2) is needed for NMII activation, likely through MLCK-mediated

phosphorylation. This novel septin-actin-NMII lattice on the SG surface provides new structure-function insights into protein-driven remodeling of micron-scale curved membranes in vivo.

M78

The interaction of FHOD1 with nesprin-2G activates a cryptic actin binding site and stimulates potent actin bundling activity: implications for nuclear movement.

S. Antoku[1], G.G. Gundersen[1];
[1]Pathology Cell Biology, Columbia University, New York, NY

The formin FHOD1 binds to specific spectrin repeats (SR11-13) in the outer nuclear membrane protein nesprin-2G and this interaction is required for formation of TAN (transmembrane actin-associated nuclear) lines and nuclear movement in fibroblasts (Kutscheidt et al, NCB, 2014). To understand the mechanistic basis for this, we tested how the interaction of nesprin-2G's SR11-13 affected FHOD1's actin polymerizing and bundling activities. Using purified recombinant proteins, we confirmed that full length (FL) FHOD1 alone did not stimulate actin polymerization by pyrene assays but rather weakly inhibited it. Addition of nesprin-2G SR11-13 activated the inhibitory effect of FL FHOD1 on actin polymerization to a similar degree as that of constitutively active FHOD1 ΔDAD. FHOD1 has reported F-actin bundling activity (Schonichen et al, JCS, 2013) and we confirmed that FHOD1 ΔDAD bundled F-actin and that this activity required both its FH2 domain and a putative second actin binding site (ABS). Low speed pelleting assays to detect actin bundling activity revealed that SR11-13 activated FL FHOD1's bundling activity (SR11-13 had no activity alone). The level of bundling with FL FHOD1 and SR11-13 was similar to that of FHOD1 ΔDAD, yet SR11-13 further stimulated FHOD1 ΔDAD's bundling activity to the low nM range making it more potent than that either fascin or α-actinin. TIRF microscopy of fluorescent actin filaments showed that actin bundles were formed by FL FHOD1 only when SR11-13 was present. To understand how SR11-13 stimulated bundling, we tested its effect on the ABS upstream of FHOD1's FH1 and FH2 domains. Using an N-terminal (NT) FHOD1 construct containing the ABS and adjacent SR11-13 binding site, we found that NT FHOD1 alone did not bind to actin filaments, but did so in the presence of SR11-13. These results show that the nesprin-2G potently activates FHOD1's actin bundling activity by activating a cryptic actin binding site and suggest that the nucleus plays an active role in strengthening it's interaction with actin bundles during nuclear movement.

Minisymposium 8: Cancer Cell Signaling, Adaptive Responses, and Metastasis

M79

Centrosome amplification triggers a non-canonical Senescence-Associated Secretory Phenotype and HIF1-α activation.

S.K. Wu[1,2], R. Picone[1,2], M.S. Levine[3], S. Papathanasiou[1,2], M. Kwon[1,2], M. Janiszewska[4], K. Polyak[4], A. Spektor[1,2], A.J. Holland[3], D. Pellman[1,2];
[1]Pediatric Oncology, Dana Farber Cancer Institute, BOSTON, MA, [2]Cell Biology, Harvard Medical School, BOSTON, MA, [3]Molecular Biology and Genetics, Johns Hopkins School of Medicine, Baltimore, MD, [4]Medical Oncology, Dana Farber Cancer Institute, Boston, MA

Centrosomes promote the assembly and organization of the microtubule, which in turn supports many cellular processes including cell division and migration. In cancer, an abnormal increase in the number of

centrosomes is commonly observed. However, tumours are extremely heterogeneous, which usually only have a subset of cells with supernumerary centrosomes. Therefore, whether centrosome amplification in a subpopulation of cells influences an entire population of cells remains an open question. Notably, centrosome amplification in a subset of cells within tissues is sufficient to promote spontaneous tumourigenesis, potentially through cell-autonomous induction of aneuploidy and invasion. But supernumerary centrosomes also trigger p53-dependent proliferation arrest in cells, thus potentially compromising their viability. Hence, how centrosome amplification promotes cancer progression despite triggering a proliferation arrest also remains incompletely understood. Here, we provide novel insights into these outstanding questions. We observe that most cells with centrosome amplification ultimately enter senescence. Senescence has long been recognized as a proliferation barrier that is activated in response to tumour initiation and a hallmark of premalignant tumours. Of note, recent evidence indicates that senescent cells can promote tumour invasion and metastasis in a cell non-autonomous manner, by up regulating the expression of cytokines and angiogenic factors - a phenomenon termed as the Senescence-Associated Secretory Phenotype (SASP). Indeed, we find that centrosome amplification increased the expression of extracellular proteins. While persistent DNA damage is the primary activator of SASP and that NF-κB is the major determinant of SASP, we show that centrosome amplification instead triggers an SASP independent of persistent DNA-damage and lacks a prominent NF-κB response. Therefore, indicating that centrosome amplification activates a non-canonical SASP program. Previously, we reported that centrosome amplification, through an increased nucleation of centrosomal microtubules, activates Rac-1 signaling and invasion in a cell-autonomous manner. Here, we find that centrosome amplification induced Rac-1 activation contributes to an increase in cellular ROS, which promotes the stabilization of HIF1α. Indeed, up regulation of cellular ROS is a common feature of senescent cells and may activate HIF1α. As most tumors only have a sub-population of cells possessing supernumerary centrosomes, we provide evidence that centrosome amplification exerts a non-cell autonomous impact by activating a non-canonical SASP and HIF1α.

M80

Extensive genetic and transcriptional variation alters the response of cancer cell lines to anticancer drugs.

U. Ben-David[1], B. Siranosian[1], G. Ha[1,2], H. Tang[1], A. Thorner[2], J. Bittker[1], R. Beroukhim[1,2], t.R. Golub[1,2];
[1]Broad Institute of Harvard and MIT, Cambridge, MA, [2]Dana Farber Cancer Institute, Boston, MA

Inconsistencies in cell line-based studies jeopardize reproducibility of cancer research. Genetic and transcriptional heterogeneity within cancer cell lines may contribute to such inconsistencies. To systematically examine this hypothesis, we performed a comprehensive genetic characterization of 27 strains of the common breast cancer cell line MCF7, which were collected from various sources. We then assessed the global gene expression profiles and the response of these strains to 321 anticancer compounds. We combined these genomic, transcriptomic and pharmacologic characterizations to explore the genetic variation within a human cell line, and its effects on gene expression and drug sensitivity. This analysis revealed extensive genetic variation across strains, at all genomic levels (point mutations, structural variation, copy number changes and aneuploidy), affecting multiple oncogenes and tumor suppressor genes. The genetic distances between strains were reflected in the distances between their global gene expression profiles and drug response patterns. Moreover, specific genetic differences could activate distinct transcriptional programs, which in turn resulted in differential drug sensitivities. Reciprocally, differential drug sensitivities could be tracked back to their transcriptional and genetic underpinnings. The variability in drug response was surprisingly high, and resistant strains were

identified for >75% of the active compounds. Moreover, gene expression-based comparison of sensitive and resistant strains correctly identified the targeted signaling pathway(s) for ~70% of the compounds. Importantly, genetic and transcriptional characterization of 23 strains of the common lung cancer cell line A549 confirmed that extensive, functionally relevant heterogeneity is a general trait of cancer cell lines. These results thus have important implications for our understanding of cell line heterogeneity and for the proper use of cell lines in basic biomedical research.

M81

The mTOR network: signal integration and metabolic regulation.

B.D. Manning[1];
[1]Department of Genetics and Complex Diseases, Harvard T.H. Chan School of Public Health, Boston, MA

The mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) is a key signaling node, universal to eukaryotic cells, which links the sensing of nutrients to the coordinated regulation of nutrient metabolism. mTORC1 has the ability to integrate signals from a variety of sources, including intracellular nutrients and secreted growth factors. The activation state of mTORC1 is tightly controlled through a small G protein switch involving the TSC1-TSC2-TBC1D7 complex (the TSC complex) and the Ras-related small G protein Rheb. The direct phosphorylation and inhibition of the TSC complex by the protein kinase Akt provides the major mechanistic link between growth factor signaling and mTORC1. Our evidence indicates that this signal is integrated with amino acid sensing pathways upstream of mTORC1 through independent spatial control over the subcellular localization of the TSC complex and mTORC1 to the surface of the lysosome. This talk will include unpublished data on additional signals that regulate mTORC1 through the TSC-Rheb circuit.
The physiological and pathological activation of mTORC1 results in a shift from catabolic processes to anabolic biosynthetic processes. Through unbiased genomic and metabolomic approaches, we have found that, in addition to its established roles in promoting protein synthesis and inhibiting autophagy, mTORC1 stimulates changes in specific metabolic pathways through transcriptional and posttranslational effects on metabolic enzymes. In this manner, mTORC1 serves to link growth signals to metabolic processes that promote the growth of cells, tissues, and tumors, including the de novo synthesis of proteins, lipids, and nucleotides. I will discuss our latest data on the role of mTORC1 in driving an integrated metabolic program that underlies cell growth and the relationship between anabolic and adaptive responses to distinct cellular signals.

M82

Macropinosomes Coordinate the Activation of PI3Kβ by Gβγ and Rac.

Z. Erami[1], B.D. Khalil[1], G.K. Salloum[1], Y. Yao[1], A. Shymanets[2], B. Nuernberg[2], A.R. Bresnick[3], J.M. Backer[1];
[1]Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, [2]Institute for Pharmacology and Toxicology, Eberhard-Karls-Universität Tübingen, Tubingen, Germany, [3]Biochemistry, Albert Einstein College of Medicine, Bronx, NY

Phosphoinositide 3-kinases (PI3Ks) are regulated by a diverse range of upstream activators, including receptor tyrosine kinases (RTKs), G-protein-coupled receptors (GPCRs), and small GTPases from the Ras, Rho and Rab families. For the Class IA PI 3-kinase PI3Kβ, which regulates thrombosis, spermatogenesis, and tumorigenesis in vivo, two mechanisms for GPCR-mediated regulation have been described: direct binding of Gβγ dimers to the C2-helical domain linker of p110β, and Dock180/Elmo1-mediated activation of Rac1, which binds to the Ras-Binding Domain (RBD) of p110β. We now show that the integration of these dual pathways is unexpectedly complex. Gβγ and constitutively active Rac1 (CA-

Rac1) additively activated PI3Kβ in breast cancer cells. GPCR-mediated activation of Akt was preserved in cells expressing a p110β RBD mutant that is deficient for Rac1 binding. However, CA-Rac1-mediated activation of PI3Kβ was blocked in cells expressing a PI3Kβ mutant that cannot bind Gβγ, and Rac1 activation of wild type PI3Kβ was inhibited by reagents that block endogenous Gβγ signaling. Moreover, Rac1 still activated PI3Kβ in cells expressing the p110β RBD mutant. These data suggest an alternative mechanism for CA-Rac1 activation of PI3Kβ that is independent of direct Rac-PI3Kβ binding. Notably, cells expressing CA-Rac1 exhibited a robust induction of macropinocytosis, which has been previously suggested to amplify signaling to PI3K by GPCRs. Consistent with this hypothesis, suppression of macropinocytosis by EIPA blocked Rac-mediated Akt activation in cells expressing wild type or Rac1-deficient p1110β. Our data suggest that Rac-stimulated macropinosomes serve as signaling platforms that enhances Gβγ coupling to PI3Kβ.

M83

Endosomal/autophagic regulation of FOXO transcription factors.

I. Meras[1], L. Chotardd[2], C.E. Rocheleau[1,2];
[1]Anatomy and Cell Biology , McGill University, Montreal , QC, [2]Research Institute of the McGill University, McGill University , Montreal, QC

FOXO proteins are a conserved family of transcription factors that regulate metabolism, stress responses, and aging. Misregulation of FOXO proteins is associated with cancer, neurodegenerative and metabolic diseases. Insulin/IGF signaling (IIS) pathway negatively regulate FOXO by activating the Akt1/2 kinase, which phosphorylates FOXO. The 14-3-3 proteins bind phospho-FOXO (*p*FOXO), sequestering it in the cytoplasm. FOXO proteins were first identified as downstream targets of IIS pathway in the nematode *C. elegans* which has a single FOXO, DAF-16, that is most closely related to human FOXO3a. During fed conditions, *C. elegans* IIS is constitutive which keeps DAF-16 in the cytoplasm. Under starvation conditions (and other stresses), there is no IIS, and DAF-16 enters the nucleus to regulate gene expression. We found that in the intestine of fed *C. elegans,* DAF-16 localizes to a subset of RAB-5 positive early, and RAB-7 positive late endosomes. DAF-16 endosomes are lost in rab-5(RNAi) and rab-7(RNAi) animals and significantly expanded in *tbc-2* mutants that result in increased RAB-5 activity. In starved animals, DAF-16 endosomes are lost, and DAF-16 localizes mainly to the nucleus, whereas re-feeding results in relocalization of DAF-16 onto endosomal membranes. These results suggest that IIS promotes DAF-16 localization to endosomes. Consistent with this hypothesis, loss of *pten*, a negative regulator of IIS, results in an increase in the number of DAF-16 endosomes, while knockdown of Akt-1, but not Akt-2, results in loss of DAF-16 endosomal localization where it accumulates mainly in the nucleus. Furthermore, we found that FTT-2 14-3-3 proteins colocalizes with DAF-16 on endosomes and knockdown of 14-3-3 protein, FTT-2, results in loss of DAF-16 endosomes. This suggests that the endosomal pool of DAF-16 is phosphorylated by Akt-1. This is conserved in human cells as we discovered that insulin treatment promotes *p*FOXO1/3a localization to Rab5 positive endosomes in HEK293 cells. We do not know the ultimate fate of *p*FOXO on endosomes, but our data shows that LC3/Atg8 engulfs DAF-16 endosomes and knockdown of LC3/Atg-8 increased the number of DAF-16 endosomes. Phosphorylated DAF-16 is shown to be ubiquitinated by RLE-1 an E3 Ubiquitin Ligase. *rle-1(RNAi)* significantly suppressed DAF-16 endosomal localization. These data suggest that DAF-16 is degraded by selective autophagy. We hypothesize that IIS pathway regulates FOXO proteins on endosomes where it can be released if needed or degraded via selective autophagy. Relevance: We believe this project will provide new insights into the regulation of FOXO proteins and open new avenues for drug development to regulate FOXO in cancer, neurodegenerative and metabolic diseases.

M84

Hypoxia and cancer stem cell activity are linked during tumor cell dissemination and metastasis in breast tumors.

E. Xue[1], V.P. Sharma[1,2], D. Entenberg[1,2,3], Y. Wang[1,2,3], B. Tang[4], L. Wakefield[4], M.H. Oktay[1,2,3,5], J.S. Condeelis[1,2,3];
[1]Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY, [2]Gruss-Lipper Biophotonics Center, Albert Einstein College of Medicine, Bronx, NY, [3]Integrated Imaging Program, Albert Einstein College of Medicine, Bronx, NY, [4]Laboratory of Cancer Biology and Genetics, National Cancer Institute, Bethesda, MD, [5]Department of Pathology, Albert Einstein College of Medicine, Bronx, NY

Hypoxia, an established hallmark of cancer, is responsible for many pro-metastatic changes that occur within agressive tumors, including increased angiongenesis, tumor cell invasion, dormancy, and dissemination. Hypoxia may be associated with the induction of cancer stem cells, a rare population thought to be responsible for progression, metastasis, recurrence and drug resistance. Here, we describe the creation of a double-reporter MDA-MB-231 cell line for the visualization of hypoxic and cancer stem cells in real time *in vivo*. The double-reporter cell line contains both our previously published novel optical reporters; for hypoxia (based upon a genetically encodable fluorescent protein, mCherry, and expressed only under hypoxic conditions) (Wang et al 2016), and for cancer stem cells (SORE6-Dscop-GFP) (Tang el al 2015). Using multiphoton microscopy, hypoxic tumor cells were found to exhibit a more persistent slow migration phenotype, associated with blood vessels, and had increased invadopodium-associated collagen degradation and intravasation activity, all as compared to normoxic cells in the same tissue location *in vivo*. In addition, hypoxic tumor cells migrated toward human epithelial growth factor gradients *in vivo* and were present in the CTC population supporting hypoxia involvement in blood vessel directed streaming migration (Leong et al 2016) and intravasation (Wang et al 2016). Since these are characteristics of dissemination and metastasis competent breast tumor cells (Gligorijevic et al 2014), we determined if hypoxic tumor cells with these phenotypes expressed the cancer stem cell reporter (SORE6-Dscop-GFP). We found that the SORE6+ cancer stem cells exhibited the hypoxic tumor cell phenotypes including slower migration than non-stem breast cancer cells (0.1 vs 1.1 µm/min, respectively), migration toward blood vessels, and enrichment for ECM degrading invasive cellular protrusions called invadopodia. Furthermore, in the primary tumor, we observed that stem cells were co-labeled with the hypoxia reporter when in association with blood vessels. Finally, we found, in spontaneous metastases in the lung, that the majority of tumor cells were SORE6+ cancer stem cells during early arrival from the primary tumor. Our results show, for the first time by intravital imaging of live tumor cells in both primary and metastatic breast tumors, that hypoxia and stemness are linked within the same migratory and disseminating population of tumors cells leading to the dissemination of tumor initiating cancer stem cells to the lung.

M85

Identification of RAN binding protein 6 (RanBP6) as an EGFR feedback regulator and a tumor suppressor in glioblastoma.

W. Hsieh[1,2], B. Oldrini[1,3], H. Erdjument-Bromage[4], P. Codega[1], M.S. Carro[5], C. Campos[1], B.S. Taylor[1,6], P. Tempst[1], M. Squatrito[3], I.K. Mellinghoff[1,7];
[1]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, [2]Department of Pharmacology, Weill Cornell Medical College, New York, NY, [3]Seve Ballesteros Foundation Brain Tumor Group, National Cancer Research Centre (CNIO), Madrid, Spain, [4]Molecular Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, [5]Department of Neurosurgery, Medical Center University of Freiburg, Freiburg, Germany, [6]Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, [7]Department of Neurology, Memorial Sloan Kettering Cancer Center, New York, NY

The epidermal growth factor receptor (EGFR) plays a critical role in many biological processes and is frequently altered or amplified in multiple cancers. Here, we examined EGFR interactome by EGFR affinity immunopurification and mass spectrometry. BioGRID and gene ontology analyses revealed that RAN Binding Protein 6 (RanBP6), a 125-kDa protein of previously unknown function, was identified as a novel EGFR interacting protein. We then characterized RanBP6 *in vitro* and showed that RanBP6 belongs to the importin β superfamily and preferentially interacts with nuclear RanGTP. To understand whether this novel EGFR interactor also regulates EGFR expression, we depleted RanBP6 by shRNA and CRISPR/Cas9 system. We found that depletion of RanBP6 raised EGFR mRNA and protein levels, and upregulated EGFR promoter activity. To mechanistically study how RanBP6 negatively regulates EGFR level, we screened a panel of transcription factors and found that RanBP6 represses EGFR transcription by promoting nuclear import of Signal transducer and activator of transcription 3 (STAT3). Depletion of RanBP6 results in reduced STAT3 binding to EGFR promoter, transcriptional derepression of EGFR, and increased EGFR pathway output. Focal deletions of the RanBP6 locus on chromosome 9p were found in a subset of glioblastoma (GBM) and silencing of RanBP6 promoted glioma growth *in vivo*. Our results introduce a novel aspect of EGFR regulation in cancer through silencing of components of the nuclear import pathway.

M86

Nedd9 influences lung cancer tumorigenesis through regulation of autophagy.

A.Y. Deneka[1,2], M.C. Kopp[3], A.S. Nikonova[2], L. Haber[2], A. Gaponova[2], A. Nagele[2], H. Hensley[2], E.A. Golemis[2];
[1]Biochemistry, Kazan Federal University, Kazan, Russia, [2]Molecular Therapeutics, Fox Chase Cancer Center, Philadelphia, PA, [3]Pulmonary, Allergy and Critical Care , University of Pennsylvania, Philadelphia, PA

Non-small cell lung cancer (NSCLC) has a low survival rate, with metastasis contributing to the vast majority of deaths. Expression of the NEDD9 (HEF1/Cas-L) protein is elevated and promotes metastasis in a large subset of NSCLC tumors and other malignancies. NEDD9 functions as a scaffold for multiple critical effectors in integrin/FAK/SRC and receptor tyrosine kinase signaling cascades, with overexpression enhancing signaling by these pro-oncogenic proteins. However, we found that a Nedd9 constitutive null genotype (Nedd9-/-) enhanced tumor growth in an inducible 129S/Sv-Krastm3Tyj/Trp53tm1Brn (KP) model in which Kras mutation is induced specifically in lung tissue by inhalation of adenovirus bearing the Cre gene. Pathological examination of tissues indicated Nedd9 null genotype also was associated with higher invasive capacity in vivo, including direct invasion to the heart,

as well as elevated proliferation rate, decreased apoptotic activity, and changes in the expression of proteins such as vimentin, associated with mesenchymal status. These results contradicted previous studies in which depletion of NEDD9 by RNAi reduced the growth and invasion of established lung cell lines and tumors, based on cell culture and xenograft assays. To gain insight into these paradoxical results, we performed Reverse Phase Protein Array (RPPA) analysis to characterize signaling in isolated tumors. This revealed numerous tumor-intrinsic changes associated with a Nedd9-/- genotype, affecting GYS1 and other proteins in the glycogen synthesis metabolic pathway, and including notable upregulation of BECLN1 and ATG3, indicative of elevated autophagy. Subsequent in vitro analysis coupled with immunohistopathological and metabolic analysis of tumor tissue and tumor-derived cells confirmed and extended these results. Overall, our data support a model in which NEDD9 provides critical support for early stages of NSCLC growth, and progression beyond this early stage in the absence of NEDD9 requires extensive metabolic reprogramming. This reprogramming includes upregulation of the AMPK signaling axis and inhibition of the mTOR pathway, resulting in activation of autophagy responses and changes in oxidative phosphorylation and metabolism. Our data for the first time identifies NEDD9 as a regulator of these processes, and emphasizes distinct requirements for NEDD9 in early tumorigenesis versus in established tumors.

M87

Dietary conjugated polyunsaturated fatty acids induce ferroptotic cell death and suppress breast cancer tumorigenesis and metastasis.

A.C. Beatty[1], L.S. Fink[1], A. Strigun[2], T. Singh[1], E. Peter[1], C.M. Ferrer[3], E. Nicolas[1], K.Q. Cai[1], T.P. Moran[1], M.J. Reginato[3], U. Rennefahrt[2], J.R. Peterson[1];
[1]Cancer Biology, Fox Chase Cancer Center, Philadelphia, PA, [2]metanomics GmbH Metanomics Health GmbH, Berlin, Germany, [3]Drexel University, Philadelphia, PA

Targeting metabolic dependencies to promote tumor cell death is an emerging therapeutic paradigm. Cancer cells have increased levels of reactive oxygen species (ROS), potentially increasing their dependence on antioxidant pathways for survival. Consistent with this, we discovered that a subset of triple-negative breast cancer (TNBC) is addicted to glutathione. We traced this addiction to glutathione consumption by the glutathione-dependent peroxidase GPX4, which neutralizes lipid hydroperoxides, a form of ROS generated from polyunsaturated fatty acids. Decreasing glutathione or GPX4 activity leads to an accumulation of lipid hydroperoxides, triggering a form of non-apoptotic death called ferroptosis. Ferroptotic propensity in vitro was linked to cellular abundance of phospholipid-linked polyunsaturated fatty acids. Furthermore, we identified that supplementing cells with conjugated polyunsaturated fatty acids was sufficient to induced ferroptosis in TNBC cells, and found these fatty acids impeded the growth and metastasis of tumor xenografts in vivo. These results identify a targetable metabolic vulnerability in TNBC, a highly refractory malignancy lacking targeted therapies, and introduce a novel therapeutic approach through the induction of ferroptosis. We introduce a novel class of small molecule ferroptotic inducers that are cancer specific and can be administered in the diet.

M88

Distinct ECM proteins of breast cancer metastatic niches in multiple organs.

J.D. Hebert[1], S.A. Myers[2], A. Naba[1], K.R. Clauser[2], S.A. Carr[2], R.O. Hynes[1];
[1]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, [2]Proteomics Platform, Broad Institute of MIT and Harvard, Cambridge, MA

Metastasis remains the cause of the vast majority of cancer-related deaths, and one of the most insidious aspects of metastatic cancer is the incredible adaptability of cells from a single primary tumor to survive in multiple, vastly different secondary sites. Nevertheless, how cells from the same primary tumor are able to adapt to all these environments is not fully understood. At each secondary site of metastasis, tumor cells create a metastatic niche, a microenvironment conducive to their survival and proliferation. A critical component of each niche is the extracellular matrix (ECM), which provides structural support, migration control, and growth and survival signals. However, a comprehensive comparison of the ECM components of metastatic niches at various secondary tumor sites has not yet been conducted. We isolated metastases from the bone marrow, brain, liver and lung, which were all derived from a common population of MDA-MB-231 breast cancer cells. We then enriched these tumor samples for ECM proteins and used quantitative mass spectrometry to analyze their ECM composition. Across all samples, 247 ECM and ECM-associated proteins were identified, of which 44 were exclusively produced by the human tumor cells and 142 were made solely by the murine stroma. Strikingly, both the tumor-derived and the induced stroma-derived ECM and ECM-associated proteins differ at each site; that is, the niches created are distinct. By abundance, most of the proteins produced only by tumor cells were secreted factors, while the proteins made only by stromal cells were mostly ECM glycoproteins. The set of proteins produced by both the tumor and stromal cells consisted mainly of collagens. Overall, greater than 90% of the total ECM protein abundance within metastases originated from stromal cells. Using these data, protein abundance was compared across all metastatic sites in order to determine which ECM proteins were most significantly elevated in each particular tissue relative to the others. By comparing all metastatic to all normal samples, proteins that were broadly elevated across all metastatic tissues were also identified. Following this analysis, ongoing work is focused on a set of ECM proteins produced by tumor cells specifically in metastases to the brain, testing whether the metastasis of MDA-MB-231 cells to the brain is dependent on the expression of these proteins by injecting knockdown cells into circulation and observing their resulting metastatic growth and tropism. This investigation will provide insight into the fundamental biology of metastatic niches, as well as provide potential markers of metastatic breast cancer at various sites for imaging and therapy.

Minisymposium 9: Ensuring Fidelity of Chromosome Segregation

M89

Role of chromatin and repetitive DNA in centromere formation and propagation.

J. Palladino[1], A. Chavan[1], A. Sposato[1], B. Mellone[1,2];
[1]Molecular and Cell Biology, University of Connecticut , Storrs, CT, [2]Institute for Systems Genomics, University of Connecticut , Storrs, CT

Centromeres are essential regions of the genome that mediate the accurate segregation of chromosomes during mitosis and meiosis. In most metazoans, centromeres are composed of simple and complex repeats and transposable elements organized into a specialized type of chromatin marked by the histone H3 variant CENP-A. For the past two decades, the centromere has been thought to be epigenetically specified, with centromeric DNA being regarded as neither necessary nor sufficient for

centromere function. However, the respective contributions of chromatin and DNA to centromere formation and propagation have remained elusive. Here, we test whether ectopic centromeres, which are devoid of centromeric DNA, can be formed and transmitted during *Drosophila* development and investigate their ability to compete with native centromeres. We induced the formation of ectopic centromeres on integrated *lacO* repeats to which the CENP-A assembly factor CAL1 is tethered via the Lac Repressor (CAL1-LacI). Ectopic centromeres formed successfully in flies with insertions at discrete chromosomal locations in both euchromatin and heterochromatin. During constitutive tethering, an ectopic centromere on the X chromosome sometimes prevailed, causing the inactivation of the endogenous centromere by either DNA loss or HP1-dependent epigenetic silencing. Upon release of CAL1-LacI, however, this inactivation did not occur. Ectopic centromeres appeared to be maintained epigenetically, suggesting that the presence of the CENP-A epigenetic mark can support centromere propagation during animal development.

M90

Condensin complexes promote chromosome movement during mitosis.

K.R. Salmon[1,2], D.A. Compton[1,2];
[1]Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH, [2]Norris Cotton Cancer Center, Lebanon, NH

Mitosis is a highly-regulated process that ensures the accurate formation of two genetically identical daughter cells. An important characteristic of mitotic cells is the compaction of genetic material into discrete chromosomes to facilitate their alignment and faithful segregation (Ono et al., 2004). This compaction is produced by the activity of two independent condensin complexes. To better understand the role of condensin complexes in chromosome movement during mitosis, we knocked down the essential condensin subunit CAP-D3 in Condensin II. This led to decreased expression of other condensin subunits in both condensin complexes. Cells depleted of condensin subunits significantly delayed the completion of mitosis and exhibited disrupted chromosome movement. Chromosome oscillations around the metaphase plate were significantly reduced with some chromosomes displaying no perceivable movement. To understand the basis for this change in chromosome motion, we examined the effect of condensin depletion on molecules with known roles in driving chromosome oscillation. We observed no change in the localization or function of the kinesin Kif18A. However, the depletion of CAP-D3 disrupted the polar ejection force generated by the chromokinesins Kid and Kif4A. Neither Kid nor Kif4A localized to mitotic chromosomes in condensin-depleted cells. Thus, the condensin complexes are required for loading of Kid and Kif4A onto mitotic chromosomes. Without these chromokinesins, chromosome movement in mitosis is disrupted, providing an explanation for the mitotic delay and the chromosome segregation errors seen in the absence of condensins.

M91

Chromokinesin Kif4 is required for faithful chromosome segregation in mammalian oocytes.

C.M. Heath[1], S.M. Wignall[1];
[1]Molecular Biosciences, Northwestern University, Evanston, IL

Spindles in female meiotic cells (oocytes) lack centrosomes, which usually serve to nucleate microtubules and organize spindle poles. Despite the importance of oocyte meiosis to successful reproduction, how acentrosomal spindles interact with chromosomes and mediate accurate segregation is poorly understood. We are investigating the contribution of a kinesin, Kif4, to the meiotic divisions in mouse oocytes. Kif4 is a plus-end directed microtubule motor in the kinesin-4 family that contributes to

chromosome organization and midzone formation in mitotically-dividing cells. However, its roles in oocyte meiosis are less well understood. Using immunofluorescence microscopy, we found that, similar to mitosis, Kif4 localizes to chromosomes from prometaphase to metaphase. However, in oocytes Kif4 displayed a dynamic localization pattern during these stages, as it was excluded from kinetochores and only on chromosomes arms during prometaphase but then became kinetochore-enriched at metaphase, the stage at which kinetochore-microtubule attachments are formed in mouse oocytes. During anaphase, Kif4 relocalized to the spindle midzone; it first appeared in stretches along microtubules and then concentrated into distinct ring-like structures that appeared to organize bundles of microtubules. Other midzone proteins, MgcRacGAP (part of the centralspindlin complex) and PRC1 (protein regulator of cytokinesis 1), also localized to these structures. During mitosis, PRC1 bundles microtubules and binds to Kif4, and our localization data suggest that this relationship is maintained in oocytes. Together, the observed Kif4 localization pattern raised the possibility that this motor could be involved in chromosome dynamics and spindle midzone formation in oocytes. Consistent with this idea, depletion of Kif4 using morpholinos resulted in severe anaphase defects, including chromosome bridges and defective midzone formation, indicating that Kif4 has important functions in oocytes. Building on these results, we also found that treatment of oocytes with AZD1152, an Aurora B/C kinase inhibitor, inhibited localization of Kif4 to metaphase chromosomes and to the anaphase spindle midzone, suggesting that Aurora B/C regulate Kif4 localization. In addition, this treatment caused severe chromosome architecture and segregation defects, some of which resemble Kif4 depletion defects. Moreover, the anaphase spindles appeared disorganized and unstable, indicating that Aurora B/C plays an important role in anaphase spindle organization and thus proper chromosome segregation. Taken together, our work has revealed essential roles for Kif4 during the meiotic divisions and forms the basis for further understanding mechanisms promoting accurate chromosome segregation in acentrosomal oocytes.

M92

Human centromeres produce non-coding alpha satellite RNAs that are chromosome-specific and required for centromere protein loading.

S.M. McNulty[1], L.L. Sullivan[1], B.A. Sullivan[1,2];
[1]Department of Molecular Genetics and Microbiology, Duke University, Durham, NC, [2]Division of Human Genetics, Duke University, Durham, NC

Centromeres serve as the chromatin foundation for the kinetochore and are essential for proper chromosome segregation. In humans, the centromere on each chromosome is defined by a homogenous, repetitive array of alpha satellite DNA. Although alpha satellite DNA was originally thought to be transcriptionally inert, the presence of histone marks at the centromere compatible with active transcription and the known role of non-coding centromeric RNA in other organisms suggest transcription and the resulting transcripts may also be involved in human centromere function. Recent studies confirmed the presence of alpha satellite transcripts, but the exact origin of these non-coding RNAs was unclear, as well as their involvement in the centromere-kinetochore assembly cascade. Using cytological and molecular techniques that allow alpha satellite arrays on different chromosomes to be molecularly distinguished from each other, we have demonstrated that each chromosome produces unique non-coding RNAs that localize *in cis* to their site of production. Alpha satellite RNAs bind at least two key centromere proteins: CENP-A, the centromere-specific histone variant, and CENP-C, a component of the constitutive centromere-associated network of proteins that bridge the centromere and the kinetochore. Targeted depletion of alpha satellite RNAs from a single chromosome leads to 30% loss of CENP-A due, at least in part, to deficient loading of new CENP-A-containing nucleosomes. CENP-C levels are also reduced by nearly half at the targeted centromere. Importantly, loss of alpha satellite

RNA from a single chromosome does not affect the centromeric RNA or protein levels at any other centromere in the cell, highlighting the *cis*-regulatory nature of these non-coding RNAs, but it does lead to cell cycle arrest prior to mitosis. Collectively, these findings implicate non-coding alpha satellite RNA as an essential player in centromere maintenance and underscore the ability of a single defective centromere to alter cell cycle progression.

M93

Kinetochore inactivation by expression of a repressive mRNA.

E. Ünal[1], J. Chen[1], A. Tresenrider[1], M. Chia[2], F. van Werven[2];
[1]Molecular and Cell Biology, UC Berkeley, Berkeley, CA, [2]Francis Crick Institute, London, United Kingdom

Differentiation programs such as meiosis depend on extensive gene regulation to mediate cellular morphogenesis. Meiosis requires transient removal of the outer kinetochore, the complex that connects microtubules to chromosomes. How the meiotic gene expression program temporally restricts kinetochore function is unknown. We discovered that in budding yeast, kinetochore inactivation occurs by reducing the abundance of a limiting subunit, Ndc80. Furthermore, we uncovered an integrated mechanism that acts at the transcriptional and translational level to repress NDC80 expression. Central to this mechanism is the developmentally controlled transcription of an alternate NDC80 mRNA isoform, which itself cannot produce protein due to regulatory upstream ORFs in its extended 5' leader. Instead, transcription of this isoform represses the canonical NDC80 mRNA expression in cis, thereby inhibiting Ndc80 protein synthesis. This model of gene regulation raises the intriguing notion that transcription of an mRNA, despite carrying a canonical coding sequence, can directly cause gene repression.

M94

Most kinetochore fibers in human cells form via mechanisms intrinsic to the kinetochores and not by capture of astral microtubules.

V. Sikirzhytski[1], F. Renda[1], I. Tikhonenko[1], B.F. McEwen[1], A. Khodjakov[1];
[1]Transnational Medicine, Wadsworth Center, Albany, NY

The basic principle of mitotic spindle assembly is known as 'search and capture' (S&C). In the classic formulation of S&C hypothesis, plus ends of 'astral' MTs (MTs) that emanate from the centrosomes (spindle poles) are captured by the kinetochores that reside at the chromosome's centromere. Repetitive captures lead to formation of MT bundles, termed 'K-fibers', that connect kinetochores with spindle poles. Live-cell microscopy proves that direct capture of astral MT by kinetochores does occur occasionally, which supports the S&C hypothesis. However, an alternative mechanism for K-fiber formation has also been observed. In this mechanism, MT bundles grow outwards from the kinetochores. Minus ends of these growing fibers are eventually captured by astral MTs and transported to the spindle poles by dynein. We seek to establish which of these alternative mechanisms dominates mitotic spindle assembly in human cells. Array tomography (fluorescence collected from serial 70-nm sections of the entire cell) reconstructions of the MT density in early prometaphase Rpe1 cells demonstrate that the density of MTs near kinetochores is higher than in those parts of the spindle where kinetochores are absent. This observation suggests that a significant number of MTs form directly at the kinetochores. Correlative light/electron microscopy analyses reveal that <2 min after nuclear envelope breakdown >75% of kinetochores are already in contact with 25-30 MTs residing <250 nm from the kinetochore. These MTs appear to emanate from the kinetochore's outer plate and they tend to orient orthogonally to the plate as expected for end-on attachment. Thus, shortly after NEB nascent K-fibers are already present at most kinetochores. Interestingly, we find no correlation between the

presence or the number of MTs within the nascent K-fiber and the amount of the checkpoint protein Mad2 at the kinetochore. Further, inhibition of Aurora B activity does not affect the number or orientation of the MTs present at the early prometaphase kinetochores. In contrast, inhibition of the kinesin CenpE known to reside at the kinetochore increases the number of MTs at the kinetochores by ~25% and changes the MT orientation. Upon CenpE inhibition, MTs orient randomly to the outer plate with both their ends residing outside of the kinetochore. Further, live-cell recordings demonstrate that inhibition of CenpE alters the pattern of chromosome movements during early prometaphase. Together, our results suggest that most kinetochores attach to short MTs that are nucleated in their spatial proximity. Motor activity of CenpE brings the plus ends of these initially disoriented MTs into the kinetochore, converting lateral interactions into end-on attachment to a K-fiber with the appropriate MT polarity.

M95

Identification and characterization of novel spindle assembly checkpoint components.

J.Z. Torres[1], Y.A. Garcia[1], I. Ramirez[1], A. Gholkar[1], Q. Yang[1], S. Senese[1], J. Ong[1], T. Falegan[1], K.S. Morillo[1], N. Filbert[1], Q. Dam[1];
[1]Chemistry and Biochemistry, University of California Los Angeles, Los Angeles, CA

Human cell division is a highly coordinated set of events that ensures the proper transmission of genetic material from one mother cell to two newly formed daughter cells. Critical to the fidelity of chromosome segregation is the multi-component spindle assembly checkpoint (SAC), which is activated when kinetochores remain unattached to microtubules or in response to nonproductive microtubule-kinetochore attachments. An active SAC arrests cells in metaphase to give time to correct these deficiencies and generate proper microtubule-kinetochore attachments before proceeding with chromosome segregation. Misregulation of the SAC can lead to premature sister chromatid separation, chromosomal instability, aneuploidy and resistance to antimitotics. Thus, understanding the SAC is critical to understanding cell division, tumorigenesis and the response of tumor cells to antimitotic drugs. Although decades of research has shed light on the components and function of the SAC, we are far from elucidating the full complement of regulatory factors involved in this complex pathway and from understanding how misregulation of this pathway can lead to tumorigenesis and chemotherapeutic drug resistance. To address these issues, we recently performed a high-throughput small interfering RNA screen for novel regulators of the SAC. This approach yielded novel kinases and phosphatases. Inactivation of these novel factors leads to inactivation of the SAC in the presence of antimitotics and to premature chromosome segregation. To our knowledge, these new SAC components have not been previously linked to SAC functioning and understanding how these components regulate the SAC is important to advance our understanding of the SAC and more broadly cell division. We have utilized complementary multidisciplinary approaches that include cell biology, biochemistry, mass proteomic and microscopy-based approaches to characterize the importance of these proteins to SAC functioning and proper chromosome segregation, to define their cell cycle dependent subcellular localization and the determinants that regulate their localization, to identify their interacting proteins, to define the substrates they modify and to determine the importance of these interactions/modifications to SAC function, and to determine how they cooperate with other SAC proteins to ensure the fidelity of chromosomal segregation. We are optimistic that our studies will increase our understanding the mechanisms that ensure proper chromosome segregation and how their misregulation can lead to tumorigenesis and chemotherapeutic resistance.

M96

The Mad1/Mad2 spindle checkpoint complex is repurposed in development to promote cell cycle progression.

P. Lara Gonzalez[1], J. Mendoza[1], M. Moyle[2], K. Oegema[1,3], A.B. Desai[1,3];
[1]Ludwig Cancer Research San Diego, La Jolla, CA, [2] Yale School of Medicine , New Haven, CT,
[3]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA

The mitotic phase of the cell cycle is controlled by Cyclin B, which activates the kinase Cdk1. Cyclin B accumulation in G2 promotes mitotic entry, whereas its degradation by the Anaphase-Promoting Complex/Cyclosome (APC/C) triggers anaphase onset and mitotic exit. Here we report that components of the spindle assembly checkpoint that inhibit APC/C activity during mitosis until all chromosomes connect to the spindle, are repurposed in *C. elegans* development to control mitotic entry. This conclusion initiated with the observation that deletion of the genes encoding two essential spindle assembly checkpoint proteins, Mad2 (*mdf-2*) and Mad3 (*san-1*), give rise to dramatically different phenotypes. A Mad2 mutant exhibits severe embryonic and post-embryonic developmental defects that are not observed in a Mad3,mutant, despite both being equally defective in the spindle checkpoint. Comparison of the phenotypic profile of Mad2 removal in the germline to a prior screen of genes required for embryo production in the same tissue revealed similarity to components required for mitotic entry (Cyclin B, Cdk1 and Cdc25), leading to the hypothesis that Mad2 promotes mitotic entry in specific developmental contexts. In agreement with this, Mad2 inhibition delayed the cell cycle of larval germ cells at the G2 stage and greatly reduced their proliferation rate. These data suggest that Mad2 is activated by specific developmental cues to promote cell cycle progression, independently of its established function in the spindle assembly checkpoint. This function requires Mad2 to form a complex with its partner, Mad1, to undergo conformational conversion following asymmetric dimerization, and to bind the mitotic activator of the APC/C, Cdc20. We propose that the ability of the Mad1/Mad2 complex to inhibit APC/C-Cdc20 has been adapted in a checkpoint-independent developmental context to promote progression into mitosis, potentially by allowing accumulation of Cyclin B to a level sufficient for mitotic entry. Interestingly, based on phenotypic analysis, Cyclin B3 appears to be the Cyclin B isoform whose accumulation requires Mad1/Mad2. Overall, this effort has revealed how a core module of the spindle checkpoint is repurposed to provide an essential function in organismal development, and should influence interpretation of phenotypes associated with checkpoint component inhibitions in metazoans as well as therapeutic approaches designed to target the checkpoint pathway in cancer.

M97

Sensing of the Magnitude of Centromeric Tension at Metaphase Elicits a Graded Cellular Response.

S. Mukherjee[1], D. Tank[1], Q. Yang[1], M.K. Gardner[1];
[1]Genetics, Cell Biology, and Development, University of Minnesota , Minneapolis, MN

During mitosis, motors associate with microtubules to exert forces that push spindle poles apart, thus establishing a mitotic spindle. These pushing forces in turn cause tension in the chromatin that connects oppositely attached sister chromatids. This tension has been hypothesized to act as a mechanical signal that allows the cell to detect chromosome attachment errors during mitosis. However, the magnitude of any changes in tension that could be detected by the cell to initiate an error correction response during mitosis have not been measured, and so the underlying mechanics of a tension-based error detection pathway remains unknown. In this study, we generated and measured a gradient in tension over multiple isogenic budding yeast cell lines by genetically altering the magnitude of motor- based spindle

forces. This allowed us, for the first time, to quantitatively elucidate the mechanics of a tension-based error detection pathway in mitosis. We found that a decreasing gradient in tension led to an exponentially increasing gradient in rates of kinetochore detachment and anaphase chromosome mis-segregration, with a corresponding gradient in metaphase times. Further, these tension-based cellular response gradients were abrogated in the absence of key error-correction pathway proteins. We conclude that the cell is exquisitely tuned to the magnitude of tension as a signal to detect potential chromosome segregation errors during mitosis.

M98

Microtubule sliding in the bridging fiber pushes kinetochore fibers apart to segregate chromosomes in human cells.

P. Risteski[1], K. Vukušić[1], R. Buđa[1], A. Bosilj[2], A. Milas[1], N. Pavin[2], I.M. Tolić[1];
[1]Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia, [2]Department of Physics, Faculty of Science, University of Zagreb, Zagreb, Croatia

During cell division, microtubules of the mitotic spindle segregate chromosomes by exerting forces on kinetochores, protein complexes on the chromosomes. The central question is what forces drive chromosome segregation. The current model for anaphase in human cells includes shortening of kinetochore fibers and separation of spindle poles. Both processes require kinetochores to be linked with the poles. Here we show, by combining laser ablation, photoactivation and theoretical modeling, that kinetochores can separate without any attachment to one spindle pole. This separation requires the bridging fiber, which connects sister kinetochore fibers. The number of bridging fibers per spindle correlates with the variable chromosome numbers in HeLa cells, suggesting a one-to-one relationship. Bridging microtubules in intact spindles slide apart together with kinetochore fibers, indicating strong crosslinks between them. We conclude that sliding of microtubules in the bridging fibers drives pole separation and pushes kinetochore fibers poleward by the friction of passive crosslinks between these fibers. Thus, sliding in the bridging fiber works together with the shortening of kinetochore fibers to segregate chromosomes.

Minisymposium 10: Lipids in Signaling and Membrane Organization

M99

Structural determinants of raft partitioning for single-pass transmembrane proteins and their effects on subcellular localization.

J.H. Lorent[1], B.B. Diaz-Rohrer[1], K.R. Levental[1], I. Levental[1];
[1]Integrative Biology and Pharmacology, Mc Govern Medical School at UT Health Science Center at Houston, Houston, TX

Eukaryotic plasma membranes (PMs) are compartmentalized into functional lateral domains, including lipid-driven membrane rafts. Rafts are involved in most PM functions by selective recruitment and retention of specific proteins. Transmembrane proteins (TMPs) reflect ~30% of the mammalian proteome and mediate almost all cellular functions. However, neither the structural determinants of TMP partitioning to raft domains, nor the functional consequences thereof, are known. We hypothesized that structural features of the transmembrane domain (TMD) of single-pass TMPs would guide raft partitioning and raft-dependent functionality. To explore TMD-dependent raft partitioning, we isolated intact cellular plasma membranes as Giant Plasma Membrane Vesicles (GPMVs), a model system that allows direct observation of protein partitioning between raft and non-raft domains by

fluorescence microscopy. We quantified raft partitioning of >100 TMPs and identified three physical features – TMD surface area, length, and palmitoylation – that independently affect raft partitioning. Specifically, palmitoylated and longer TMDs with smaller surface areas partitioned efficiently to the more ordered raft domains. These findings were rationalized with a mechanistic, physical model wherein raft affinity is determined by the interfacial energy between a protein TMD and the surrounding lipid matrix. This model was shown to be capable of correctly predicting raft affinity solely from protein sequence. Using bioinformatics, we generated proteome-wide predictions of raft affinity and observed that PM proteins have higher predicted raft affinity than those of intracellular membranes, consistent with raft-mediated PM sorting. These predictions were confirmed by cellular experiments in which PM localization was dependent on raft affinity for a variety of unrelated TMPs. Thus, our experimental observations and physical model establish general rules for raft partitioning of TMDs and support the central role of rafts in membrane traffic.

M100

SNX-BAR mediated retrograde trafficking of yeast synaptobrevin/Snc1 is conferred by its transmembrane domain.

M. Ma[1], L. Purushothaman[2], M. Babst[3], C. Ungermann[2], C.G. Burd[1];
[1]Department of Cell Biology, Yale University, New Haven, CT, [2]Department of Biology/Chemistry, University of Osnabrueck, Osnabrück, Germany, [3]Department of Biology, University of Utah, Salt Lake City, UT

Synaptobrevin/VAMP2 is an essential SNARE protein that has been extensively studied in its role in synaptic vesicle fusion. However, how synaptobrevin is sorted for trafficking within the cell is not well understood. A unique structural feature of synaptobrevin is a conserved aromatic motif on the cytosol-proximal interface of the juxtamembrane region that has been proposed to confer a 35° tilt angle in the transmembrane domain, allowing conserved basic residues in the transmembrane domain to interact with the membrane surface. The aromatic motif has been shown to promote binding of calmodulin and phospholipids, and to facilitate SNARE complex assembly. In yeast, the synaptobrevin homologue Snc1 has been shown to require the aromatic motif for proper trafficking. We demonstrate that the basic residues on the cytosol-proximal surface of the Snc1 transmembrane domain play an important role in promoting sorting of Snc1 into retrograde trafficking pathways. Additionally, we demonstrate that the Snx4-Atg20 SNX-BAR dimer, which functions in retrograde trafficking of Snc1, binds preferentially to anionic membranes and we propose this contributes to Snc1 recognition and sorting out of the endosome. We further characterize Snc1 trafficking pathways in yeast and propose a bifurcated trafficking model for Snc1 where endocytosed Snc1 and Snc1 on the late/pre-vacuolar endosome are returned to the Golgi via two different retrograde pathways.

M101

A role of an inositol 5-phosphatase in ER architecture.

R. Dong[1,2,3], T. Zhu[4], L. Benedetti[1,2,3], S. Gowrishankar[1,2,3], H. Deng[4], X. Wang[4], K. Shen[5,6], P. De Camilli[1,2,3,7];
[1]Department of Neuroscience, Yale University School of Medicine, New Haven, CT, [2]Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT, [3]Department of Cell Biology, Yale University School of Medicine, New Haven, CT, [4]Institute of Biophysics, Chinese Academy of Sciences, Beijing, China, [5]Department of Department of Biology and Department of Pathology , Stanford University School of Medicine, Stanford, CA, [6]Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA, [7]Kavli Institute for Neurosciences, Yale University School of Medicine, New Haven, CT

INPP5K, a member of the inositol 5-phosphatase family, consists of the phosphatase domain followed by a SKICH domain. Partial loss of function mutations of INPP5K were recently reported to result in congenital muscular dystrophy. How INPP5K localizes and functions in the ER remains unknown. Here we report that recruitment of INPP5K to the ER relies on ARL6IP1, an ER membrane protein which shares features of ER shaping proteins and whose dysfunction results in hereditary spastic paraplegia. The interaction of INPP5K with ARL6IP1 requires a cooperation of both its phosphatase and SKICH domains. Both INPP5K and ARL6IP1 are localized throughout the tubular ER network, but are not present in ER sheets. In addition, they are more concentrated, relative to generic ER proteins (e.g. Sec61b, VAPB), in newly formed tubules that undergo rapid extension along microtubule tracks. Depletion of either INPP5K or ARL6IP1 results in decreased abundance of ER tubules and in the expansion of ER sheets, possibly due to impaired formation of new ER tubules. Supporting these findings, a forward genetic screen aimed at the identification of genes controlling the ER architecture in PVD neurons of C. elegans, led to the isolation of cil-1 mutants, which are deficient in the function of the INPP5K orthologue. In these mutants, the complexity of ER tubule network and the extension of ER tubules into dendritic branches was impaired. These defects were rescued by expression of WT CIL-1, but not of catalytically inactive CIL-1or SKICH domain-deleted CIL-1. Our results implicate an ER localized inositol-5-phosphatase in the control of the ER architecture.

M102

Regulation of sites for organelle biogenesis at the endoplasmic reticulum.

p. carvalho[1], S. Wang [1], F. Idrissi[1];
[1]Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom

The endoplasmic reticulum (ER) is the largest membrane bound compartment in cells and supplies lipids and membrane proteins to almost all other organelles. These molecules are delivered through COPII vesicle trafficking, membrane contact sites or sorted directly from specific ER domains into a new organelle, as in the case of lipid droplets (LDs) and peroxisomes. While our understanding of COPII vesicle formation and of ER contact sites has been steadily growing, how ER domains specialized in organelle biogenesis are established is unknown. Also, it is not known whether distinct domains are required for the biogenesis of LDs and peroxisomes, which are structurally different organelles. While peroxisomes consist of a phospholipid bilayer enclosing a dense matrix rich in metabolic enzymes, LDs have a unique phospholipid monolayer enclosing a hydrophobic core made up of neutral lipids. Recent work from us and others showed that seipin is a major component of the ER-LD interface with key roles in LD biogenesis across eukaryotes. We now find that seipin cooperates with the ER shaping protein Pex30. In the absence of both proteins LDs fail to bud resulting in neutral lipid accumulation in abnormal

ER structures. The double mutant *pex30 seipin* is also defective in peroxisome biogenesis suggesting a link between LD and peroxisome budding from the ER. Moreover, a well-established assay to monitor *de novo* peroxisome biogenesis show that Pex30 and seipin co-localize with peroxisomal membrane proteins to discrete ER domains. Thus, our data indicate that LDs and peroxisomes form in ER domains of similar composition and that are stabilized by seipin and Pex30. Additional genetic and lipidomics experiments indicate that these domains specialized in organelle budding have a distinct lipid composition from the bulk ER. Altogether, we propose a model in which seipin and Pex30 establish and stabilize ER domains of defined lipid composition that is permissive for biogenesis of structurally unrelated organelles such as LDs and peroxisomes.

M103

Lipid droplet emergence from the ER membrane is mediated by phospholipids.

V. Choudhary[1], G. Golani[2], A. Joshi[1], M.M. Kozlov[2], W. Prinz[1];
[1]Laboratory of Cell and Molecular Biology, NIH/NIDDK, Bethesda, MD, [2]Department of Physiology and Pharmacology, Tel Aviv University, Tel Aviv, Israel

Lipid droplets (LDs) store fats and play critical roles in lipid and energy homeostasis. They form between the leaflets of ER membranes and consist of a neutral lipid core wrapped in a phospholipid monolayer with proteins. Two types of ER-LD architecture are thought to exist and be essential for LD functioning. Maturating LDs either emerge from the ER into the cytoplasm, remaining attached to the ER by a narrow membrane stalk, or stay embedded in the ER and are surrounded by ER membrane. Here, we identify a lipid-based mechanism that controls ER-LD architecture. Using a combination of theoretical modeling and experimentation with mutants, we show that lipids with negative intrinsic curvature favor LD embedding, while lipids with positive intrinsic curvature support LD emergence. We find that lipid droplets emerge from specialized domains of the ER that have a unique lipid composition that affects ER-LD architecture. Our findings suggest that cells regulate LD integration in the ER membrane by modulating ER lipid composition, particularly at sites of LD biogenesis.

M104

Mechanism of Targeting of Amphipathic Helix-Containing Proteins to Lipid Droplets.

C. Prévost[1,2,3], M. Cohen[4,5,6], N. Kory[1,2,3], Q. Lin[1,2,3], G.A. Voth[4,5,6], R.V. Farese, Jr.[1,2,3], T.C. Walther[1,2,3,7];
[1]Department of Genetics and Complex Diseases, Harvard T. H. Chan School of Public Health, Boston, MA, [2]Department of Cell Biology, Harvard Medical School, Boston, MA, [3]Broad Institute of Harvard and MIT, Cambridge, MA, [4]Department of Chemistry, The University of Chicago, Chicago, IL, [5]James Franck Institute, The University of Chicago, Chicago, IL, [6]Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL, [7]Howard Hughes Medical Institute, Boston, MA

Cytosolic lipid droplets (LDs) are the main storage organelles for metabolic energy in most cells. They comprise a core of neutral lipids bounded by a monolayer of phospholipids decorated with specific proteins. These proteins notably perform crucial functions in cellular energy and lipid metabolism. However how proteins localize to LDs is not well understood. In particular, binding of cytosolic proteins to LDs is typically mediated by amphipathic helices (AHs), which are well-known membrane-binding motifs. How these AHs distinguish the LD monolayer from the ER membrane, which has a similar phospholipid composition, is unknown. Using a multidisciplinary approach, we demonstrate that the physicochemical properties of the LD surface makes it ideally suited to recruit AHs containing large hydrophobic residues. First, using molecular dynamic simulations, we investigated the structure of the LD surface and the initial steps of AH binding with atomic resolution. These simulations revealed that

the LD surface displays defects in the packing of phospholipids that are larger and more frequent than those on bilayer membranes. Further, we found that model AHs initially anchor to the surface of LDs through one or several large hydrophobic residues which insert in such packing defects. AHs containing this type of residues preferentially bound to the LD monolayer in in vitro systems comprising LD and membrane mimics. Finally by expressing AHs from both LD and non-LD proteins in Drosophila cells, we confirmed that targeting to LDs is dependent on large hydrophobic residues in amphipathic helical sequences. Our work elucidates the unusual features of LD surfaces that influence protein targeting, identifies properties of AHs that recognize these surface features, and suggests how these mechanisms contribute to shaping the LD proteome.

M105

Pattern formation and stochastic geometry sensing in a lipid kinase-phosphatase competitive reaction.

S.D. Hansen[1,2], W. Huang[3], Y. Lee[3], P. Bieling[1,4], J.T. Groves[1,3];
[1]California Institute for Quantitative Biosciences, University of California Berkeley, Berkeley, CA, [2]Chemistry and Biochemistry, University of Oregon, Eugene, OR, [3]Chemistry, University of California Berkeley, Berkeley, CA, [4]Systemic Cell Biology, Max Planck Institute of Molecular Physiology, Dortmund, Germany

Symmetry breaking and the establishment of spatial patterns of molecules in a dynamic, fluid environment is a hallmark of cellular organization in living systems. Central to many of these cellular signal pathways are phosphorylation reactions, driven by competing lipid kinases and phosphatases. Here, we describe the biochemical reconstitution of a lipid kinase-phosphatase reaction system that drives the interconversion of PI(4)P and PI(4,5)P2 lipids on planar membrane surfaces. This system can be tuned to exhibit bistability and spontaneous pattern formation. Using micropatterned membranes to spatially confine the competitive reaction, we discover that final reaction outcome can be modulated by the geometric size and shape of the membrane environment. The physical mechanism of this process, which we call stochastic geometry sensing, is based on the system's asymmetrical response to stochastic compositional fluctuations in enzyme copy number and the corresponding local changes lipid density. The fundamental finding is that stochastic geometry sensing provides a relatively simple route for a biologically relevant chemical reaction to form patterns and differentially respond to the geometric constraints of the reaction environment.

M106

ER-Golgi contact sites are maintained through redundant and essential roles of ORPs and use FAPP1 to control Golgi PI4P.

R. Venditti*[1], M.C. Masone*[1], L.R. Rega*[1], M. Santoro[1], G. Di Tullio[1], E.V. Polishchuk[1], R. La Montagna[1], M. De Matteis[1,2];
[1]TIGEM, Telethon Foundation, Pozzuoli, Italy, [2]Molecular Medicine and Medical Biotechnology, Federico II University, Naples, Italy

Despite their early identification, there are still many unknowns regarding the functions and composition of ER-Trans Golgi Network (TGN) membrane contact sites (MCSs) due to the lack of visualization methods with optical microscopy. We developed a FRET-based strategy to follow ER-TGN MCSs by optical microscopy and identified the VAPA and VAPB proteins as structural determinants for this class of contact sites. The VAPs were used as positive controls in a High Content Screening campaign to identify additional components that are required to maintain the ER-TGN MCSs. We screened the full

repertoire of proteins that possess a dual targeting motif for both the Golgi and ER (OSBP1, ORP9, ORP10, ORP11, CERT, FAPP1, and FAPP2) and found that CERT and FAPPs are dispensable, some ORPs have a redundant tethering role while other ORPs have an essential role due to their lipid exchange activity in maintaining ER-TGN MCSs. Destabilizing the ER-TGN MCSs causes an increase in the levels of PI4P in the TGN due to the inability of the ER-localized 4-phosphatase Sac1 to access its substrate, PI4P, at the Golgi. We demonstrate that Sac1 can act in trans on PI4P at the TGN thanks to the intervention of the PI4P-binding protein FAPP1, which binds both Sac1 and VAPs and acts as a PI4P sensor that sets the levels of PI4P at the Golgi.

M107

PI(4,5)P2 controls the level of its precursor, PI4P, in the plasma membrane by regulating PI4P/PS transport at ER-plasma membrane contact sites.

M. Sohn[1], M. Korzeniowski[1], G. Hammond[2], E. Boura[3], T. Balla[1];
[1]National Institute of Child Health and Human Development , National Institutes of Health , Bethesda , MD, [2]Department of Cell Biology , University of Pittsburg , Pittsburg , PA, [3]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague , Czech Republic

Phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] is a minor but critical regulatory phospholipid playing a role in signal transduction, endocytosis, exocytosis, actin dynamics as well as ion channel activity in the plasma membrane (PM). Little is known about how cells keep PI(4,5)P2 levels within normal range. Here we found that PM PI(4,5)P2 controls its own precursor supply at the PM by regulating the activities of the oxysterol-binding protein related proteins (ORP) 5 and ORP8 at ER-PM contact sites. ORP5/8 was previously known to transfer PI4P from the PM to the ER in exchange for phosphatidylserine (PS) which is transported in the reverse direction. We found that while ORP5 is already active at basal levels of PI4P and PI(4,5)P2, ORP8 shows little activity due to its poor membrane interaction. This difference is caused by the different membrane binding affinities of the N-terminal PH domains of ORP5/8. Importantly, interaction of ORP5 with the PM was found to depend both on PI4P and PI(4,5)P2 and decreases in either phosphoinositides levels caused dissociation of ORP5 from the PM. In contrast, PM interaction of ORP8 was strongly enhanced by expression of a PIP-5-kinase (PIP5K), which synthesizes PI(4,5)P2 in the PM. PIP5K overexpression also caused a massive reduction in PM PI4P and PS levels and only moderately increased the level of PI(4,5)P2. Thus, enhanced PI(4,5)P2 production facilitates the PI4P/PS exchange to the point where PM PI4P levels are depleted and no more able to support PS transport to the PM. This regulatory mechanism helps cells maintain PI(4,5)P2 levels by controlling the PI4P flux between PI4P/PS transfer and PI(4,5)P2 generation.

M108

SAC1 degrades its lipid substrate PtdIns4P in the ER to maintain a steep electrochemical gradient on donor membranes.

J.P. Zewe[1], S.J. Sangappa[1], R.C. Wills[1], B. Goulden[1], G.R. Hammond[1];
[1]Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA

Phosphatidylinositol 4-phosphate (PtdIns4*P*) is one of the most functionally diverse molecules utilized by eukaryotic cells. It is both a metabolic hub for other crucial signaling lipids such as PtdIns(4,5)P_2 and PtdInsP_3, and a key molecule for recruitment of membrane and lipid transport proteins in its own right. Most recently, it has been proposed to form a “phosphoinositide-motive force”, whereby transport of PtdIns4*P* molecules down their concentration gradient from the plasma membrane (PM) or Golgi to the ER drives counter transport of other lipids up their own concentration gradients. A critical requirement

for this model is that the main PtdIns4*P* degrading enzyme, the integral ER phosphatase SAC1, hydrolyzes PtdIns4*P* in the ER in a "cis" configuration. Alternatively, it has been suggested that other functions of PtdIns4*P* are regulated by SAC1 hydrolyzing PtdIns4*P* directly in the PM in a "trans" configuration at membrane contact sites (MCS). However, such activity would surely disrupt lipid counter transport. Therefore, we sought to determine whether SAC1 acts in "cis", "trans", or both in mammalian cells. Acute chemical ablation of SAC1 activity drives ectopic accumulation of PtdIns4*P* in the ER, revealing "cis" activity. Furthermore, endogenous or ectopically expressed SAC1 localizes to the ER and Golgi, but does not constitutively or dynamically enrich at ER-PM MCS. Forced recruitment of SAC1 to experimentally induced MCS does not produce robust "trans" activity on PM PtdIns4*P* . However, "trans" activity can be induced by adding an approximately 6 nm long helical linker between the ER anchor and the catalytic domain. Together, our results reveal that SAC1 operates in a "cis" configuration. This ensures a "phosphoinositide-motive force" for lipid transport is effective, and also implies regulation of PM phosphoinositide signaling is tightly linked to non-vesicular traffic of PtdIns4*P* at ER-PM MCS.

Minisymposium 11: Multicellular Interactions: Tissue Regeneration and Mechanisms of Disease

M109

Probing the physiological purpose of tissue damage-induced inflammation in zebrafish.

C. Huang[1], P. Niethammer[1];
[1]Cell Biology, Sloan Kettering Institute, New York, NY

Neutrophil recruitment into damaged organs is a devastative, clinical complication of many tissue degenerative diseases, including ischemia, drug-induced liver injury, cancer, and others. It is unclear why professional antimicrobial cells, such as neutrophils, possess the dangerous ability to seek out tissue damage given the many negative, clincial repercussions. By live imaging of neutrophil migration in transparent zebrafish larvae, we show that tissue damage signals are essential for neutrophil recruitment to infection sites and post-infection survival of animals. We illuminate underlying signaling circuits. Our data suggest that neutrophils primarily respond to tissue damage, with microbial signals acting as costimulatory amplifiers of this response. Our results suggest that tissue damage signaling provides a selective advantage after microbial infection.

M110

Building a barrier: survival of the fittest in the developing skin.

S. Ellis[1], E. Fuchs[1];
[1]Laboratory of Cell Biology and Genetics, The Rockefeller University, New York, NY

A key feature of animal embryogenesis is the creation of a permeability barrier that protects internal organs and tissues from physical damage, dehydration, pathogen invasion, and other harmful environmental stresses. In mammals, this function is fulfilled by the epidermis, a tissue comprised of stratified layers of specialized epithelial cells. During embryonic development, the epidermis expands dramatically to accommodate the growth and increase in surface area of the organism. Concomitantly, the skin undergoes a differentiation program from a monolayer of progenitors to become a functionally specialized, multi-layered permeability barrier at birth. This rapid process of patterning and growth is vulnerable to deleterious genetic errors and epigenetic and/or post-translational misregulation that could be propagated throughout the tissue and thus compromise both barrier function and the fitness

of the organism. Therefore, we sought to understand the quality control mechanisms that operate during the complex process of skin development.
Cell competition is a phenomenon by which less fit “loser” cells are eliminated by more fit “winner” cells. Could such a mechanism exist in embryonic skin to ensure that only the fittest progenitor cells persist to establish and maintain a functional epidermal barrier? Using a combination of functional genetics and time-lapse imaging, we establish a model for cell competition in the mouse embryonic epidermis. Early in epidermal development, loser cells are eliminated from the growing epithelium via apoptosis and engulfment by surrounding winner cells. Strikingly, we observe a developmental switch in loser cell elimination mechanisms; upon the emergence of stratified tissue layers, loser cells are eliminated via hastened differentiation rather than apoptosis. Abrogating the function of factors required for loser cell clearance leads to compromised barrier integrity and differentiation defects. Altogether, our studies raise the intriguing possibility that cell competition acts as a selective force during epidermal development to maximize tissue fitness. Moreover, our data illuminates how the strategy a tissue uses to preserve fitness can evolve as architectural complexity increases during morphogenesis.

M111

Src oxidation directs cell polarity to promote rapid embryonic wound healing.

M.V. Hunter[1,2], J. Woszczyk[3,4], P. Laprise[3,4], R. Fernandez-Gonzalez[1,2,5,6];
[1]Cell and Systems Biology, University of Toronto, Toronto, ON, [2]Ted Rogers Centre for Heart Research, University of Toronto, Toronto, ON, [3]Centre de Recherche sur le Cancer, Université Laval, Quebec, QC, [4]Oncologie, Centre de Recherche du CHU de Québec - Université Laval, Quebec, QC, [5]Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, [6]Developmental and Stem Cell Biology Program, Hospital for Sick Children, Toronto, ON

Embryos exhibit a striking ability to rapidly repair wounds without inflammation or scarring. Embryonic wound healing is a conserved process, driven by redistribution of the cytoskeleton and cell-cell junctions around the wound. Immediately after wounding, both actin and the molecular motor myosin become polarized in the cells around the wound, accumulating at the wound margin to form a supracellular cable. The rapid contraction of this cable draws the surrounding cells together to close the wound. We recently showed that polarized endocytosis of the junctional protein E-cadherin from the wound margin is a prerequisite for actomyosin cable assembly and wound closure. However, the upstream signal that triggers redistribution of junctions and the cytoskeleton around wounds is unknown. Using quantitative time-lapse microscopy in *Drosophila* embryos, we measured a burst of reactive oxygen species (ROS) production immediately after wounding, exclusively in the cells that are eventually enclosed by the actomyosin cable. Blocking ROS production significantly inhibited wound healing, and severely impaired junctional trafficking and actomyosin accumulation around the wound. Together, our results suggest that ROS production drives wound healing by promoting E-cadherin trafficking and subsequent polarized actomyosin assembly. ROS can post-translationally modify proteins by oxidizing electron-rich cysteine residues. The *Drosophila* ortholog of Src kinase, Src42A, contains several putative redox-sensitive cysteines, and has been implicated in E-cadherin trafficking during morphogenesis. Src42A^{C471} is a conserved cysteine that is oxidized in zebrafish leukocytes upon wounding. We mutated Src42A^{C471} to generate an oxidation-resistant form of Src42A (Src42A^{OR}). Src42A^{OR} exhibited reduced kinase activity *in vitro*, and, importantly, embryos expressing Src42A^{OR} displayed impaired wound healing. We are currently investigating the phosphorylation targets of Src42A and how Src activation promotes wound healing. We propose that ROS act as a critical wound signal, orchestrating the polarity of junctions and the cytoskeleton through Src to drive rapid wound healing.

M112

Hepatokine induction mediates anti-inflammatory actions of Colchicine.

J. Weng[1], R. Jiang[1], H. Tu[2], T.J. Mitchison[1];
[1]Department of Systems Biology, Harvard Medical School, Boston, MA, [2]Alnylam Pharmaceuticals, Inc, Cambridge, MA

Inflammation recognizes and eliminates danger causing by invading pathogens or endogenous signals. However, uncontrolled inflammation also damages tissues. Colchicine, an anti-inflammatory medication that binds to tubulin and blocks microtubule polymerization, is frequently prescribed for gout, Familial Mediterranean Fever, pericarditis, and is being clinically tested for a wide spectrum of diseases. Understanding how an anti-microtubule drug blocks inflammation would help us design safer future drugs. Colchicine inhibits extravasation of neutrophils to inflamed tissues in man. In culture, it directly inhibits neutrophil chemotaxis and maturation of the pro-inflammatory cytokine, IL-1b, but the required concentrations are 30-300x higher than plasma therapeutic concentrations, which calls into question the textbook model of direct action on leukocytes. Colchicine shows rapid clearance from blood (t1/2 ~ 0.3 hour), followed by selective accumulation in the liver, and slow elimination via bile (t1/2 ~ 1 day). This pharmacology leads us to propose an alternative “Liver Selective Action” model. By measuring biomarkers of microtubule perturbation and using hepatocyte-specific siRNAs, we identified hepatocytes as the main target of colchicine at low doses. Colchicine induced secretion of a divergent member of the TGF-b family, GDF15, from hepatocytes in mice. Conditioned serum from colchicine-dosed mice contained GDF15, blocked leukocyte adhesion, and inhibited IL-1b maturation. By comparing wild-type vs GDF15-/- mice, we found that GDF15 was required for colchicine treatment of crystal-induced peritonitis (a gout model). Our results identify GDF15 as a colchicine-induced hepatokine and show that colchicine treats inflammation by selectively accumulating in hepatocytes, triggering GDF15 expression, which globally inhibits neutrophil extravasation.

M113

The generation and sensation of fluid flow by cells: roles in development and disease.

D.T. Grimes[1], R.D. Burdine[1];
[1]Molecular Biology, Princeton University, Princeton, NJ

Like classical signal transduction cascades, the microscopic flow of fluids within biological systems provides cues critical for development, growth, and homeostasis. Organisms are permeated with networks of fluid-filled tubes while embryos contain cavities within which flows convey patterning information. Flows are critical for kidney morphogenesis, heart and vascular development, the migration of neurons, and the establishment of left-right asymmetry. However, we know very little about how flows are generated and sensed by cells. This remains a pressing concern given the increasing number of diseases associated with flow abnormalities including structural birth defects, polycystic kidney disease, heart disease, and cancer progression. Cilia, microtubule-based organelles that project from the cell surface, are central players in flow biology. Motile cilia on specialized cells beat to generate fluid flow across epithelial sheets, while primary cilia, which are found on nearly all vertebrate cells, respond to external cues including flow forces.
We use forward and reverse genetics, as well as a range of cell biological approaches, in zebrafish to interrogate the mechanisms of flow generation and sensation by cells and the roles of these processes in development and disease. I will first report data which demonstrates a molecular link between the pathways that control cilia motility, cilia polarization, and the planar cell polarity of multi-ciliated cells centering on the human disease-associated protein C21ORF59.

Next, I will discuss our recent work that demonstrates a requirement for cilia motility in the brain/spine of zebrafish in maintaining spine straightness during growth. We used a thermogenetic approach to modulate protein function at distinct developmental times which identified a critical window when cilia motility is required for spine maintenance. Absence of cilia motility during this window results in the development of severe spinal curves. We are currently systematically ablating distinct cell populations within the brain and spine to ascertain which motile ciliated cells are involved in preventing spinal curves.
Our zebrafish work has led us to hypothesize a novel cell biological mechanism to explain the human disease Idiopathic Scoliosis (IS), a common spinal curvature that impacts up to 3% of the worlds children. I will discuss the implications of our zebrafish data in the context of IS and suggest potential avenues for future work and disease treatments.

M114

The Cell Biology of Regeneration Initiating Factors.

E.M. Tanaka[1], T. Sugiura[1], P. Murawala[1];
[1]Research Institute for Molecular Pathology, Vienna, Austria

Salamander limb regeneration involves the rapid growth of the wound epidermis over the amputation surface. This wound epidermis is essential tissue for supporting limb regeneration. We have previously identified a novel function for MARCKs-Like-Protein (MLP) as an extracellular transducer of the first cell cycle during regeneration. MLP is expressed by the wound epidermis, and our studies suggest that the phosphorylation status of MLP determines whether it is secreted by tissues like the wound epidermis, or remains in the cytoplasm as in the mature skin.
In separate studies, we show that induction of cell migration is a key part of establishing the regeneration blastema.

M115

Acoel regeneration mechanisms indicate ancient and widespread role for muscle in regenerative patterning.

A.A. Raz[1], M. Srivastava[2], P.W. Reddien[1];
[1]Biology, MIT/Whitehead Institute, Cambridge, MA, [2]Organismal and Evolutionary Biology, Harvard University, Cambridge, MA

Regeneration - the replacement of lost body parts – is widespread in the Metazoa. The formation and patterning of new tissues during regeneration requires positional information. How this positional information is harbored in adult tissues is poorly understood. In the planarian *Schmidtea mediteranea*, positional control genes (PCGs) are hypothesized to convey positional information during regeneration, and are regionally and constitutively expressed in adult planarians. Planarian PCGs are predominately expressed in a single differentiated tissue type: the musculature. PCGs exist in non-planarian regenerative species as well. Acoels are early-diverging bilaterians, cladistically distinct from both deuterostomes and protostomes and separated from all other bilaterians by over 550 million years of evolution. We demonstrate here that PCGs expressed in the basal acoel *Hofstenia miamia* coexpress with one another in a common differentiated, subepidermal cell type, consistent with a single primary source of adult positional information. Strikingly, analysis by both *in situ* hybridization and single-cell qRT-PCR demonstrates that all known *Hofstenia* PCGs are specifically expressed in muscle cells during both homeostatic tissue maintenance and regeneration. The vast majority of *Hofstenia* muscle cells express one or more PCGs, suggesting expression of positional information is a major feature of adult

Hofstenia muscle. PCG expression changes dynamically in pre-existing muscle cells after injury, consistent with the known roles for many of these genes in guiding regeneration outcomes. This data demonstrating an instructive positional role for muscle in *Hofstenia* suggests that true muscle originated at the base of the Bilateria as not only a contractile tissue, but also as the source of positional information guiding adult regeneration.

M116

NIX functions as a mitophagy receptor in human keratinocytes during epidermal differentiation.

C.L. Simpson[1], E.L. Holzbaur[2];
[1]Dermatology, University of Pennsylvania, Philadelphia, PA, [2]Physiology, University of Pennsylvania, Philadelphia, PA

Mitophagy allows cells to isolate, degrade, and recycle damaged mitochondria. However, mitophagy can also occur in a programmed manner to drive clearing of mitochondria during specialized processes of differentiation. In the epidermis, keratinocytes form a multi-layered epithelium in which they undergo wholesale degradation of organelles in the uppermost layers during terminal differentiation, but the mechanisms regulating this process are not well understood. Interestingly, in normal human skin, we found that expression of NIX, a mitochondrial membrane-tethered mitophagy receptor, is restricted to the outer epidermal layers, which are poised to initiate organelle clearing. Thus, we hypothesized that initiation of NIX expression may drive mitochondrial clearing in the outer layers of the epidermis to permit terminal differentiation. To establish that NIX functions as an epidermal mitophagy receptor, we showed that expression of NIX-GFP in live primary human keratinocytes permits targeting of depolarized mitochondria into LC3-positive autophagosomes, though this was not true for optineurin, NDP52, or NBR1, which function as mitophagy receptors in other cell types. To analyze mitophagy dynamics during epidermal differentiation in vitro, we combined confocal microscopy with a live three-dimensional stratified model of human skin that replicates normal epidermal histology and organelle clearing. Imaging of stratified keratinocytes transduced with mitochondria-targeted dsRed revealed that mitochondria undergo a dramatic morphologic change in the outer layers of the epidermis, transitioning from an interconnected tubular network to spherical organelle fragments. Co-transduction of keratinocytes with NIX-GFP revealed robust localization of this mitophagy receptor to the periphery of mitochondrial fragments. Moreover, ectopic expression of NIX via viral transduction of undifferentiated keratinocytes was sufficient to induce marked fragmentation of mitochondria. Finally, we demonstrated that premature expression of NIX in organotypic epidermis led to aberrant morphogenesis and differentiation of stratified cultures. These data lead us to propose a model in which NIX expression is temporally and spatially regulated in the epidermis to initiate mitochondrial clearing in the upper layers in order to drive keratinocyte terminal differentiation and promote proper tissue morphogenesis.

M117

Collective epithelial intravasation in breast cancer metastasis.

V.L. Silvestri[1], A. Wong[2,3], P. Searson[2,3,4], A.J. Ewald[1,5,6];
[1]Cell Biology, Johns Hopkins University School of Medicine , Baltimore, MD, [2]Institute for Nanobiotechnology (INBT), Johns Hopkins University , Baltimore , MD, [3]Department of Materials Science and Engineering, Johns Hopkins University , Baltimore, MD, [4]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, [5]Oncology, Johns Hopkins University School of Medicine , Baltimore, MD, [6]Biomedical Engineering, Johns Hopkins University School of Medicine , Baltimore, MD

A key goal in cancer research is to understand the underlying mechanisms of the metastatic process, by which cancer cells spread from a primary tumor to other sites and form secondary tumors. Indeed, metastasis is responsible for around 90% of cancer patient mortality. Despite its clinical importance, metastatic dissemination is an inefficient process. In experimental animal models, less than 0.02% of the single tumor cells leaving the primary tumor ultimately develop into metastatic lesions. Our lab has found that cells from primary tumors sometimes escape as clusters and that cell clusters are more efficient in forming lung metastases than single cancer cells.
We now focus on a key step of the metastatic process: intravasation. This process is facilitated by molecular changes that promote the ability of cancer cells to cross endothelial cell barriers that form the walls of microvessels. Previous studies have shown that tumor cell clusters are able to travel through pulmonary circulation *in vivo*. However, the mechanism by which tumor clusters gain access to the systemic circulation is still unknown. Our lack of understanding of this mechanism is due to the difficulties in establishing tumor models in which tumor-vessel interactions can be visualized and characterized. To overcome this barrier, we use a microfluidic approach with a physiologically and morphologically realistic vasculature along with 3D organotypic culture of mammary gland tumor organoids embedded in collagen-I gels. This approach allows us real-time and quantitative assessment of tumor-vessel interactions under *in vivo* like conditions. Preliminary evidence shows tumor organoids pushing through the tumor-vessel boundary resulting in the disruption of the vessel structure and function. We observed in real-time an intravasation event that preceded the disruption of a vessel. Our current work focuses on imaging optically cleared primary tumors to correlate the tumor-vessel structures observed *in vivo* with those observed *in vitro*. We anticipate that deeper understanding of the mechanisms of tumor cell cluster intravasation will provide new insights for targeted anti-metastatic treatments.

M118

Discovering the embryonic origins and cell cycling behavior of the mesodermal stem cells using live-imaging in a marine annelid.

B. Ozpolat[1,2], M. Handberg-Thorsager[3], M. Vervoort[1], G. Balavoine[1];
[1]Institut Jacques Monod, CNRS, Paris, France, [2]Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Institute, Woods Hole, MA, [3]Molecular Cell Biology and Genetics, Max Planck Institute, Dresden, Germany

Segmented worms (annelids) grow continuously throughout their lives by adding new segments from a posterior growth zone. A ring of cells within the growth zone expressing germline/multipotency genes, have been suggested to be the stem cells that give rise to tissues of the newly-made segments. Despite their significance in the segmented worm life cycle, precise embryonic origins of the growth zone and the characteristics of the cells that make up the zone remain largely unknown. We developed methods

for live-imaging the embryos and larvae of the marine annelid *Platynereis dumerilii*. Using single-cell-resolution live-imaging and cell lineage analysis, we determined the embryonic origins of the mesodermal component of the growth zone in the early larvae. We also utilized a live-cell cycle reporter and analyzed the cell cycle characteristics of this population of cells. We found that they are not quiescent but exhibit significantly slower cycling compared to other mesodermal lineages that precede the growth zone lineage. These findings provide a foundation and necessary tools for starting to understand the nature of the stem cell populations in this highly regenerative organism, and will allow for evolutionary comparisons to stem cells and regenerative mechanisms in other research organisms. Studies are in progress for establishing transgenic lines expressing the cell cycle reporter along with cell membrane and cell nucleus markers, in order to extend these analyses into post-larval growth and regeneration.

Minisymposium 12: Protein Folding, Misfolding and Neurodegeneration

M119

Prion-specific Hsp40 functions promote amyloid diversity in yeast: reciprocal roles for J-proteins in promoting and preventing amyloid elimination.

J.K. Hines[1];
[1]Chemistry, Lafayette College, Easton, PA

Yeast prions are protein-based genetic elements, most of which are heritable amyloid aggregates of functional proteins. Their propagation to subsequent cell generations is dependent upon the fragmentation of these aggregates by a core set of chaperone proteins including the J-protein/Hsp40 Sis1, Hsp70s, and Hsp104. Prions form distinct amyloid structural polymorphisms, called ‘strains’ in mammalian systems and ‘variants’ in yeast, that dictate the intensity of yeast prion-associated phenotypes and stability in mitosis. Recently we and others have uncovered significant complexity in the chaperone requirements of various yeast prions and prion variants, demonstrating that, in contrast to Hsp104 and Hsp70, which have general roles, J-proteins represent a prion-specific component of the prion-propagation machinery. Most notable has been a direct demonstration that the persistence of alternative conformations of prions is dependent on the action of different molecular chaperones. Because J-proteins often act as targeting factors for Hsp70s, they may constitute the first chaperone response to the presence of amyloid, but a critical barrier to advancing the understanding of chaperone function in prion biology is that the fundamental chaperone requirements for most yeast prions remain unidentified, precluding a comprehensive understanding of how protein sequences give rise to amyloids with distinct patterns of chaperone interaction. Here we will present our newest undergraduate-generated data regarding: 1) the unusual role of the J-protein Swa2 in the propagation of the [URE3] prion and our proposal of a novel chaperone complex involving Hsp90, 2) the conservation of the prion-maintaining J-protein functions of both Sis1 and Swa2 in plants, and 3) our recent discovery of reciprocal roles for the J-proteins Sis1, Apj1 and Ydj1 in the elimination of the prion [PSI+] by Hsp104. These findings suggest that amyloid structures of alternative prion conformations have unique features that are differentiated by chaperone proteins, revealing a previously unappreciated level of additional complexity that may be exploitable for therapeutic intervention.

This work was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R15GM110606. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

M120

Potentiated Hsp104 Variants to Counter Protein Misfolding.

M.E. Jackrel[1];
[1]Chemistry, Washington University, St. Louis, MO

There are no therapies that reverse the proteotoxic misfolding events that underpin fatal neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD). Hsp104, a conserved hexameric AAA+ protein from yeast, solubilizes disordered aggregates and amyloid but has no metazoan homolog and only limited activity against human neurodegenerative disease proteins. We have reprogrammed Hsp104 to rescue TDP-43, FUS, and a-synuclein proteotoxicity by mutating single residues in the middle domain or nucleotide-binding domain 1 of Hsp104. Strikingly, we have uncovered numerous missense mutations throughout these regions that potentiate Hsp104. Hsp104 potentiation stems from loss of amino acid identity rather than introduction of specific interactions. Potentiated Hsp104 variants enhance aggregate dissolution, restore proper protein localization, suppress proteotoxicity, and in a *C. elegans* PD model attenuate dopaminergic neurodegeneration. We now demonstrate that Hsp104 variants can also suppress the toxicity of dipeptide repeat proteins and SOD1 aggregates implicated in ALS, as well as other proteins harboring prion-like domains. Our work establishes that disease-associated aggregates and amyloid are tractable targets and that enhanced disaggregases can restore proteostasis and mitigate neurodegeneration.

M121

Role of Polyphosphate in Amyloidogenic Processes.

U. Jakob[1], C.M. Cremers[1], D. Knoefler[1], M.J. Gray[1], N. Yoo[1], J. Dahl[1], L. Xie[1], J. Lempart[1];
[1]MCDB, University of Michigan, Ann Arbor, MI

Absolutely conserved, highly abundant, and present in all cells and organisms studied, polyphosphates (polyPs) are one of the most ancient macromolecules found on earth. They consist of long chains of phosphates, linked by high-energy phosphoanhydride bonds. PolyP has been shown to play crucial roles in bacterial pathogenesis, biofilm formation, stress resistance and blood clotting, and has been implicated in signaling processes and cancer. Despite these important functions, however, little is known about the mechanism(s) by which polyP influences these diverse processes. Based on our most recent discoveries, we now postulate that polyP affects this wide and seemingly unrelated range of biological functions by using a single, unifying mechanism: serving as a scaffold that stabilizes protein folding intermediates. This mechanism explains how polyP confers resistance to stress conditions that cause protein unfolding and accelerates processes, such as biofilm formation, which involve the stabilization of amyloid-like proteins in a fiber-forming conformation. We have now investigated the mechanism by which polyP influences these processes using a combination of genetic, biochemical, and structural approaches. We discovered that polyP serves as an effective nucleation source for amyloidogenic processes, triggering rapid in vitro polymerization of amyloids at physiologically relevant amyloid concentrations, and generating shedding-resistant amyloid fibrils of unique morphology. In vivo, the amyloid-stimulating and fibril-stabilizing effects of polyP have wide-reaching consequences, increasing the rate of biofilm formation in pathogenic bacteria and mitigating amyloid toxicity in differentiated neurons and C. elegans strains that serve as models for human folding diseases. These results suggest that we have discovered a widely conserved, abundantly present, and cytoprotective modifier of amyloidogenic processes.

M122

Rapid heat-shock response depends on intracellular pH.

C.G. Triandafillou[1], A.R. Dinner[2], D.A. Drummond[3];
[1]Biophysical Sciences Graduate Program, University of Chicago, Chicago, IL, [2]James Franck Institute, University of Chicago, Chicago, IL, [3]Biochemistry and Molecular Biology, University of Chicago, Chicago, IL

Single-celled organisms must be able to dynamically sense and respond to changes in the protein-folding environment, such as temperature, to ensure proteostasis and survival. The budding yeast S. cerevisiae grows best in acidic (low-pH) conditions, yet during logarithmic growth maintains a resting cytosolic pH just above neutral, expending a considerable portion of cellular ATP resources to do so. When cells experience acute stresses such as high temperatures or energy depletion, intracellular pH drops. Although changes in intracellular pH have been shown to influence the cellular response to starvation, in the case of heat shock little is known about the interplay between changes in pH and other, more well-studied processes such as the transcription and translation of the heat shock genes, and the behavior of the protein homeostasis machinery. In order to understand how changes in intracellular pH might influence the heat shock response, we dictate intracellular pH with an ionophore, use a pH-sensitive GFP derivative to measure intracellular pH, and simultaneously measure the production of heat shock proteins using quantitative, multicolor flow cytometry.
We find that cells that are held at the pre-shock pH during heat shock almost completely fail to synthesize certain heat shock proteins in an interval where acidified cells do so robustly. We measure relative growth rates and demonstrate that the intracellular pH profile during stress also alters cellular fitness during recovery. Finally, we correlate production of molecular chaperones to intracellular pH during recovery at the single-cell level. These findings implicate pH regulation as a key aspect of the cellular stress response.

M123

A prion domain that promotes cellular fitness by pH-regulated protein phase separation.

T. Franzmann[1], M. Jahnel[1], S.W. Grill[1], A.A. Hyman[1], S. Alberti[1];
[1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Single cell organisms are frequently exposed to stressful environments. They respond to stress by arresting the cell cycle, shutting down metabolism and inducing stress-protective pathways. Upon cessation of stress, they must be able to rapidly reprogram their metabolism and restart growth and division. Phase separation provides a rapid way for cells to respond to metabolic changes. This is because phase separation itself is sensitive to small changes in physico-chemical conditions, such as the cytosolic pH, which are a read-out of metabolic state. Recent work has shown that prion-like sequences can drive phase separation of protein compartments in cells. However, it remains to be shown whether prion-like domains sense changing metabolic conditions and promote stress adaptation by compartment formation. Here, we demonstrate that concomitant with acidification of the cytosol under energy stress, the prion domain of the translation termination factor Sup35 undergoes pH-sensitive liquid phase separation. The liquid compartments formed subsequently solidify into a more gel-like state, sequestering the translation termination factor. pH-sensing is conserved among different yeast species and encoded within its charged middle domain, which couples Sup35 gelation to the cytosolic pH. Cessation of stress raises the pH, which dissolves the gels, thereby releasing the translation termination factor and allowing restart of translation. Our results demonstrate that prion-like domains can function

as environmental sensors that regulate protein phase behavior. This suggests that prion-like domains are stress-adaptive devices that facilitate the adaptation in unstable environments.

M124

Tardigrade intrinsically disordered proteins mediate desiccation tolerance.

T.C. Boothby[1], S. Piszkiewicz[1], A. Mehta[1], A.H. Brozena[2], H. Tapia[3], D.E. Koshland[3], A.S. Holehouse[4], R.V. Pappu[4], B. Goldstein[5], G.J. Pielak[1];
[1]Chemistry, UNC, Chapel Hill, NC, [2]Chemical Engineering, NC State, Raleigh, NC, [3]Molecular and Cell Biology, UC Berkeley, Berkeley, CA, [4]Biomedical Engineering, Washington University in St. Louis, St. Louis, MO, [5]Biology, UNC, Chapel Hill, NC

Tardigrades (water bears) are a phylum of tiny, extremotolerant animals renowned for their ability to survive desiccation, freezing, boiling temperatures, intense irradiation, and even prolonged exposure to the vacuum of outer space. The functional mediators, and their mechanisms of protection, used by tardigrades to survive these extremes have not been fully elucidated. Tardigrades possess at least three families of novel intrinsically disordered proteins (IDPs). We found that members of one of these IDP gene families are upregulated during, and are required for tardigrades to survive, desiccation. Additionally, these proteins increase the desiccation tolerance of heterologous systems (yeast and bacteria), stabilize protein structure, preserve enzyme function during desiccation and form gels. Gelation increases their beta-sheet content. Structural simulations, bioinformatics analyses, and empirical biochemical experiments indicate that these proteins, while disordered, are comprised of three discrete regions, two terminal regions bridged by an extended linker that together resemble a dumbbell. The terminal regions are ‘sticky’ and display beta-sheet propensity. Based on experimental evidence and simulations, we propose that this dumbbell-like confirmation inhibits intraprotein interactions, but facilitates gelation via interprotein interactions. Upon desiccation, these gels vitrify forming glass-like solids, and the vitrification of these proteins appears mechanistically essential as disruption of this vitreous state correlates with a loss of their protective capabilities. Our results identify the first functional mediators of tardigrade desiccation tolerance, and reveal a potential mechanism of action. More broadly, our findings provide insight into how changes in the conformation and function of disordered proteins can be influenced by environmental factors. These studies provide a platform for pursuing novel methods for stabilizing sensitive biomedical material and engineering stress tolerant crops.

M125

Characterization of p97 mutations causing multisystem proteinopathy support a gain-of-function model for pathology.

E.E. Blythe[1], A. Martin[2], R.J. Deshaies[1];
[1]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, [2]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA

Valosin-containing protein (VCP, or p97) is an ATPase essential in numerous protein quality control (PQC) pathways, such as ER-associated degradation. p97 functions as a segregase, extracting ubiquitylated proteins from membranes or complexes so they can be degraded by the proteasome. However, the complexity of native p97 PQC substrates has stymied the detailed biochemical study of this function. Previously, to address this problem, we developed an in vitro p97 substrate based on an ubiquitin fusion degradation (UFD) pathway substrate, Ub-GFP, and showed that the unfolding of this substrate by p97 is dependent upon extensive substrate ubiquitylation, the p97 adaptors NPLOC4-

UFD1L, and ATP hydrolysis. Here, we make use of this system, employing an updated version of this substrate, to explore how mutations in p97 that cause multisystem proteinopathy (MSP) affect substrate processing. Previous studies have shown that MSP mutants have higher basal ATP rates than wild type yet cause deficiencies in many p97-dependent pathways, creating controversy as to whether these dominantly inherited mutations cause disease through a gain-of-function or a loss-of-function. We have now analyzed seven distinct MSP mutants, all of which showed modestly improved unfolding of our model substrate over wild type p97, providing evidence that the increased ATPase activity leads to a gain-of-function. Furthermore, we showed evidence that p97 inhibitors may restore proper p97 function to MSP mutants, suggesting a potential treatment strategy for p97 diseases.

M126

Protein arginylation targets alpha synuclein, facilitates normal brain health, and prevents neurodegeneration.

J. Wang[1], X. Han[2], N.A. Leu[1], S. Sterling[1], S. Kurosaka[1], M.E. Fina[1], V.M. Lee[3], D.W. Dong[1,4], J. Yates iii[2], A.S. Kashina[1];
[1]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, [2]The Scripps Research Institute, La Jolla, CA, [3]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [4]Institute for Biomedical Informatics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Alpha synuclein (α-syn) is a central player in neurodegeneration, but the mechanisms triggering its pathology are not fully understood. Here we found that α-syn is a highly efficient substrate for arginyltransferase ATE1 and is arginylated in vivo by a novel mid-chain mechanism that targets the acidic side chains of E46 and E83. Lack of arginylation leads to increased α-syn aggregation and causes the formation of larger pathological aggregates in neurons, accompanied by impairments in its ability to be cleared via normal degradation pathways. In the mouse brain, lack of arginylation leads to an increase in α-syn's insoluble fraction, accompanied by behavioral changes characteristic for neurodegenerative pathology. Our data show that lack of arginylation in the brain leads to neurodegeneration, and suggests that α-syn arginylation can be a previously unknown factor that facilitates normal α-syn folding and function in vivo.

M127

Poly (ADP-ribose) modulates phase separation of the ALS-associated protein TDP-43.

L. McGurk[1], E. Gomes[2], L. Guo[2], J. Mojsilovic-Petrovic[3], V. Tran[1], R.G. Kalb[3], J. Shorter[2], N.M. Bonini[1];
[1]Department of Biology, University of Pennsylvania, Philadelphia, PA, [2]Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, [3]Department of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA

Stress granules are membraneless organelles that form by liquid to liquid phase separation of RNA-protein complexes. In amyotrophic lateral sclerosis and frontal temporal dementia, pathological protein aggregates of phosphorylated TDP-43 are thought to arise from persistent stress granules, however, the nucleation of this process is unknown. In *Drosophila*, we identified poly (ADP-ribosylation) as a potent genetic modifier of TDP-43-associated toxicity. We establish that polymers of poly (ADP-ribose), PAR, nucleates liquid-liquid phase separation of TDP-43 *in vitro*. We show that disease-associated protein fragments of TDP-43, which lack the PAR-binding region, have impaired phase-separation dynamics and fail to respond to PAR *in vitro*. In mammalian cells, TDP-43 undergoes stress-induced phase separation and we uncover that localization to stress granules is dependent upon PAR binding. The disease-

associated fragments of TDP-43 undergo stress-induced phase separation *in vivo* but unlike the normal form of TDP-43, fail to fuse with stress granules. Finally, we show that phase-separated droplets of TDP-43 that are excluded from stress granules take on the molecular marks of pathological aggregates. We propose a model whereby the nucleation of disease-associated pathology occurs when TDP-43 droplets are excluded from the protective environment of stress granules.

M128

Small molecules for modulating protein driven liquid-liquid phase separation in neurodegenerative disease.

R.J. Wheeler[1], H. Lee[1], A. Pal[2,3], A. Murthy[4], I. Poser[1], L. Marrone[3], T. Doeleman[2,3], J. Sterneckert[3], M. Bickle[1], A. Hermann[2,3], S. Alberti[1], A.A. Hyman[1];
[1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany, [2]Department of Neurology, Dresden, Germany, [3]Center for Regenerative Therapies, Dresden, Germany, [4]Department of Molecular Pharmacology, Physiology, and Biotechnology, Brown University, Providence, RI

New approaches for identifying drug candidates for amyotrophic lateral sclerosis (ALS) are urgently needed as current therapies do little to reduce mortality. The physiological function of the unstructured low complexity domains of ALS-associated RNA binding proteins, including fused in sarcoma (FUS), has recently been shown to be formation of liquid compartments by phase separation. We asked whether this protein phase separation is a druggable target. We developed cellular and *in vitro* screens to find compounds which modulate FUS phase separation *in vitro* and in cells. We identified three active compound classes which we then tested for their ability to rescue mutant FUS phenotypes associated with familial ALS; FUS droplet propensity to aggregate into fibres *in vitro* and axonal transport defects in motor neurons *ex vivo*. This showed self-interaction of unstructured domains driving liquid-liquid demixing of proteins is a druggable target and our new screening approaches are effective at identifying candidate compounds. By combining multiple new screening methods we target multiple aspects of ALS pathology, which we suggest improves predictivity for beneficial effects *in vivo*. We have therefore developed new screens for potential ALS therapeutics, identified a new compound family as therapeutic candidates for ALS, and are extending these screening methods to larger compound libraries.

Minisymposium 13: Subcellular Organization of Neural Cells

M129

Airway mechanoreceptors that control breathing.

R. Chang[1], D. Strochlic[1], K. Tanaka[2], S. Woo[2], A. . Patapoutian[2], S. Liberles[1];
[1]Cell Biology, Harvard Medical School, Boston, MA, [2]Molecular and Cellular Neuroscience, The Scripps Research Institute, La Jolla, CA

The airways contain a myriad of sensory cells that mediate olfaction, control breathing, and protect the respiratory system from damage and disease. The vagus nerve is a major conduit between lung and brain required for normal respiration, and some vagal sensory neurons detect airway expansion that occurs with every breath. Mechanical inflation of the lungs and airways triggers a protective respiratory reflex termed the Hering-Breuer inspiratory reflex, characterized by acute apnea. How vagal sensory neurons detect increases in airway volume was unknown at a molecular level. We initiated a molecular and genetic analysis of the sensory vagus nerve, identifying cell types that innervate the lung and powerfully control breathing. Moreover, we identified a critical role for the ion channel Piezo2 in airway mechanoreception. Optogenetic activation of vagal afferents containing Piezo2 causes apnea, trapping

animals in a state of exhalation. Moreover, conditional deletion of Piezo2 from nodose ganglion-derived sensory neurons eliminates sensory neuron responses to airway stretch as well as the classical Hering-Breuer inspiratory reflex. These findings indicate a key role for Piezo2 as an airway stretch sensor critical for establishing efficient respiration at birth and maintaining respiratory homeostasis in adults. Understanding the sensory biology of respiratory control neurons in the vagus nerve may provide therapeutic targets for airway disease intervention.

M130

Apical cell-cell adhesions reconcile symmetry and asymmetry in zebrafish neurulation.

C. Guo[1], X. Wei[1];
[1]Ophthalmology, University of Pittsburgh, Pittsburgh, PA

The symmetric tissues and body plans of animals are paradoxically constructed with asymmetric cells. To understand how the yin-yang duality of symmetry and asymmetry are reconciled, we asked whether or not apical polarity proteins orchestrate mirror-symmetric neural tube development in zebrafish by hierarchically modulating apical cell-cell adhesions. We found that apical polarity proteins localize by a pioneer-intermediate-terminal order. Pioneer proteins establish the mirror symmetry of the neural rod by initiating two distinct types of apical adhesions: The parallel apical adhesions (PAAs) cohere cells of parallel orientation, and the novel opposing apical adhesions (OAAs) cohere cells of opposing orientation. Subsequently, intermediate proteins selectively enhance the PAAs when the OAAs dissolve by endocytosis. Finally, terminal proteins inflate the neural tube by generating osmotic pressure. Our findings suggest a general mechanism to construct mirror symmetric tissues: Tissue symmetry can be automatically established by simply aligning cellular asymmetry opposingly via adhesions.

M131

The Golgi Outpost Protein TPPP Mediates Uniform Microtubule Polarity and Branching in Oligodendrocytes.

M. Fu[1], J.A. Oses-Prieto[2], C. Lee[1], N.L. Saw[3], R. Shi[1], M. Nori[1], M. Shamloo[3], A. Burlingame[2], B.A. Barres[1];
[1]Neurobiology, Stanford University, Stanford, CA, [2]Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA, [3]Neurosurgery, Stanford University, Stanford, CA

Oligodendrocytes are specialized glial cells in the central nervous system that produce myelin, the fatty layers of insulation that wrap around axons to facilitate efficient action potential conduction. Unlike Schwann cells in the peripheral nervous system that ensheath a single axonal segment, one oligodendrocyte can ensheath multiple axonal segments and consequently extends multiple processes. These microtubule-rich processes are elaborate and highly branched, yet it is unclear how they are organized and how they form. We now show using live-cell imaging that microtubules in oligodendrocytes have uniform polarity, with growing EB3-labeled plus ends directed away from the cell body. Interestingly, though polarity is consistent throughout oligodendrocyte differentiation, speeds of polymerization vary at different developmental time points. In addition, we now show by immunostaining that oligodendrocyte processes contain Golgi outposts, which may act as a source of acentrosomal microtubule nucleation at sites that are far from the cell body. Previous experiments in Drosophila neurons have demonstrated roles for Golgi outposts in microtubule nucleation and dendrite branching. In order to screen for candidate Golgi outpost interactors, we used our lab's RNA-Seq database to identify microtubule-associated proteins that are highly and specifically expressed in oligodendrocytes. We identify TPPP (tubulin polymerization promoting protein) and show that it selectively localizes to Golgi outposts but not to Golgi bodies in the cell body. Knockdown of TPPP

results in aberrantly mixed microtubule polarity and increased branching in oligodendrocytes, but does not alter the speed of EB3-labeled plus ends, suggesting that contrary to its name, TPPP does not actually mediate microtubule polymerization in cells. An alternative possibility is that TPPP stabilizes microtubules by binding at the minus end and we are currently addressing this by using polarity marked microtubules and in vitro kinesin motility assays. In addition, we are using mass spectrometry to identify the Golgi outpost proteome, by purifying Golgi from rat pup brains followed by immunoprecipitation against TPPP. Finally, preliminary data from mice indicate that TPPP knockout may lead to sensorimotor deficits and anxiety-like behavior. Together, our data demonstrate that TPPP is required for uniform microtubule polarity and process branching in oligodendrocyte development.

M132

A Wnt Signaling Pathway Acts as a Master Coordinator of Microtubule Regulators at Dendrite Branch Points.

A.T. Weiner[1], D.Y. Seebold[1], N.L. Michael[1], M.A. Guignet[1], B. Follick[1], C. Feng[1], C. Kozlowksi[1], D.J. Barbera[1], E. Jones[1], C.T. Folker[1], B.A. Yusko[1], N. Wasilko[1], M. Patel[1], P.G. Torres[1], M.M. Rolls[1];
[1]BMB, The Pennsylvania State University, University Park, PA

Mature neurons contain non-centrosomal microtubule arrays with different arrangements of microtubules in axons and dendrites. In Drosophila dendrites microtubule nucleation sites are concentrated at dendrite branch points, and local nucleation contributes to minus-end-out microtubule polarity. Golgi outposts have been suggested to house nucleation sites (Ori-McKenney, Jan and Jan, Neuron 2012), however, we found that removal of the Golgi from dendrites did not reduce targeting of gamma-tubulin, the core microtubule nucleator, to dendrite branch points (Nguyen et al, MBoC 2014). To generate an alternative hypothesis about what might localize nucleation sites to branch points, we started with the clue that another microtubule regulator, Apc2, is also concentrated at dendrite branch points. Apc2 recruits Apc so it can link the plus-tip protein EB1 to kinesin-2 and steer growing microtubules along stable ones at branch points. As Apc2 is a scaffolding protein with many known interaction partners, we performed a candidate screen to identify proteins required to position Apc2-GFP. We found that mitochondrial energy production, ankyrin2 and its membrane partner neuroglian, branched actin, heterotrimeric G proteins and axin were all required for Apc2 targeting. We next tested whether any of these proteins were also required to localized gamma-tubulin to branch points. Surprisingly we found that the membrane proteins frizzled and frizzled2 act with the heterotrimeric G protein GalphaO to localize axin to branch points, and in turn axin is required to localized gamma-tubulin. Thus a modified wnt signaling pathway is an essential regulator of gamma-tubulin localization in dendrites. To determine the functional consequences of these wnt pathway members on microtubule organization in dendrites, we performed two additional assays. First, we assayed microtubule polarity and found that these pathway members are required to maintain minus-end-out polarity. Second, we assayed the increase in microtubule nucleation triggered by axon severing, and again found that frizzleds, G proteins and axin were required for this response. Thus this alternate wnt signaling pathway acts as a master regulator to localize both Apc2 and gamma-tubulin, and is required to control local microtubule nucleation in dendrites.

M133

A microtubule-associated septin maintains neuronal polarity by directing motor-cargo traffic in dendrites.

E.P. Karasmanis[1], C. Phan[1], D. Angelis[1], I. Kesisova[1], C.C. Hoogenraad[2], R.J. McKenney[3], E.T. Spiliotis[1];
[1]Biology, Drexel University, Philadelphia, PA, [2]Cell Biology, Utrecht University, Utrecht, Netherlands, [3]Molecular and Cellular Biology, University of California Davis, Davis, CA

Neuronal function requires axon-dendritic membrane polarity, which depends on sorting of membrane traffic during entry into axons. Dendrites receive vesicles from the axon and cell body without apparent capacity for directional sorting due to a microtubule network of mixed polarity. Here, we found that a microtubule-associated septin (SEPT9), which localizes specifically in dendrites, impedes and reverses the entry of axonally-destined cargo of the microtubule motor kinesin-1/KIF5 such as the amyloid precursor protein (APP) and Bassoon. In contrast, SEPT9 promotes anterograde transport of kinesin-3/KIF1 cargo (e.g., low density lipoprotein receptor; postsynaptic density protein 95) from proximal to distal dendrites. In live neurons and single molecule cell-free motility assays, microtubule-associated SEPT9 suppresses kinesin-1/KIF5 motility and enhances kinesin-3/KIF1 specifically, regulating the trafficking and localization of their cargo to axons-dendrites. We show that this traffic control is critical for the growth of axons and dendrites in developing neurons. Thus, membrane traffic is spatially sorted and directed in dendrites by a septin-mediated mechanism, which maintains neuronal polarity and growth.

M134

Neuronal membrane remodeling machinery controlling the traffic of extracellular vesicle cargoes.

A. Yeh[1], Z. Zhao[1], K. Koles[1], A.A. Rodal[1];
[1]Biology, Brandeis University, Waltham, MA

Extracellular vesicles (EVs) including exosomes have emerged as an important trafficking pathway in the nervous system. EVs are small membrane bound compartments that traffic through endosomal pathways. They carry a variety of cargoes including lipids, proteins, and RNAs, and can impact the physiology of distant target cells. However, the vast majority of studies on EVs have been conducted in vitro, and many questions remain regarding the mechanisms of EV biogenesis and trafficking in neurons. Using the Drosophila melanogaster neuromuscular junction as an in vivo model synapse, we generated tools to examine the traffic of endogenous and exogenous EV cargoes. We conducted a targeted genetic screen of synaptic membrane remodeling machinery, and identified multiple pre- and post-synaptic candidates whose loss-of-function alters the abundance and/or distribution of neuronally derived EVs at the NMJ. We found that neuronal endocytic proteins localizing to the presynaptic periactive zone, including dynamin, synaptojanin, endophilin, and the F-BAR protein Nervous Wreck, are specifically and locally required to maintain normal levels of EV cargo at donor cell axon terminals. EVs are released from the NMJ in response to neuronal activity, and this process is strongly reduced in periactive zone mutants. Since periactive zone membrane remodeling machinery is subject to activity-dependent regulation, it may be the regulatory substrate for controlling activity-dependent EV release. EVs are thought to contribute to many human health conditions, including the spread of pathological proteins in neurodegenerative disease. Given the conserved nature of synaptic membrane trafficking machinery, our findings and tools will lay the foundation for new insights into endogenous EV traffic the healthy and diseased nervous system.

M135

ER-shaping proteins form functionally distinct microdomains in the ER membrane of neurons.

J. Nixon-Abell[1,2], C.J. Obara[2], J. Lippincott-Schwartz[2], C. Blackstone[1];
[1]NINDS, National Institutes of Health, Bethesda, MD, [2]Janelia Research Campus, Ashburn, VA

The endoplasmic reticulum (ER) is a complex membranous structure present in all eukaryotic cells, stretching from the nuclear envelope to the plasma membrane. This remarkably versatile network plays crucial roles in numerous cellular functions while simultaneously orchestrating interactions with, and between, almost every other subcellular organelle. The regulation of ER structure and function appears to be of particular importance in neuronal tissue, as almost all neurodegenerative pathways converge on ER-associated processes. The complex structural arrangement of the ER is thought to be governed, in part, by a subset of ER-shaping proteins (ERSPs). Mutations in genes encoding many of these proteins result in abnormal ER morphologies and underlie various neurodegenerative disorders. While many of these proteins are conserved throughout eukaryotes and ubiquitously expressed across tissue types, disease phenotypes are primarily restricted to neurons. For this reason, ERSPs provide a compelling model to better understand the role ER structure plays in influencing neuronal function in health and pathogenesis.
We employed a variety of superresolution imaging techniques in conjunction with gene editing technology to study the localization and function(s) of ERSPs at endogenous levels in human iPSC-derived neurons. A single iPSC line was CRISPR-edited to target multiple representative ERSPs with small-epitope tags. Multiplexed single molecule localization microscopy (SMLM) subsequently demonstrated the existence of ERSP microdomains that correlate not only with specific structural components of the ER, but also coincide with distinct biological processes. We suspect that these signature microdomain localizations are disturbed in the presence of pathogenic ERSP mutations which in turn leads to the disruption of ER-affiliated cellular processes and consequential pathogenesis. This is a particularly appealing model as it might help to explain why such a wide range of cellular phenotypes are observed in many neurodegenerative diseases. Studying the differentiation cascade from iPSC through to motor neuron, we further describe a morphogenic role of ERSPs in contributing towards the ability of a cell to generate neuronal morphology. Using a combination of correlative RNA-seq, quantitative proteomics, and SMLM we show which ERSP-containing microdomains are important for the differentiation process at different stages.
Here we provide the first evidence that ERSPs, at endogenous levels, exist in microdomains throughout the ER membrane and correlate with specific biological processes. Further, our data suggests that ERSP microdomains play a crucial role in supporting the growth, development and function of neurons.

M136

Expression of WIPI2B counteracts age-related decline in autophagosome biogenesis in neurons.

A.K. Stavoe[1], E.L. Holzbaur[1];
[1]Physiology, University of Pennsylvania, Philadelphia, PA

Autophagy defects are implicated in multiple late-onset neurodegenerative diseases. Since aging is the most common risk factor in neurodegeneration, we examined autophagy in aged neurons. Autophagosome biogenesis is known to occur preferentially at the distal tips of axons, and autophagosomes mature as they transit to the soma. We compared autophagosome biogenesis in neurons from young adult and aged mice, identifying a significant decrease in the biogenesis of autophagosomes during aging. While nucleation and initiation rates did not change during aging, we observed the frequent production of stalled Atg-13-positive, LC3-negative isolation membranes in

neurons from aged mice. These stalled structures exhibited aberrant membrane morphology and failed to resolve into LC3-positive autophagosomes. Further, the majority of stalled autophagosomal structures were Atg9-positive, while autophagic vesicles that successfully recruited LC3 did not retain Atg9. To identify the underlying molecular defect, we queried expression levels of autophagy proteins and identified a specific reduction in the PI3P-binding protein WIPI2 in aged mouse brain. WIPI2 depletion in young neurons was sufficient to stall autophagosome biogenesis, phenocopying aged neurons. Importantly, reconstituting WIPI2 expression effectively restored autophagosome biogenesis in aged neurons. We additionally determined that the PI3P and Atg16L1 binding domains of WIPI2 were required for WIPI2-induced restoration of autophagosome biogenesis. Together, these data suggest a novel therapeutic target in age-associated neurodegeneration.

M137

The ataxia disease gene VPS13D plays an essential role in mitochondrial morphology and transport in Drosophila neurons.

R. Insolera[1], E. Seong[2], L.M. Rivera-Perez[1], D. Lozano[1], M. Burmeister[2,3,4,5], C.A. Collins[1];
[1]MCDB, University of Michigan, Ann Arbor, MI, [2] Molecular and Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI, [3] Department of Human Genetics, University of Michigan, Ann Arbor, MI, [4] Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, [5]Department of Psychiatry, University of Michigan, Ann Arbor, MI

The long term maintenance and survival of neurons is highly dependent on the proper functioning of mitochondria. This is evident by the disproportional representation of mitochondrial genes associated with neurodegenerative disorders. Originally discovered in yeast, vacuolar sorting protein 13 (vps13) has multiple variants in metazoans, three in Drosophila melanogaster (A, B, and D) and four in mammals (A through D). Human mutations in VPS13A, B, and C have all been linked to various forms of neurodegenerative diseases. Recent genetic studies in humans have associated mutations in VPS13D with ataxia. In order to understand the cell biology underlying the defects associated with dysfunction of the vsp13d gene in the nervous system, we are using Drosophila melanogaster as a model system. Loss-of-function mutations in vps13d lead to severe defects in mitochondrial morphology in multiple tissues, and early larval lethality. Targeted knockdown of vps13d in the nervous system circumvents this early lethality to allow further analysis, and reveals similar cell-autonomous mitochondrial defects in neurons. In larval motoneurons lacking vps13d, we observed defects in trafficking of mitochondria to axons and synaptic terminals. Loss of vps13d induces the formation of oversized, atypical mitochondria, some of which contain mitochondrial inner membrane proteins but lack markers targeted to the matrix. Most interestingly, targeted knockdown of vps13d in neurons leads to the accumulation of comparably atypical mitochondria in neighboring supportive glial cells. We are currently characterizing the nature of the cell-autonomous mitochondrial defects in neurons, and the origin of the non-cell autonomous effect on mitochondria in glia. An exciting possibility that we are testing is whether there is transfer of atypical mitochondria from neurons to glia. Altogether, these results suggest that vps13d plays an essential role in mitochondrial biology, and is required in neurons for the proper distribution of mitochondria to distal regions. In addition, vps13d disruption in neurons reveals a previously uncharacterized neuron-glia interaction which may be relevant for its roles in neurodegenerative disease.

M138

A requirement for Mena, an actin regulator, in local mRNA translation in developing neurons.

M. Vidaki[1], F.B. Gertler[1];
[1]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA

During development, the exquisitely regulated process of axon guidance establishes the circuitry necessary for a properly functioning nervous system (NS) in the adult. Aberrant axonal navigation results in defective connectivity and multiple neurodevelopmental disorders including, among others, epilepsy, intellectual disabilities, autism and schizophrenia. One key mechanism required for proper axon growth, guidance and maintenance is local mRNA translation. Although local protein synthesis has been extensively studied in synapse formation and plasticity, its regulation during axon development remains poorly understood. Here, we uncover a novel role for the actin-regulatory protein Mena in the formation of a ribonucleoprotein complex (RNP) that involves translational inhibitors (HnrnpK, PCBP1), and regulates local translation of specific mRNAs in developing axons. We find that translation of dyrk1a, a Down Syndrome- and Autism Spectrum Disorders- related gene, is dependent on Mena, both in steady state conditions as well as upon BDNF stimulation. In particular, after BDNF stimulation we find that the Mena-RNP dissociates, releasing the mRNA of dyrk1a for translation in the axon/growth cone. We identify hundreds of additional mRNAs that associate with the Mena-complex, suggesting it plays broader role(s) in post-transcriptional gene regulation. Interestingly, this function is Mena-specific, as we could not detect any of the other Enah/VASP family members (VASP and EVL) being associated with mRNAs in the brain. Our work establishes a dual role for Mena in neurons, raising the intriguing possibility that it could act as a regulatory node that coordinates and balances actin polymerization and local protein synthesis in response to specific cues during neuronal development and, potentially, in adult neuroplasticity.

Microsymp 7: Spindle Architecture, SAC and Meiosis

E43

Kinesin-binding protein (KBP) is an inhibitory regulator of the mitotic kinesins Kif18A and Kif15.

H.L. Malaby[1], M. Dumas[2], J. Stumpff[1], R. Ohi[2];
[1]Molecular Physiology and Biophysics, The University of Vermont, Burlington, VT, [2]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN

Accurate organization and segregation of genetic material during cell division is essential for the survival of all organisms. As such, mitosis has evolved into a complex process relying heavily on the regulated activity of microtubule-dependent motors from the kinesin superfamily. While many keystone players governing kinesin control have been identified, teasing out the vast spatiotemporal puzzle remains an ongoing effort even as new modes of regulation are still being discovered. Recently, kinesin-binding protein (KBP) was shown to inhibit the activities of Kif1A and Kif18A in neurons. In addition, KBP was shown to interact with a broader set of kinesins, suggesting that it may be a general kinesin inhibitor with unexamined roles in multiple processes. Here, we investigate a potential role of KBP in regulating the activities of two mitotic kinesins, Kif18A and Kif15. We show that KBP blocks the abilities of both Kif15 and Kif18A to bind and power microtubule movement in gliding filament assays. Interestingly, KBP inhibits Kif18A with much higher potency, suggesting that the ability of KBP to inhibit kinesins can vary depending on the strength of KBP-motor interactions. In cells, alteration of KBP expression produces phenotypes consistent with a major function in regulating Kif18A activity in mitosis: high KBP protein

levels cause Kif18A mislocalization within the spindle and defects in metaphase chromosome alignment. Overexpression of KBP does not measurably alter Kif15 localization, but it does reduce Kif15 driven spindle stabilization. Conversely, loss of KBP attenuates chromosome oscillations, an observation consistent with Kif18A hyperactivity. Overall, these findings are consistent with a model in which KBP functions as a novel multi-protein regulator of both Kif18A and Kif15 activity in mitosis.

E44

Cell Type-Specific Response to Spindle Misorientation and Effects on Tissue Growth.

A.S. Parra[1], C.A. Johnston[1];
[1]Biology, University of New Mexico, Albuquerque, NM

Coordination of cell polarity and spindle orientation with cell growth and proliferation ensures mitotic fidelity and thus proper animal development. Mitotic errors have been associated with aberrant tissue growth in both epithelial cells and stem cells. Mutations in cell cycle-promoting genes in neural stem cells cause a mild increase in the Drosophila central nervous system (CNS). Conversely, identical mutations in Drosophila imaginal wing discs (IWD), terminally differentiated cells, lead to massive tissue overgrowth. The mechanisms underlying the differential responses of these cells to errors in cell division, however, are unknown. Here we seek to build a stem cell model and a differentiated cell model to elucidate the varied tissue- specific responses. We found that mutated growth-promoting genes cause substantial overgrowth in epithelial cells, while no significant change in the CNS was observed when expressed in neural stem cells. Additionally, loss of Mud, a gene essential for proper mitotic spindle orientation, results in apoptosis-mediated inhibition of IWD growth in response to mutated growth-promoting genes. Our results further highlight the differential response of epithelial cells and stem cells to mutated growth-promoting genes. Taken together, these results point to an overgrowth-inhibitory mechanism in epithelial cells stemming from errors in spindle orientation caused by these mutations. Further analysis will provide a better understanding of the cell signaling pathways that govern tissue level responses to defective cell division, which will be important to improving our understanding of the underlying molecular bases for numerous human diseases.

E45

Investigating in vivo variation in the strength of the spindle assembly checkpoint.

A.R. Gerhold[1], J. Labbé[1], P.S. Maddox[2];
[1]Institute for Research in Immunology and Cancer, University of Montreal, Montreal, QC, [2]Department of Biology, University of North Carolina, Chapel Hill, Chapel Hill, NC

The spindle assembly checkpoint (SAC) is a highly conserved mitotic regulator that prevents chromosome segregation errors during cell division, thereby maintaining genome stability. Variation in the strength of the SAC (i.e. the ability to delay mitotic progression in the presence of spindle assembly defects) is a widespread, but poorly understood feature of checkpoint regulation. Using in situ live imaging, we have shown that C. elegans germline stem cells have a stronger SAC than somatic embryonic blastomeres, providing an excellent opportunity to examine variability in SAC activity in vivo and to uncover cell fate-specific adaptations. Here we show that the embryonic progenitors of the adult germline, the P lineage blastomeres, also display a stronger SAC, relative to their somatic peers. Using both Nocodazole treatment of permeabilized embryos and a novel, inducible monopolar spindle assay, we find that cells in the germline lineage exhibit longer mitotic delays than cells with a somatic fate. These differences are entirely dependent on an intact checkpoint and only partially attributable to differences in cell size, which has recently been reported to impact SAC strength in C. elegans embryos.

In the 2-cell embryo, we find that roughly half of the difference in checkpoint strength, between the larger somatic AB and the smaller germline P1 blastomere, is due to cell size, with the remainder requiring the asymmetric partitioning of cytoplasmic cell fate determinants downstream of the PAR polarity proteins. To further dissect lineage-specific adaptation of SAC strength, we are using live imaging and a semi-automated tracking and fluorescence quantification approach to monitor the levels and dynamics of key kinetochore and SAC proteins in different cell lineages during embryogenesis. Altogether our results suggest that germline cell fate correlates with enhanced SAC activity, raising the interesting possibility that variation in SAC strength is adaptive, and reveal a novel relationship between asymmetric cell division and SAC regulation.

E46

Aurora B association with nucleosomes, not transcription, regulates its centromere localization and proper SAC response in human cells.

C. Ferras[1], M. Cruz[1], M. Alba Abad[2], N. Galjart[3], J. Arulanandam[2], H.J. Maiato[1];
[1]Chromosome Instability Dynamics Lab, IBMC/I3S, Porto, Portugal, [2]Cell Biology, Wellcome Trust Centre for Cell Biology, Institute of Cell Biology, Edinburgh, , United Kingdom, [3]Genetics, Erasmus MC, Rotterdam, Netherlands

Despite the prevailing dogma that transcription in higher eukaryotes is repressed during mitosis, recent studies provided evidence that some level of transcription might still take place. In line with this hypothesis, the master mitotic regulator Cyclin B1 has been suggested to be actively transcribed during mitosis and to be required for a robust spindle assembly checkpoint (SAC), a signaling mechanism that controls the fidelity of mitosis by detecting unattached kinetochores. Furthermore, in vitro studies using Xenopus oocyte extracts have suggested that the localization and activation of Aurora B (required for proper SAC response and error correction) is dependent on centromeric mitotic transcription. However, whether active transcription is required for spindle assembly checkpoint response and Aurora B localization/activity in animal somatic cells remains unknown. Here we use deep sequencing and live cell imaging of human cells in culture to investigate the role of transcription during a prolonged mitosis. Our initial analysis using a well-known transcription inhibitor (Actinomycin D) that intercalates DNA revealed that Aurora B was indeed de-localized from the centromeres. This loss affected mitotic timing and cell fate after nocodazole treatment. However, inhibition of transcription independently of DNA intercalation did not significantly alter cell fate, mitotic duration nor Aurora B localization. Moreover, we found other DNA intercalating agents phenocopied the Actinomycin D effect. In vitro electrophoretic mobility shift assay using reconstituted nucleosome core particles (NCPs) from recombinant histones showed that DNA intercalating agents reduced the interaction of CPC core complex (that include the Aurora B) with nucleosomes. Finally, RNA-seq analysis of cells treated with Actinomycin D failed to reveal de novo transcription of mitotic genes during a prolonged mitosis. Overall our data suggest that centromere structure, rather than transcription, regulates Aurora B localization and SAC response in human cells.

E47

Negative regulatory network between the three aurora kinases protects mouse gamete euploidy.

A.L. Nguyen[1], A. Gentilello[1], K. Schindler[1];
[1]Genetics, Rutgers University, Piscataway, NJ

The Aurora kinases (AURKs) are conserved serine/threonine kinases that regulate chromosome segregation. Mitotic cells require two AURKs (A, B), while mammalian germ cells require a third: (C). The meiosis-specific expression of AURKC has lead to a hypothesis that AURKC functionally replaced AURKB in oocytes. Whether AURKB is required for female meiosis remains unknown. To determine if AURKB is required during female meiosis we utilized an oocyte-specific Cre driver (GDF9) to excise exons 2-6 in the murine Aurkb locus. We compared phenotypes of wild-type mice to that of AURKB or AURKC single knockouts. These studies revealed that AURKB is required for meiosis. In contrast to oocytes lacking Aurkc, oocytes lacking Aurkb were aneuploid. Surprisingly these Aurkb-/- oocytes had increased AURKC activity in addition to abnormal AURKC localization on the chromosome arms in metaphase II, suggesting a requirement for AURKB to restrict the activity of AURKC in vivo. To test this hypothesis, we generated mice with one copy of Aurkc in the Aurkb-/- oocyte background to ask if reducing AURKC levels could rescue the failure phenotype; utilizing oocytes without AURKB and AURKC (double knockout) as controls. Reduction of Aurkc in half partially rescued the number of eggs that were aneuploid but did not rescue the Met II mis-localization of AURKC. The aneuploidy defect and mis-localization of AURKC seen in Aurkb-/- oocytes could only be rescued by complete removal of Aurkc, supporting our hypothesis that AURKB is a negative regulator of AURKC. Surprisingly, double knockout oocytes were euploid and indistinguishable from WT. Here we show that AURKA, the homolog restricted to spindle poles, compensates for the loss of AURKB and AURKC by binding to the Chromosomal Passenger Complex and by phosphorylating AURKB/C substrates. Interestingly, this compensation is dependent on the absence of AURKC as its endogenous expression restricts AURKA to spindle poles. Therefore, these data also imply that AURKC negatively regulates AURKA by preventing its localization to chromosomes in wild-type oocytes. We speculate that AURKB has been retained in oocytes to ensure that AURKC activity is spatially restricted and that this restriction keeps the balance of counteracting AURKA/AURKC activity in check. These studies show, for the first time, the ability for AURKA to functionally complement AURKB/C in vivo. Importantly, these data shed new light on a negative regulatory network among the kinases, which may be critical for generating euploid gametes. This work was supported by grants from the NIH (F31HD089591: A.L.N.; R01GM112801-02: K.S.).

E48

Histone dynamics during oocyte meiosis in *C. elegans*.

S. Rosu[1], P. Thepmankorn[1], O. Cohen-Fix[1];
[1]LCMB, NIH-NIDDK, Bethesda, MD

During meiosis, the process through which gametes are formed, homologous chromosomes must pair, undergo recombination, and segregate from each other. Chromatin dynamics, which have been studied extensively in somatic cells, have been less explored in the context of meiosis. To gain new insights into this process, we are using live imaging in *C. elegans* to examine chromatin dynamics during various stages of meiotic prophase. Unexpectedly, we have found widespread histone H2B exchange in the late stages (diplotene and diakinesis) of oocyte meiotic prophase. We have confirmed this observation in three different ways: 1) by the complete recovery of mCherry::H2B fluorescence after photobleaching in a strain carrying a mCherry::H2B transgene (indicating new unbleached histones are loaded onto

chromatin), 2) by the disappearance of photoconverted Dendra2::H2B in a strain overexpressing the Dendra2::H2B transgene in the germline (indicating that converted histones are unloaded, and new unconverted histones are loaded onto chromatin), and finally, 3) by the redistribution of photoconverted H2B::Dendra2 from a sub-region of chromatin to the entire chromatin in a strain where the Dendra2 tag was inserted at an endogenous H2B locus by CRISPR. This suggests that H2B histones are widely unloaded from chromatin at the diplotene and diakinesis stages in meiosis. Furthermore, our data suggests that when the nucleoplasmic pool of the tagged H2B is limited (as in the endogenously tagged strain), the converted histones are reloaded, and when the nucleoplasmic pool is large (as in the over-expressing transgenic strain), they are degraded. This widespread exchange is specific to meiosis, as we have not observed this phenomenon in terminally differentiated somatic cells in the timeframe assayed. The histone exchange at this stage of meiosis is surprising, as no replication is occurring and transcription is thought to be shut down by diakinesis. The mechanism and role of histone exchange at this stage are unknown, but possible roles include enabling changes in chromosome configuration important for the meiotic divisions, and/or resetting of chromatin marks. In future work, we will screen candidate histone chaperones and chromatin remodelers to find factors involved in this meiotic histone exchange. We will also examine the consequence of blocking histone exchange, which may lead to defects in meiosis or embryo development.

E49

Interplay between microtubule bundling and sorting factors ensures acentriolar spindle stability during *C. elegans* oocyte meiosis.

T.J. Mullen[1], S.M. Wignall[1];
[1]Molecular Biosciences, Northwestern University, Evanston, IL

During mitosis, duplicated centrosomes nucleate microtubules and form the spindle poles. However, oocytes of most species lack centrioles, so spindles assemble using a different pathway. Recently, we reported that acentriolar spindle assembly in *C. elegans* oocytes proceeds by: 1) formation of a cage-like microtubule array inside the disassembling nuclear envelope, 2) sorting of microtubule minus ends to the periphery of the array, and 3) focusing of these ends into nascent poles that coalescence until bipolarity is achieved. Moreover, we demonstrated that the kinesin-12 family motor KLP-18 acts during the sorting step to force the minus-ends outwards where they can be organized into the spindle poles (1). Now, we have uncovered additional insights into the principles underlying this specialized form of cell division, through studies of KLP-15 and KLP-16, two highly homologous members of the minus-end-directed kinesin-14 family. Fixed and live imaging of KLP-15/16-depleted oocytes revealed that following nuclear envelope breakdown, microtubules form a transient cage-like structure, but then microtubules collapse into a disorganized ball-shaped structure surrounding the chromosomes. These results suggest that KLP-15/16 bundle and organize microtubules during acentriolar spindle assembly, and consistent with this proposed function, these proteins localize to spindle microtubules during the cage stage and remain microtubule-associated throughout the meiotic divisions. However, despite the severe spindle assembly defects observed following KLP-15/16 depletion, we were surprised to find that these disorganized microtubules were then able to reorganize into a spindle capable of segregating chromosomes during anaphase, revealing the existence of additional mechanisms that can act to bundle and organize spindle microtubules. This phenotype therefore enabled us to identify factors promoting microtubule organization and assembly during anaphase, whose contributions are normally imperceptible in wild-type worms. First, we discovered that SPD-1 (PRC1), which loads onto microtubules in early anaphase, is required for the formation of anaphase microtubule bundles in the absence of KLP-15/16. Moreover, we found that KLP-18, which sorts microtubules during spindle

assembly, can then function during anaphase to sort these bundles into a functional orientation capable of mediating chromosome segregation. Therefore, our studies have revealed an interplay between distinct mechanisms that together promote spindle formation and chromosome segregation in the absence of structural cues such as centrioles. (1) Wolff, et.al., (2016) MBoC

Microsymp 8: Cytoskeleton and Motility

E50

Composition of LAT clusters regulates their movement within actomyosin networks.

J.A. Ditlev[1,2], A.R. Vega[3], D.V. Koester[2,4], X. Su[2,5], R.D. Vale[2,5], S. Mayor[2,4], K. Jaqaman[3], M.K. Rosen[1,2];
[1]Department of Biophysics and Howard Hughes Medical Institute, UT Southwestern Medical Center , Dallas, TX, [2]Howard Hughes Medical Institute Summer Institute, Marine Biological Laboratory, Woods Hole, MA, [3]Department of Biophysics, UT Southwestern Medical Center, Dallas, TX, [4]National Centre for Biological Sciences, Bangalore, India, [5]Department of Cellular and Molecular Pharmacology and Howard Hughes Medical Institute, University of California San Francisco, San Francisco, CA

In activated T cells, phase separated LAT clusters move from the edge to the center of the immune synapse. Recent studies have used super-resolution microscopy to describe two networks of actin within the immune synapse; a region of actin retrograde flow that exists near the synapse edge and a region of formin-generated actin bundles that exists adjacent to the region of retrograde flow, near the center of the synapse. LAT clusters move through these two regions at velocities that are tightly correlated with actin movement, suggesting that cluster movement is directly linked with actin movement. However, the molecular mechanism that regulates cluster movement through the different actin cytoskeletal networks within activated T cells was unknown. Using Jurkat T cells that fluorescently expressed components of LAT clusters, we observed that the molecular composition of LAT clusters in activated Jurkat T cells changes as clusters move from the edge to the center of the synapse. We hypothesized that this composition change may alter the ability of clusters to interact with the surrounding actin cytoskeletal network. To test our hypothesis, we reconstituted LAT phase separated clusters within two types of active actomyosin networks on supported lipid bilayers. Within a steady-state actomyosin network, in which LAT clusters of varying compositions formed amid a constantly moving actin network, we observed that cluster composition regulated the mechanism by which clusters were actively moved by the actomyosin network. Within a contractile actomyosin network, in which LAT clusters of varying compositions were formed within an existing actin network prior to Myosin II-induced actin network contraction, cluster movement was tightly correlated with actin movement depending on the composition of LAT clusters. Using our reconstituted system, we discovered a molecular clutch that links LAT cluster movement with actin filament movement in active actomyosin networks. The biochemical results from our reconstituted system explain how the movement of LAT clusters is correlated with the movement of both actin retrograde flow and contractile actomyosin bundles within different regions of an activated Jurkat T cell.

E51

The kinesin-3 KIF1C is a processive dimer and activated by a scaffold phosphatase.

A. Bachmann[1,2], N. Siddiqui[1,2], D. Roth[1,2], I. Kaverina[3], A. Straube[1,2];
[1]Centre for Mechanochemical Cell Biology, University of Warwick, Coventry, United Kingdom, [2]Warwick Medical School, University of Warwick, Coventry, United Kingdom, [3]Cell and Developmental Biology, Vanderbilt University, Nashville, TN

KIF1C is a microtubule motor that transports α5β1-integrins and is therefore required for the regulation of cell adhesion structures. We have shown previously that KIF1C contributes to the maturation of focal adhesions and the formation of podosomes. Mutation of KIF1C in humans causes spastic paraplegia and cerebellar dysfunction, suggesting its importance in neuronal long-distance transport.
Here we identify the scaffold phosphatase PTPN21 as a positive regulator of KIF1C-mediated transport. PTPN21 is essential for podosome formation, a function that does not require its catalytic activity. PTPN21 has an N-terminal FERM domain that is similar to cytoskeletal-associated proteins including band 4.1, ezrin, radixin and moesin. The FERM domain binds directly to the KIF1C tail region and is sufficient to activate KIF1C transport both in migrating epithelial cells as well as in primary hippocampal neurons.
Hydrodynamic analysis of recombinant full-length KIF1C and crosslinking mass spectroscopy reveals that KIF1C is a stable dimer that adopts an autoinhibited conformation by interaction of its tail with the motor domain. KIF1C is a processive plus-end directed motor in single molecule assays. In the presence of PTPN21 FERM domain, the fraction of motile KIF1C motors increases, suggesting that PTPN21 is not required for loading KIF1C onto the microtubule, but to activate its motility.
Our data are consistent with a model whereby PTPN21 relieves KIF1C autoinhibition. This regulation is likely to be important for KIF1C's long distance transport function and might act as a KIF1C activity switch during bidirectional cargo transport.

E52

Rapid and dynamic arginylation of the leading edge β- actin is required for cell migration.

I. Pavlyk[1], N.A. Leu[1], P. Vedula[1], A. Kashina[1], S. Kurosaka[1];
[1]Department of Biomedical Sciences, University of Pennsylvania, Philadelphia, PA

β- actin plays key roles in cell migration and is one of the most ubiquitous, abundant, and essential proteins in eukaryotic cells. Our previous work demonstrated that β- actin in migratory non-muscle cells is N-terminally arginylated and this is required for normal lamellipodia extension. Mouse embryonic fibroblasts lacking arginyltransferase (Ate1) show impairments in migration rates and leading edge function, but the exact role of beta actin in this process is not understood. Here we examined the function of β- actin arginylation in cell migration. We found that arginylated β- actin is concentrated at the leading edge of lamellipodia and that this enrichment is abolished after serum starvation as well as in contact-inhibited cells in confluent cultures, suggesting that arginylated β- actin at the cell leading edge is coupled to active migration. Arginylated actin levels exhibit dynamic changes in response to cell stimuli, lowered after serum starvation and dramatically elevating within minutes after cell stimulation by re-addition of serum or lysophosphatidic acid (LPA). This dynamic changes are not seen in confluent contact-inhibited cell cultures and require active translation. Microinjection of arginylated actin antibodies into cells severely inhibits their migration rates. Together, these data strongly suggest that arginylation of β- actin is a tightly regulated dynamic process that occurs at the leading edge of locomoting cells in response to stimuli and is integral to the signaling network that regulates cell migration.

E53

Coordination of acto-myosin contractility and mitochondrial positioning during neutrophil migration in live animals.

N. Melis[1], B. Subramanian[1], D. Chen[1], C. Parent[1], R. Weigert[1];
[1]Laboratory of Cellular and Molecular Biology, National Cancer Institute - NCI, Bethesda, MD

Cell migration is a fundamental process which occurs during a variety of physio-pathological events such as embryonic development, wound healing, immune response, and cancer. This multi-step process requires an extensive remodeling of the cellular membranes, which is mediated by the constant re-arrangement of the actin cytoskeleton. Neutrophil migration has been extensively used as a paradigm to investigate cell motility, due to its important role in the innate immune response. However, most of the studies have been carried out in *in-vitro* model systems (2-D or 3-D cell cultures) and only limited work has been performed on dissecting the molecular mechanisms that link membrane remodeling to cell motility *in-vivo* .
To this end, here we use intravital subcellular microscopy (iSMIC) and focus on understanding the mechanisms underlying the coordination among acto-myosin cytoskeleton, mitochondrial metabolism, and plasma membrane remodeling during neutrophil migration in the ear skin of live mice following laser-mediated injury. Specifically, neutrophils are isolated from the bone marrow of donor mice expressing selected fluorescently-tagged proteins, labeled with additional fluorescent dyes highlighting specific sub-cellular compartments, and injected subcutaneously in the ear of recipient mice. Our preliminary results show that the actin-based motor Myosin IIA (NMIIA) is not only present at the rear of the migrating cells, as previously described in vitro, but also at the leading edge. Moreover, we find that sharp changes in the direction of migration are preceded by the assembly of sub-populations of NMIIA filaments on the side of the cells, perpendicularly to the new direction. Finally, we observe that mitochondria: 1) constantly reposition in order to match the localization of NMIIA, most likely in order to supply the ATP required to sustain the contractile activity; and 2) change their polarization during the various phases of migration. In summary, we have begun to highlight a novel correlation between mitochondria repositioning and activity, and the dynamic rearrangement of the actomyosin complex in vivo.

E54

An investigation on the potential of Fidgetin-like 2, a microtubule severing enzyme, as a target to induce angiogenesis and heart regeneration.

A.H. Kramer[1], V. Paulino[2], Y. Wang[3], P. Nacharaju [1], J.M. Friedman[1], B. Zhou[3], R.N. Kitsis [2], D.J. Sharp[1];
[1]Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY, [2]Wilf Family Cardiovascular Research Institute, Albert Einstein College of Medicine, Bronx, NY, [3]Genetics, Albert Einstein College of Medicine, Bronx, NY

Recovery from heart disease requires regeneration and remodelling of the heart, specifically at infarct zones (regions of cell necrosis). Failure to revascularize these diseased regions is associated with decreased survival and recurrent angina. Interestingly, regulators of microtubule (MT) dynamics have become a new area of focus as they have been shown to play an important role in repair processes, including angiogenesis. Our group has previously shown that knockdown of the MT severing enzyme Fidgetin-Like 2 (FL2) greatly enhances the directionality and velocity of cell migration in vitro. Knockdown studies of FL2 show an increase in the rate of angiogenic tube formation of induced Human Umbilical Vein Cells (HUVECs) in culture. HUVECs display a similar cortical localization of FL2 previously

reported in other cells, which is specifically diminished after FL2 siRNA treatment. Immunofluorescence analysis also shows a difference in MT organization and a shift towards less stable MTs. In in vitro angiogenesis assays, the number and length of tip cell filopodia were found to increase with FL2 knockdown, perhaps as a result of a change of actin dynamics in response to increased MT dynamics. Furthermore, we have shown that FL2 knockdown affects endogenous expression of VEGFA and VEGFR2, genes known to play crucial roles in angiogenesis. Translational studies using ex vivo and in vivo heart models suggest that FL2 plays a role during heart development and regeneration. In a severe myocardial infarction model, ejection fractions were higher in FL2 nanoparticle treated hearts indicating healthier cardiac function. The treatment also enhanced recovery and resulted in more vessel regrowth than in control hearts. Based on these findings, we hypothesize that FL2 activity normally works to suppress angiogenesis and may be feasible a target to induce heart regeneration and angiogenesis.

E55

Evolutionarily Conserved Mechanisms Drive Sarcomere Assembly in Cardiomyocytes.

A.M. Fenix[1], N. Taneja[1], M.R. Visetsouk[2], B.R. Nixon[3], A. Manalo[1], J.R. Becker[3], S.W. Crawley[4], D. Bader[1,3], M.J. Tyska[1], J.H. Gutzman[2], D.T. Burnette[1];
[1]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, [2]Department of Biological Sciences, Cell and Molecular Biology, University of Wisconsin, Milwaukee, WI, [3]Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, [4]Department of Biological Sciences, The University of Toledo, Toledo, OH

The sarcomere is the basic contractile unit within cardiomyocytes driving heart muscle contraction. Mechanisms governing sarcomere assembly, however, are poorly understood. Complicating this, there are no manipulable assays to test mechanisms of de novo sarcomere assembly. Here, we develop a novel assay using human cardiomyocytes to test de novo sarcomere assembly. Using this assay, we report non-muscle like stress fibers (NMLSFs) are essential sarcomere precursors, and evolutionarily conserved mechanisms drive sarcomere assembly in human cardiomyocytes. We show sarcomeric actin filaments arise directly from NMLSFs, which requires formin-mediated actin polymerization and non-muscle myosin IIB (NMIIB). We demonstrate formin-mediated sarcomere assembly is driven by the formin FHOD3, knockdown of which stops the NMLSF to sarcomere transition. Interestingly, we show NMIIB is required for the assembly of de novo sarcomeres but not maintenance of existing sarcomeres. We confirmed NMIIB is required in vivo for sarcomere assembly, as Zebrafish NMIIB knockdown animals fail to form sarcomeres. Furthermore, we show muscle myosin II filaments concatenate to form the A-band through a mechanism that is completely dependent on organized actin filaments. Thus, we provide a robust model of de novo cardiac sarcomere formation based on evolutionarily conserved mechanisms between non-muscle and muscle cells. This model offers new insight into the mechanisms governing sarcomere formation in human cardiomyocytes, and provides a testable platform to investigate normal sarcomere assembly during development or aberrant sarcomere assembly during disease states, such as cardiomyopathies.

E56

EFHC1 and EFHC2 are necessary for motile cilia function and A-tubule MIP recruitment.

B.A. Bayless[1], D. Stoddard[2,3], Y. Zhao[4], J. Gaertig[5], D. Nicastro[2,3], M. Winey[1];
[1]Molecular and Cellular Biology, University of California, Davis, Davis, CA, [2]Biology, Brandeis University, Waltham, MA, [3]Cell Biology and Biophysics, University of Texas Southwestern Medical Center, Dallas, TX, [4]Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO, [5]Cellular Biology, University of Georgia, Athens, GA

Motile cilia are essential cellular structures that beat to move extracellular fluid in a unified direction. Disruption of ciliary beating has dire consequences on a number of essential processes including the generation of cerebral spinal fluid movement, mucus clearance from the lungs and movement of the egg down the fallopian tube during ovulation. The structural organization of thousands of motor proteins and regulators along a microtubule-based scaffold is critical for the function of motile cilia. They are composed of nine sets of modified doublet microtubules arranged radially around a central pair of microtubules. Ciliary doublet microtubules are unique in that they are extremely stable and resistant to the mechanical forces associated with ciliary beating. Recent advances in cryo-electron tomography have made it possible to map the structure of doublet microtubules with molecular resolution. We now know that there are a number of densities on the luminal surfaces of the doublet microtubules termed Microtubule Inner Proteins (MIPs), however, their composition and function remain unexplored. We have identified the role of two proteins, EFHC1 and EFHC2, in motile cilia function and MIP formation. We find that Tetrahymena thermophila homologs of EFHC1 and EFHC2, Bbc73 and Bbc60, respectively, localize to the doublet microtubules of both basal bodies and cilia. Strains with single or double null alleles of BBC73 and BBC60 exhibit motile cilia beating and assembly defects. Interestingly, when axonemes are visualized using cryo-electron tomography, knockout of either Bbc73 or Bbc60 results in independent loss of a number of A-tubule MIPs. Furthermore, we find that introduction of Bbc60-GFP into Bbc60 knockout cells rescues the loss of MIPs associated with Bbc60 absence and imparts and extra density in the region of MIP4. We hypothesize that this extra density corresponds to the additional density of the GFP tag on Bbc60 and represents the first evidence of the identification of a MIP protein. To identify other MIPs associated with EFHC proteins we used mass spectrometry to identify axoneme proteins lost in EFHC mutant Tetrahymena cells. CAPS was identified as a potential MIP protein and Tetrahymena cells that are heterozygous for CAPS show swimming defects that are consistent with deficiencies of motile cilia beating. Overall, our data reveal a major role for EFHC proteins in the function of motile cilia through the recruitment of a complex network of MIPs to the A-tubule of doublet microtubules.

Microsymp 9: Organelle Structure and Dynamics

E57

An image-based subcellular map of the human proteome.

P.J. Thul[1], L. Åkesson[1], D. Mahdessian[1], A. Bäckström[1], F. Danielsson[1], C. Gnann[1], M. Hjelmare[1], R. Schutten[1], C. Stadler[1], D.P. Sullivan[1], C.F. Winsnes[1], M. Uhlén[1], E. Lundberg[1];
[1]Science fir Life Laboratory, Royal Institute of technology (KTH), Stockholm, Sweden

Compartmentalization of biological reactions is an important mechanism to allow multiple cellular reactions to occur in parallel. Resolving the spatial distribution of the human proteome at a subcellular level increases our understanding of human biology and disease. A high-resolution map of the human cell has been generated, the Cell Atlas (1), part of the open access Human Protein Atlas database (2,3).

Using an antibody and imaging-based approach 12,003 proteins have been localized to 32 subcellular structures, enabling the definition of 13 major organelle proteomes. The high spatial resolution allowed identification of novel protein components of fine structures such as the midbody, nuclear bodies as well as rods & rings. An integrative approach was used including strict antibody validation criteria using gene silencing, paired antibodies, and fluorescently tagged proteins (4,5). Deep learning approaches and a citizen science approach was employed for refined pattern recognition in images, the mini-game "Project Discovery" integrated into an massively-multiplayer online game that has engaged more than 200,000 players world-wide (6).
We show that half of all proteins localize to multiple compartments. On one level, it can be a spatial confinement to control the timing of the molecular function in one compartment. On another level, such proteins may have context specific functions and 'moonlight' in different parts of the cell, thus increasing the functionality of the proteome and the complexity of the cell from a systems perspective. We further reveal 16% of the proteome to show single cell expression variation in terms of protein abundance or spatial distribution. Finally we show that current protein-protein network models benefit from integration of the Cell Atlas localization data as spatial boundaries.
Here we present the most comprehensive subcellular map of the human proteome and discuss the importance of spatial proteomics for single cell biology.

1. Thul (2017), Science
2. www.proteinatlas.org
3. Uhlén (2015), Science
4. Stadler (2013), Nat Methods
5. Uhlén (2016), Nat Methods
6. Peplow (2016), Nat Biotechnol

E58

VLDL Secretion from Hepatocytes is Controlled by Phosphatidic Acid.

M. Kumar[1], P. Rai[1], P. Barak[1,2], S.S. Kamat[3], R. Mallik[1];
[1]Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, India,
[2]Department of Physiology, Anatomy and Genetics, Oxford University, Oxford, United Kingdom,
[3]Departments of Biology Chemistry, Indian Institute of Science Education and Research, Pune, India

Lipid droplets (LDs) are catabolized in hepatocytes to provide lipids for assembling VLDL particles, which are then secreted to peripheral tissues. This requires extensive physical interactions between LDs and endoplasmic reticulum (ER). We found that this interaction requires LDs to be transported towards the smooth ER by motor proteins, resulting in lipid and protein exchange between these two organelles. How do metabolic signals control the activity of motors on LDs to facilitate catabolism of lipids? Here, using targeted lipidomics, in-vitro reconstitution, organelle motility and biochemical assays, we have attempted to solve this puzzle. We found that insulin via phospholipase D activity increases the phosphatidic acid level on LDs in fed state of an animal. This recruits the small GTPase Arf1 and the kinesin motor to LDs, which are transported to the ER followed by LD catabolism. Phosphatidic acid levels on LDs are reduced after fasting. This removes Arf1 and kinesin from LDs to reduce ER-LD physical contacts, and in turn decreases lipid availability for VLDL lipidation. This mechanism appears to control triglyceride secretion from the liver across feeding/fasting cycle and ensures that serum triglyceride levels are maintained approximately constant.

E59

A new model for COPII-mediated cargo export from the endoplasmic reticulum.

O. Shorn[1], I. Nevo-Yassaf[1], T. Aviad[1], J. Shepchelovich[1], Y. Yaffe[1], E. Perlson[2], A. Yeheskel[3], M. Pasmanik-Chor[3], G.H. Patterson[4], C. Kaether[5], K. Hirschberg[1];
[1]Pathology, Tel Aviv University, Tel Aviv, Israel, [2]Physiology and Pharmacology, Tel Aviv University, Tel Aviv, Israel, [3]Bioinformatics Unit, G.S.W. Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel, [4]Section on Biophotonics, NIBIB, National Institutes of Health, Bethesda, MD, [5]Leibniz Institute for Age Research, Fritz Lipmann Institute, Jena, Germany

Both COPII and COPI are considered as analogous sets of coat protein complexes mediating the formation of membrane vesicles translocating in opposite directions to different destinations within the secretory pathway. Here we provide evidence for a fundamentally different model for the function of the COPII coat during protein export from the endoplasmic reticulum (ER). We used live cell microscopy combined with a range of well-defined pharmaceutical and genetic perturbations of ER-Golgi transport. Primarily, ER to Golgi transport of a cargo membrane protein was visualized in living intact cells coexpressing fluorescently tagged Sec24 isoforms. Further COPII localization and function were analyzed and characterized in living intact cells by uncoupling vesicle fission from the preceding cargo sorting and accumulation processes in ERESs (using BFA and Nocodazole treatment). Finally, perturbation at the interface of COPII-cargo using mutagenesis of Sec24B was applied resulting in cargo accumulation in the ER, functionally establishing the localization of COPII at the ER-ERES boundary. Together, these data support the hypothesis that rather than generating coated vesicles, COPII mediates the sorting of transport-competent proteins by dynamic binding to stable elongated membranes that comprise the ER-ERES boundary. The finding that COPII is absent from nascent vesicles and thus does not limit carrier size or shape, resolves the dispute of how large cargo molecules or complexes can be accommodated in ER-derived vesicle. We propose that cargo sorting and concentration is driven by ER to ERES-directed treadmilling of the membrane bound COPII coat-cargo complex. This treadmilling movement is generated by restricting COPII recruitment to the ER side of the boundary by the activated GTP-bound ER-localized Sar1 GTPase. Cargo-protein interaction with its neighboring membrane environment contributes to direct this process. These findings transform our understanding of the role of coat proteins in ER to Golgi transport.

E60

Myosin-Va is required for preciliary vesicle transportation to the mother centriole during ciliogenesis.

C. Wu[1,2], H. Chen[1], T.K. Tang[1,2];
[1]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [2]Taiwan International Graduate Program in Interdisciplinary Neuroscience , National Yang-Ming University and Academia Sinica, Taipei, Taiwan

Primary cilia are microtubule-based organelles protruding from the apical cell surface to sense different chemical signals. Defects in ciliogenesis can lead to a number of genetic disorders collectively known as ciliopathies. Pre-ciliary vesicle accumulation at the distal appendages of mother centrioles to form a larger ciliary vesicle is critical in early step of ciliogenesis. What is the marker protein for the preciliary and ciliary vesicle? Where do the ciliary vesicles come from? How do the ciliary vesicles transport to the distal appendages of centrioles? The above questions remain largely unknown. Here, using three-dimensional structured illumination microscopy and correlative light and electron microscopy we first demonstrate that the actin-based motor protein Myosin-Va localizes to pre-ciliary vesicles, ciliary

vesicles, and ciliary sheath. Moreover, the myosin-Va-associated ciliary vesicle is not only detected in the intracellular pathway-based ciliogenesis like RPE1 cells but also the extracellular pathway based ciliogenesis like IMCD3 cells. CRISPR/Cas9-mediated Myosin-Va gene knockout in cells dramatically inhibits not only the attachment of the pre-ciliary vesicles to the distal appendages of mother centriole but also cilia assembly, implying that Myosin-Va is required for regulating the ciliary vesicle trafficking. By comparing with other early ciliary membrane protein, we found that Myosin-Va functions upstream of EHD1- Smo- and Rab11-mediated ciliary vesicle formation. Importantly, through disrupting the function of microtubules and dynein, we further demonstrate that, Myosin-Va-associated ciliary vesicles are derived from the post-Golgi membrane, and transported to the pericentrosomal region by dynein-mediated transportation along microtubules. Finally, disturbing the centrosome-based branched actin filament by Arp2/3 inhibitor, CK-666 or siRNA-mediated knockdown of Arp2 dramatically inhibits the formation of large ciliary vesicle at the distal appendages but does not affect the transportation of the pre-ciliary vesicle from the post-Golgi to the pericentrosomal region. These results indicate that Myosin-Va takes over and transports pre-ciliary vesicles from pericentrosomal region to the mother centriole along the centrosomal branched-actin network. Together, our results indicate that Myosin-Va is the first marker to identify the preciliary and ciliary vesicle and Myosin-Va-regulated preciliary vesicle transportation from the post-Golgi to the distal appendages of mother centriole is the earliest step of ciliogenesis.

E61

Axonemal Lumen Dominates Cytosolic Protein Diffusion inside the Primary Cilium.

W. Luo[1], A. Ruba[1], D. Takao[2], L.P. Zweifel[3], R.Y. Lim[3], K.J. Verhey[2], W. Yang[1];
[1]Biology, Temple University, Philadelphia, PA, [2]Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI, [3]Biozentrum and the Swiss Nanoscience Institute, University of Basel, Basel, Switzerland

Transport of membrane and cytosolic proteins in primary cilia is thought to depend on intraflagellar transport (IFT) and diffusion. However, the relative contribution and spatial routes of each transport mechanism are largely unknown. Although challenging to decipher, the details of these routes are essential for our understanding of protein transport in primary cilia, a critically affected process in many genetic diseases. By using high-speed super-resolution microscopy, we have mapped the 3D transport routes for various cytosolic proteins in the 250-nm-wide shaft of live primary cilia with an unprecedented spatiotemporal resolution of 2 ms and < 16 nm. Our data reveal two spatially distinguishable transport routes for cytosolic proteins: an IFT-dependent path along the axoneme, and a passive-diffusion route in the axonemal lumen that escaped previous studies. While all cytosolic proteins tested primarily utilize the IFT path in the anterograde direction, differences are observed in the retrograde direction where IFT20 only utilizes IFT, and approximately half of KIF17 and one third of □–tubulin utilizes diffusion besides IFT.

E62

Two-Color STORM Reveals that Disruption of Ciliary Transition Zone Architecture Causes Joubert Syndrome.

X. Shi[1], G. Garcia[2,3], J.F. Reiter[2,3], B. Huang[1,2];
[1]Pharmaceutical Chemistry, University of California - San Francisco, San Francisco, CA, [2]Biochemistry and Biophysics, University of California - San Francisco, San Francisco, CA, [3]Cardiovascular Research Institute, University of California - San Francisco, San Francisco, CA

Diverse human ciliopathies, including nephronophthisis (NPHP), Meckel syndrome (MKS) and Joubert syndrome (JBTS), can be caused by mutations affecting components of the transition zone, a ciliary domain near its base. The transition zone controls the protein composition of the ciliary membrane, but how it does so is unclear. To better understand the transition zone and its connection to ciliopathies, we defined the arrangement of key proteins in the transition zone using two-color stochastic optical reconstruction microscopy (STORM). This mapping revealed that NPHP and MKS complex components form nested rings comprised of nine-fold doublets. The NPHP complex component RPGRIP1L forms a smaller diameter transition zone ring within the MKS complex rings. JBTS-associated mutations in RPGRIP1L disrupt the architecture of the MKS and NPHP rings, revealing that vertebrate RPGRIP1L has a key role in organizing transition zone architecture. JBTS-associated mutations in TCTN2, encoding an MKS complex component, also displace proteins of the MKS and NPHP complexes from the transition zone, revealing that RPGRIP1L and TCTN2 have interdependent roles in organizing transition zone architecture. To understand how altered transition zone architecture affects developmental signaling, we examined the localization of the Hedgehog pathway component SMO in human fibroblasts derived from JBTS-affected individuals. We found that diverse ciliary proteins, including SMO, accumulate at the transition zone in wild type cells, suggesting that the transition zone is a waypoint for proteins entering and exiting the cilium. JBTS-associated mutations in RPGRIP1L disrupt SMO accumulation at the transition zone and the ciliary localization of SMO. We propose that the disruption of transition zone architecture in JBTS leads to a failure of SMO to accumulate at the transition zone, disrupting developmental signaling in JBTS.

E63

Retrograde diffusion of kinesin-II facilitates flagellar length control in *Chlamydomonas*.

A. Chien[1], S. Shih[2], R. Bower[3], D. Tritschler[3], M.E. Porter[3], A. Yildiz[1,2];
[1]Biophysics, University of California, Berkeley, Berkeley, CA, [2]Physics, University of California, Berkeley, Berkeley, CA, [3]Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, Minneapolis, MN

To assemble and maintain functional cilia, kinesin-II and dynein-1b motors power intraflagellar transport (IFT) between the cell body and the ciliary tip. IFT has been implicated in ciliary length control, but the underlying mechanism remained unclear. Using Photogate microscopy, we visualized the full range of movement of single IFT trains and motors in *Chlamydomonas* flagella. We observed that, unlike dynein-1b, kinesin-II detaches from IFT trains at the flagellar tip and returns to the cell body by diffusion. As the flagellum grows longer, diffusion delays return of kinesin-II to the basal body, depleting kinesin-II available for anterograde transport due to its large accumulation in flagella. When the flagellum is elongated via Li+ treatment, or when the cell regrows flagella in the presence of protein synthesis inhibitor, the base is further depleted of kinesin-II. In all cases, the flagellar length correlates strongly with the amount of kinesin-II accumulated in the flagella, and the equilibrium length is reached upon depletion of kinesin-II at the basal body. These results suggest that kinesin-II's accumulation in the

flagellum acts as a negative-feedback mechanism to limit the number of kinesin-II available for moving cargo on IFT trains, and limits the growth of flagella when they reach longer lengths.

Microsymp 10: Cell Adhesion and Motility

E64

Invadopodia are limited to the G1 phase of cell cycle.

B. Bayarmagnai[1], L. Perrin[1], K. Esmaeili Pourfarhangi[1], B. Gligorijevic[1,2];
[1]Bioengineering, Temple University, Philadelphia, PA, [2]Cancer Biology Program, Fox Chase Cancer Center, Philadelphia, PA

Invadopodia are dynamic, actin-rich, proteolytically active protrusions of motile cancer cells, which are necessary for degrading the surrounding extracellular matrix (ECM). We have previously shown that in vivo, invadopodia assemble around major blood vessels in tumors. Their elimination led to abolishment of perivascular ECM degradation, intravasation and metastasis. Understanding the coordination of cancer cell motility with cell cycle progression has been a subject of much attention. Over a decade ago, the Go-or-Grow hypothesis was proposed, suggesting that proliferation and invasion are mutually exclusive. However, molecular explanation for this dichotomy is still missing. In this study, we explore how invadopodia-mediated cell motility relates to cell cycle progression. Using invadopodia- and cell cycle- fluorescent markers, we show that in 2D/3D cultures, as well as in vivo, cells form invadopodia and invade into the surrounding ECM during the G1 phase of the cell cycle. Furthermore, cells arrested in G0/G1 degrade at significantly higher levels during 0-20 hours post-release from the G0/G1 arrest. Consistent with this, mRNA and protein levels of the invadopodia core (Cortactin, Tks5, MT1-MMP) peak at 14 hours post-release. We further demonstrate that invadopodia are controlled by cell cycle regulators Cyclin D1 and Cip/Kip family of proteins. Taken together, these findings suggest that invadopodia formation is tightly linked to cell cycle progression and that the coordination of invasion and cell cycle must be considered when designing chemotherapies.

E65

ECM cross-linking regulates invadopodia dynamics.

K. Esmaeili Pourfarhangi[1], A. Bergman[2], B. Gligorijevic[1];
[1]Bioengineering Department, Temple University, Philadelphia, PA, [2]Systems Computational Biology Department, Albert Einstein College of Medicine, New York, NY

Invadopodia are dynamic membrane protrusions assembled by invasive cancer cells in contact with extracellular matrix (ECM). Invadopodia are rich in structural proteins, such as actin and cortactin as well as metalloproteases, whose function is to degrade the surrounding ECM. During cancer metastasis, cumulative degradative activity of invadopodia leads to the breach of the collagen and basement membrane adjacent to blood vessels and consequent cancer cell intravasation. While signaling pathways involved in the assembly and function of invadopodia are well studied, there are only few studies striving to understand the dynamics of invadopodia and how it relates to ECM. To this purpose, we recorded time-lapse movies of invasive cancer cells (MTLn3) plated on ECM and observed that a switch from migration to stationary invadopodia assembly and ECM degradation. To explore this switch and predict the relationship between motility states and ECM properties, we have built a phenomenological mathematical model. The model provided us with a testable hypothesis, that the dynamics of invadopodia assembly will non-monotonically respond to ECM cross-linking ratio. We have screened across wide range of ECM crosslinking ratios, measuring the cumulative degradation by invadopodia in

2D and 3D. Results showed a peak in degradation at cross-linking ratio of 0.39 for three breast carcinoma cell lines- Hs-578T, MDA-MB-231, and MTLn3. Similarly, in 3D, the peak degradation occurred at cross-linking ratio of 0.36. At selected cross-linking ratios, we further investigated three dynamic features of invadopodia assembly: 1. Speed of invadopodia protrusion-retraction into the ECM; 2. Frequency of cytosolic calcium spikes and 3. Frequency of protease delivery to invadopodia. Collectively, our results demonstrate that at the intermediate level of ECM cross-linking, there is a higher frequency of, protrusion-retraction cycles, calcium spikes and MT1-MMP delivery to the membrane, which together result in more efficient ECM degradation. We further investigated the role of ß1 integrin in translating effects of ECM cross-linking variations into alterations in invadopodia dynamics. Results indicated that partial blocking of ß1 integrin not only abrogates invadopodia activity, but also disrupts the balance between migratory and degrading states lifetime by diminishing the time span that a cell spends for degrading the ECM and increasing the migration time. Results of this study suggest that small differences in external physical environment non-linearly translate to differences in dynamics and activity of cancer cells.

E66

Stiff microenvironments promote multinucleation via failure of midbody abscission downstream of Snail.

A.K. Simi[1], A.A. Anlas[1], S.X. Zhang[1], T. Hsia[1], D.C. Radisky[2], C.M. Nelson[1,3];
[1]Chemical Biological Engineering, Princeton University, Princeton, NJ, [2]Cancer Biology, Mayo Clinic Cancer Center, Jacksonville, FL, [3]Molecular Biology, Princeton University, Princeton, NJ

Tumorigenesis coincides with tissue stiffening, which induces changes in the organization and proliferation of cells. Here we investigated how tissue stiffness regulates multinucleation, a phenotype associated with invasion, chemotherapeutic resistance, and increased tolerance for mutation. Importantly, multinucleated cells act as an intermediate to aneuploidy, a known driver of tumorigenesis. We investigated the effects of substratum stiffness on multinucleation in mammary epithelial cells, where genome doubling precedes other genomic abnormalities in 45% of breast tumors. Cells cultured on “stiff” substrata, representing tumor tissue, showed a 10-fold increase in multinucleation compared to cells cultured on “soft” substrata, representing normal tissue. We found that multinucleation was regulated in part by signaling downstream of matrix metalloproteinase-3 (MMP3), which is commonly upregulated in cancer and known to induce epithelial-mesenchymal transition (EMT). This signaling included expression of the key EMT effector and transcription factor, Snail. Similarly, we found that transforming growth factor-beta (TGF-b), another EMT-inducer, also caused multinucleation downstream of Snail. Under all conditions, cells cultured on soft substrata maintained a low frequency of multinucleation. We further found that multinucleation on stiff substrata primarily resulted from failure of midbody abscission. A soft microenvironment protected the stability of the genome by preventing midbody persistence, which resulted from upregulation of septin-6, a novel target of Snail. Taken together, these data suggest that tissue stiffening during tumorigenesis synergizes with oncogenic signaling to promote genomic abnormalities that drive cancer progression. Further, our results suggest that EMT-related signaling pathways are associated with disease progression not only because they induce metastasis, but also because they contribute to genomic instability.

E67

Aquaporin-5 in carcinogenesis: expression decreases levels of cell:cell adhesion proteins in MDCK cells.

F.H. Login[1], H.H. Jensen[1,2], J.J. Morgen [1,2], G.A. Pedersen[1,3], J.S. Koffman[4], J. Palmfeldt[1], P. Bross[1], M. Parsons[3], L.N. Nejsum[1,4];
[1]Department of Clinical Medicine,, Aarhus University, Aarhus, Denmark, [2]Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark, [3]King's College, London, United Kingdom, [4]Interdisciplinary Nanoscience Center,, Aarhus University, Aarhus, Denmark

Aquaporins (AQPs) are water channels that facilitate transport of water across the plasma membrane. Moreover, increasing evidence indicates oncogenic properties of some AQPs, especially AQP5: AQP5 is overexpressed in several cancers and overexpression correlates with poor prognosis. In cell cultures, ectopic expression induces cell migration and loss of epithelial cell markers.When AQP5 is phosphorylated on S156 by protein kinase A (PKA), major signaling pathways such as NF-κB and Ras are activated. To further elucidate the role of AQP5 in carcinogenesis, we investigated the effects of AQP5 on proteins involved in cell adhesion. We found that AQP5 expression in MDCK cells affected both mRNA and protein levels of several junctional proteins. Immunofluorescence showed that AQP5 expression decreased protein levels of β-catenin, ZO1, p120 catenin and plakoglobin (γ-catenin) and fluorescent in situ hybridization analysis showed a drastic decrease in β-catenin and ZO1 transcripts. Surprisingly, the AQP5S156A mutant that is impaired in phosphorylation by PKA also promoted low levels of adhesion proteins, indicating a Ras independent pathway. The Wnt pathway feedback inhibitor Axin2, a β-catenin/TCF-regulated gene, was also down-regulated in AQP5 expressing cells. Inhibition of glycogen synthase kinase-3β (GSK-3β) with LiCl partially restored β-catenin levels in AQP5 expressing cells. This indicates that AQP5 expression may interfere with the Wnt signaling pathway by activating or preventing inhibition of GSK-3β.
Moreover, pull-down and mass spectrometry analysis revealed that AQP5 interacted with β-catenin, plakoglobin and ZO1. Thus, AQP5 may downregulate adhesion proteins via interference with the Wnt signaling pathway as well as interactions with adhesion proteins, which triggers degradation. Our findings suggest that AQP5 may contribute to carcinogenesis by several mechanisms including interference with the Wnt pathway and degradation of adhesion proteins.

E68

Co-regulation of Rac and Rho Signalling in Cell Motility by a Scaffold RhoGAP BPGAP1.

C.Q. Pan[1], P.J. Chua[2], T.W. Chew[1], S.Y. Er[1], P. . Chaudhuri[1], D.C. Wong[1], A. Salim[3], A. Thike[4], C. Koh[5], C. Lim[6], P.H. Tan[4], B.H. Bay[2], A.J. Ridley[7], B.C. Low[1];
[1]Biological Sciences, Mechanobiology Institute, National University of Singapore, Singapore, Singapore, [2]Department of Anatomy, Yong Loo Lin School of Medicine, National University Health System, National University of Singapore, Singapore, Singapore, [3]Department of Mathematics and Statistics, La Trobe University, Bundoora, Australia, [4]Department of Pathology, Singapore General Hospital, Singapore, Singapore, [5]Division of Molecular Genetics Cell Biology, School of Biological Sciences, , Nanyang Technological University, Singapore, Singapore, [6]Department of Biomedical Engineering, , Mechanobiology Institute, National University of Singapore, Singapore, Singapore, [7]Randall Division of Cell and Molecular Biophysics, King's College London, London, United Kingdom

Cancer metastasis comprises multiple steps of cell migration that requires active remodeling of the cytoskeleton for cells to invade the surrounding tissues. Rho small GTPases are key molecular switches that control such processes and are known to contribute to cancer progression. Among them, RhoA,

Rac1 and Cdc42 specifically promote actomyosin contractility, lamellipodia and filopodia formation, respectively. They are activated by guanine exchange factors (GEFs) and inactivated by GTPase-activating proteins (GAPs). Mutations in Rho are frequent only in a few cancer types, but deregulation of Rho signaling is often associated with tumorigenesis at the level of their gene expression or activation through their regulators or downstream effectors. Intriguingly, RhoA and Rac1 often act antagonistically and have distinct spatiotemporal activity profiles in lamellipodia. However, it is unclear whether the distinct spatiotemporal activity of Rho/Rac can be synchronized by a single protein that is common to both GTPases, which would help in facilitating a more efficient control in cell dynamics. We hypothesize that such an integrator for Rho/Rac signaling could exist by targeting and regulating both RhoA and Rac1 in close proximity.
Expression of the BPGAP1 (or ARHGAP8) is often elevated in primary colorectal tumors, invasive cervical and breast cancer. It is a multi-domain RhoGAP that inactivates RhoA and induces cell protrusions and cell migration via the interplay of its BCH domain, Proline-Rich Region and RhoGAP domain. Consistent with its pro-metastatic potential, BPGAP1 translocates the actin regulator, cortactin, to lamellipodia where BPGAP1 also interacts with the inactive form of Rac1, raising the possibility that BPGAP1 could coordinate and integrate Rac1 and RhoA signaling in cell motility. Mechanistically, stimulation by growth factor EGF releases BPGAP1 autoinhibition, exposing its BCH domain to recruit the RacGEF, Vav, to activate Rac1 and promote polarized cell motility, cell spreading, invadopodia formation and cancer extravasation. Importantly, BPGAP1 controls local Rho activity that influences Rac1 binding to BPGAP1 and its subsequent activation by Vav1. BPGAP1 therefore acts as a dual-functional scaffold that recruits Vav to activate Rac1 while inactivating RhoA to synchronize both Rho and Rac signaling in cell motility. As EGF receptor, Vav, Rho, Rac and BPGAP1 are all associated with cancer metastasis, BPGAP1 offers a crucial checkpoint for EGFR-BPGAP1-Vav-Rac-Rho signaling axis and possible regimes in cancer intervention.

E69

Apoptosis Signal-Regulating Kinase 1 (ASK1) is a novel regulator of heparin-induced thrombocytopenia and thrombosis in mice.

P. Patel[1], Y. Zhou[1], S. McKenzie[1], U. Naik[1];
[1]Cardeza Foundation for Hematologic Research and Center for Vascular Biology Research, Department of Medicine, Thomas Jefferson University, Philadelphia, PA

Heparin-induced thrombocytopenia and thrombosis (HITT) is a life-threatening (20-30% mortality risk) disease in which IgG antibodies against the heparin-PF4 complex bind and activate platelets via FcγRIIA. Our laboratory has identified that the Apoptosis Signal-Regulating Kinase (ASK1), a MAP3K, is present in both human and murine platelets and potentiates many platelet functions. Given that ASK1 regulates platelet function, and that platelets are known to play a major role in the pathogenesis of HITT, we hypothesized that ASK1 is a novel regulator of HITT.
To establish if ASK1 is activated downstream of FcγRIIA, we first stimulated washed human platelets with anti-CD9 (700ng/mL). Anti-CD9 induces the activation of FcγRIIA. We found that anti-CD9 induced a robust activation of ASK1, as measured by phosphorylation of ASK1 Thr^{845} (a marker of ASK1's kinase activity). ASK1 exclusively activates p38 in platelets; therefore we also measured phosphorylation of p38 a marker of ASK1's signaling activity. We found that anti-CD9 also induced a robust phosphorylation of p38.
To determine the role ASK1 plays in platelet-FcγRIIA signaling and HITT, we crossed $Ask1^{-/-}$ mice to $FcγRIIA^{+/+}$(hFcR) mice. We found that genetic ablation of Ask1 did not have any effect on anti-CD9-induced activation of PLCγ2 or Syk in hFcR/$Ask1^{-/-}$ (KO) platelets compared to hFcR/$Ask1^{+/+}$ (WT).

However, loss of Ask1 did result in the complete absence of p38 activation in KO platelets following activation of FcγRIIA by anti-CD9 (500ng/mL). Further, we observed that anti-CD9-induced integrin $\alpha_{IIb}\beta_3$ activation, α-granule secretion, and platelet aggregation were all significantly attenuated in KO platelets ($P<0.01$). These in vitro results strongly suggested that ASK1 plays a prominent role in FcγRIIA-mediated platelet activation.
To further investigate the role of ASK1 in HITT pathogenesis, WT and KO mice were subjected to an in vivo model of HITT by injecting anti-CD9 IgG. Platelet counts were measured in samples of whole blood collected at various time points post-injection, and compared to resting platelet counts taken before HITT was initiated. We found that KO mice were significantly ($P<0.01$) protected from thrombocytopenia compared to WT mice when injected with 250μg/kg anti-CD9. However, when injected with 500μg/kg anti-CD9, both WT and KO mice displayed the same level of thrombocytopenia. Despite not protecting from thrombocytopenia, when observed for signs of shock, we found that KO mice were significantly ($P<0.01$) protected from anti-CD9 induced shock compared to WT mice regardless of the dose.
Taken together these in vitro and in vivo data strongly suggest that ASK1 regulates FcγRIIA-mediated platelet activation, and that ASK1 plays a key role in the pathogenesis of HITT.

E70

A novel Twist1-PKD1 axis promotes epithelial dissemination.

D. Georgess[1], O.K. Sirka[1], G. Frid[1], A. Choi[1], N.M. Neumann[1], A.J. Ewald[1];
[1]Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD

Metastasis is initiated by tumor cells adopting an invasive, migratory program that allows them to disseminate and colonize distant organs. The minimal molecular requirement for metastasis is incompletely understood. We focused on elucidating the molecular mechanisms of cell detachment and migration downstream of Twist1, a transcription factor that is overexpressed in metastatic breast cancers and correlates with poor prognosis.
Using 3D organotypic culture of the mammary epithelium, we show that gain of Twist1 expression drives single cell dissemination without loss of epithelial identity. RNAseq of Twist1+ versus WT organoids revealed that Twist1 upregulates the expression of 107 genes, 9 of which are targetable with commercially available small molecules. We tested these inhibitors in our Twist1-induced dissemination assay. In particular, inhibition of protein kinase D1 (PKD1) blocked dissemination completely without affecting growth of WT epithelium. Knockdown of PKD1 in Twist1+ organoids confirmed its specific requirement for dissemination. We then investigated the cellular processes controlled by PKD1. Timelapse microscopy revealed that early pharmacologic inhibition of PKD1 prevented Twist1+ cells from protruding into the extracellular matrix (ECM) and detaching from the epithelium, while late PKD1 inhibition in disseminated cells abrogated their motility. The requirement of PKD1 for invasion and dissemination was also validated in organoids generated from a breast cancer mouse model (C3(1)-Tag) and in organoids isolated from primary human tumors.
To study signaling downstream of the Twist1-PKD1 axis, we utilized phosphoprotein arrays which showed that PKD1 promotes the phosphorylation of the oncogene Myc, the tumor suppressor Brca1, the adherens junction protein β–catenin, the microtubule-associated protein Tau, and integrin partner FAK. Immunofluorescence staining confirmed that β-catenin phosphorylation (but not expression) increased in cells as they detached from the epithelium. Inhibition of FAK revealed that its activity was required for cells to disseminate. Moreover, PKD1 activity converged with microtubule depolymerization, likely through Tau phosphorylation. Microtubule depolymerization itself had a positive feedback on PKD1 as treatment with nocodazole, but not paclitaxel, weakened the anti-disseminative effect of the PKD1 inhibitor.

Our data proposes a new working model whereby Twist1 drives epithelial dissemination by triggering PKD1 expression. In turn, PKD1 hijacks the cytoskeleton, thus forcing the cell to invade the ECM, then detach from the epithelium, and finally migrate with high persistence into the surrounding stroma. These results also highlight PKD1 as a new potential target for anti-metastatic therapy.

Microsymp 11: Cell Migration, Stem Cells, and Disease

E71

Epithelial cells spatiotemporally coordinate molecular activities and mechanical forces to drive radial intercalation during ductal elongation.

N.M. Neumann[1], M.C. Perrone[2,3], J.H. Veldhuis[2,3], R.J. Huebner[1], H. Zhan[1], P.N. Devreotes[1], G.W. Brodland[2,3], A.J. Ewald[1];
[1]Cell Biology and Center for Cell Dynamics, Johns Hopkins University School of Medicine, Baltimore, MD, [2]Centre for Bioengineering and Biotechnology, University of Waterloo, Waterloo, ON, [3]Civil and Environmental Engineering, University of Waterloo, Waterloo, ON

During pubertal development, the mammary epithelium undergoes extensive elongation and bifurcation to form a ductal network. Elongation is led by a stratified, low-polarity structure known as the terminal end bud, which transitions to a polarized duct at the end of puberty. This process is regulated, in part, by receptor tyrosine kinases (RTKs). We sought to determine how cells coordinate the molecular activities in single cells with mechanical forces during elongation.
Using organotypic culture and real-time confocal imaging, we first used fluorescent molecular biosensors to test whether the molecular logic of cell migration is conserved in cells within a tissue by visualizing the downstream signaling events of the RTK pathway. We observed individual cells asymmetrically enriching Ras activity, PIP3, and F-actin in protrusions, consistent with a migratory molecular polarity. In the protrusions, both Ras activity and PIP3 enriched concurrently at the site of F-actin enrichment. Inhibiting actin dynamics prevented protrusion formation and morphogenesis, but did not prevent PIP3 enrichment.
For a duct to elongate, the basal surface must expand, which solely internal migration cannot accomplish. We therefore sought to examine the dynamics of migration as a cell approached the basal surface. We observed ductal elongation driven by radial intercalation in which migratory internal cells insert into the most superficial cell layer. We observed enrichments of Ras activity, PIP3, and F-actin in protrusions during intercalation. These dynamics are consistent with a transition from migratory front-rear polarity back to a stationary apico-basal polarity.
Next, we used the Cellular Force Inference Toolkit in 3D to analyze force balance equations at each cellular junction within an organoid. We found that migration through a tissue requires specific ratios of protrusion tension and posterior interfacial tension gradients. For intercalation, the duct requires high basal tension and a time-varying, increasing posterior interfacial tension gradient as protrusions are initiated. Finally, using finite element modeling, we generated in silico organoids with cells undergoing migration and radial intercalation to elongate a duct, solely based on mechanical alterations.
We conclude that ductal elongation results from an epithelial motility program that uses directed enrichments of Ras activity, PIP3, and F-actin in protrusions to drive migration and radial intercalation. These molecular dynamics create a spatial gradient of tensions across cells and time-dependent changes within the tissues that together accomplish morphogenesis. We now seek to understand how cancer cells re-access these developmental epithelial motility programs during metastasis.

E72

Long-range intercellular communication in collective cell migration.

A. Zaritsky[1,2,3], Y. Tseng[4], M. Rabadán[4], S. Krishna[4], M. Overholtzer[4], A. Hall[4], G. Danuser[1,3];
[1]Cell Biology, UT Southwestern Medical Center, Dallas, TX, [2]Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel, [3]Bioinformatics, UT Southwestern Medical Center, Dallas, TX, [4]Cell Biology, Memorial Sloan-Kettering Cancer Center, New York, NY

Collective cell migration of cohesive groups involves intercellular mechanical communication transmitted between adjacent cells through cell-cell contacts to eventually drive long-range communication. We discovered how local mechanical fluctuations induce long-range communication and identified some potential molecular players driving this mechanism.
By designing and applying new analytical methods to migrating monolayers of epithelial cells, we find that cells at the front transmit mechanical cues by inducing normal and shear strains on neighboring follower cells. Accumulation and propagation of these mechanical cues over time and space create groups of cells that migrate and exert forces in a coordinated manner. Such motion patterns direct cells from within the monolayer toward the sites of shear-strain-induced motion at the monolayer front. These results provide a model of long-range mechanical communication between cells, in which local alignment of velocity and stress translates local mechanical fluctuations into globally collective migration.
Efficient collective migration depends on a balance between contractility, cytoskeletal rearrangements, and adhesions, all controlled by GTPases of the RHO family. Spatiotemporal analysis revealed a surprising role of the RHO GTPase RhoA in regulating long-range communication that was mirrored upon slight down-regulation of myosin-II contractilty. A comprehensive screen uncovered a group of RhoA-activator guanine nucleotide exchange factors (GEFs) that are implicated in intercellular communication. Downregulation of these GEFs differentially enhanced propagation of guidance cues through the group, defining two functional clusters: RHOA-ARHGEF18 and ARHGEF3-ARHGEF28-ARHGEF11, with RHOC as an intermediate between them. We conclude that for effective collective migration the RhoA-GEFs/RhoA/C/actomyosin pathways must be optimally tuned to compromise between generation of motility forces and restriction of intercellular communication.

E73

High-throughput Screening Platform for Treatment Optimization using Cardiac Tissues derived from Patient's iPSCs.

S. Park[1], W. Jung[1], C. Ong[2], M. Li[1], N. Hibino[2], Y. Chen[1];
[1]Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD, [2]Division of Cardiac Surgery, Johns Hopkins University, Baltimore, MD

The mechanical forces are required for many biological processes, including heartbeats, body movements, and intestine peristalsis, among others. With the advances in tissue engineering, it is critical to assess the mechanical properties and function of the engineered tissues, or the impact of various drugs on them, before they are implemented clinically. Currently there is no adequate method to measure forces in 3D tissues with high-throughput capacity. We designed and developed an ultrafast personalization-ready high-throughput screening (HTS) platform to serve the purpose, where 96 samples can be measured in 40 seconds. As a proof of concept, we tested the performance of our prototype system using 3D cardiac tissues consisting of iPSC-derived cardiac tissues fabricated by bio-3D printing, which can rapidly create patient-specific 3D cardiac tissues in large quantities. The contractile force magnitude/frequency and stress map were computed. The viscosity of the medium was

modulated to reflect the varied mechanical resistance of the arteries corresponding to individual patients, as well as to suppress the detectable random Brownian motion due to thermal fluctuation. We found the cardiac tissues derived from patients' iPSCs exhibited spontaneous contractility at the rate between 0.5 Hz and 1 Hz, similar to the heart rate in vivo. Functional comparison was performed between normal and diseased tissues derived from Brugada syndrome patients. The result showed that diseased tissue retained the beating rate but exerted less force manifested in contractility. It was also verified and quantitated that drugs inhibiting myosin activity such as Y27632 and Blebbistatin slowed down the contraction rate. Overall we successfully demonstrated that our system is suitable for fast, personalized drug screening for tissue functions and/or cardiotoxicity of drugs. Compared to the conventional methods, our system addresses the issues of tissue size/geometry restrictions, lengthy and complicated sample preparation, prohibitive costs and other technical difficulties, in addition to drastic improvement in measurement speed by at least 500 folds, thus permitting HTS in miniaturized engineered cardiac tissues.

E74

Self-organization of brain tumors: oncostreams determine growth, invasion, and malignity of brain tumors.

P.R. Lowenstein[1,2], A. Comba[2], D. Zamler[2], A. Argento[2], F. Nunez-Aguilera[2], S. Motsch[3], M.G. Castro[1,2]; [1]Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, [2]Neurosurgery, University of Michigan, Ann Arbor, MI, [3]Mathematical and Statistical Sciences, Arizona State University, Tempe, AZ

Biological self-organization is when a process or a biological multicellular structure forms at the macroscopic level as a result of interactions among lower-level components, i.e., individual cells. Interactions among the individual components only utilize local information. Cancer growth is mostly understood as random. The role that stereotypical structures may have in tumor growth has not been carefully considered. During the study of both rodent and human brain tumors we determined the existence of multicellular structures which we named oncostreams. Oncostream are elongated cells 5-20 cells wide and of variable length. Oncostreams express proteins that are typical of brain tumors, i.e., GFAP, nestin, twist, MMP2, retinoblastoma, olig2, amongst others. The density of immunocytochemically distinct proteins varies between oncostream and non-oncostream glioma cells. The function of oncostreams was explored utilizing cell biological, molecular, and mathematical modeling experiments. Oncostreams mediate glioma invasion, as they form tight fingers of parallel elongated cells that push into surrounding normal brain. Oncostreams can also serve as preferential highways for the movement of glioma cells throughout the brain. Injection of slowly moving human brain glioma stem cells into rapidly streaming rodent glioma cells results in the alignment of the human glioma cells to the oncostreams, and their fast distribution throughout the tumor along these structures. Oncostreams may also limit immune cells' entry into gliomas as they appear to be excluded from these structures. To test the hypothesis that oncostreams are formed solely from interactions between individual glioma cells, we built a series of agent-based mathematical models. These allowed us to discover that only elongated cells form structures resembling oncostreams. Circular cells never do so. The length:width ratio of the elongated oncostream-forming cells is not significantly different from the ratio measured in vivo. Finally, to understand whether oncostreams differ molecularly from surrounding glioma cells, we dissected oncostreams and surrounding glioma tissue using laser scanning microdissection, RNA-Seq and bioinformatics. The set of gene expression patterns differs significantly between the two areas, thus enabling us to identify the molecular individuality of oncostreams. Network analysis has identified fyn and STAT-1 as highly connected nodes. Deletion studies are now allowing us to demonstrate that fyn appears to play a role in oncostream formation and malignant tumors'

phenotype. In conclusion, we demonstrate that oncostreams are a novel cancer structure with individual molecular makeup and function, and have an important role in determining the phenotype of malignant brain gliomas.

E75

Live cell FLIM-PLIM microscopy reveals proliferative and metabolic heterogeneity of intestinal organoids.

R.I. Dmitriev[1], T. Foley[2], D.B. Papkovsky[1], I.A. Okkelman[1];
[1]School of Biochemistry and Cell Biology, University College Cork, Cork, Republic of Ireland, [2]Department of Anatomy and Neuroscience, University College Cork, Cork, Republic of Ireland

Small intestinal organoids gain popularity in stem cell research, tissue engineering and translational applications. The complex 3D composition of organoids includes Lgr5+ stem cell niche(s), enterocytes and other cell types, which form distinct epithelial cell monolayer (villi and crypt regions) and lumen compartments. Organoids are relatively easy to produce within days, by simple growing them in 3D matrices and yielding functional micro-tissues. However, static growth conditions, matrix viscosity, active proliferation and metabolism of organoids impact the supply of nutrients, exchange of excretion products and other factors in complex manner. So far, only few studies addressed organoid physiology and micro-heterogeneity in their live state, with single cell resolution. We combined live cell imaging by fluorescence (FLIM) and phosphorescence (PLIM) lifetime imaging microscopy approaches, to study oxygenation and proliferation of organoids in growing cultures. First, we traced cell cycle S phase by FLIM-based Hoechst 33342-BrdU quenching method [1], which allowed visualization of proliferating (incorporating BrdU) cell zones, most likely representing stem cell niches. Interestingly, the number of proliferation zones in organoids correlated well with the number of crypts. Secondly, using cell-penetrating phosphorescent O_2 probe Pt-Glc and PLIM detection [2], we monitored oxygenation of organoids in real-time. We found strong variability of oxygenation between and within organoids, ranging 27~ 92 µM, or 2.8~9.7% O_2. In some organoids we detected trans-epithelial O_2 gradients of up to 20-25 µM O_2, which indicate their active aerobic metabolism. Importantly, oxygenation of organoids was dynamic and could be modulated by activation/inhibition of mitochondria. Oxygenation also differed between passages, implying the changes in organoid metabolism during culturing. Collectively, these results prove that monitoring of organoid metabolic and proliferative status is important for proper interpretation of physiological responses to drugs and other stimuli. Further studies on monitoring / modulating of stem cell niche metabolism and proliferation are required in order to properly characterize and use this experimental model.
References: [1] Okkelman IA, Dmitriev RI, Foley T, Papkovsky DB. Use of Fluorescence Lifetime Imaging Microscopy (FLIM) as a Timer of Cell Cycle S Phase. PLOS ONE. 2016;11:e0167385. [2] Dmitriev RI, Kondrashina AV, Koren K, Klimant I, Zhdanov AV, Pakan JMP, et al. Small molecule phosphorescent probes for O_2 imaging in 3D tissue models. Biomaterials Science. 2014;2:853-66.

E76

In vivo cellular reprogramming to restore respiratory function after SCI.

S. Fernandes[1], L.V. Zholudeva[1], M.A. Lane[1], P.W. Baas[1], L. Qiang[1];
[1]Neurobiology and Anatomy, Drexel University College of Medicine, PHILADELPHIA, PA

The injured adult mammalian spinal cord is incapable of significant repair. This limitation is due in part to two major neuropathological consequences of spinal cord injury (SCI): i) the formation of growth-inhibitory glial scars, of which activated astrocytes are a key component, and ii) the destruction of

intraspinal neuronal connectivity, contributed to by the loss of the interneurons in the spinal circuitry. Previous cell-based strategies have traditionally been focused on transplantation of various neural stem cells into the injury site to replace lost neurons, improve the inhibitory environment and modulate inflammation; however, there are significant hurdles to their applications. For example, obtaining sufficient amounts of purified cells for transplantation may be difficult; the procedure often requires the use of immuno-suppression, which has detrimental effects on the host; and successful implementation of such a strategy needs to address the challenges of cell survival and appropriate cell differentiation without formation of tumors. Here we applied an in vitro and in vivo direct conversion strategy to reprogram activated astrocytes to potential functional interneurons by introducing into the injured site a single lentiviral vector encoding Ascl1, which serves as a major neurogenic regulator CNS interneurons. Our in vitro data revealed that Ascl1 is a potent reprogramming factor that was able to convert more than 40% of the activated astrocytes into functional neurons in cultures. These cells not only were stained positive for pan-neuronal markers, but also were mature enough to bear typical neuronal electrophysiological properties. Successful in vivo conversions in adult rats were also achieved using the same strategy, yet with a much more diminished efficiency (On average, 4 converted neurons from each animal were identified) 6 weeks after the viral injection followed by the contusive cervical SCI. As we expected, functional examinations such as plethysmography and terminal diaphragm electromyography showed no significant improvement. However, a substantial reduction of the glial scars was identified in Ascl1 treated animals. Based on the data obtained so far, we posit that to achieve more efficient in vivo conversions, a longer duration may be required following the viral injection. Despite the lack of functional recovery from our current treatment strategy for SCI, our pilot study serves as a proof-of-concept for its potential translational applications. Meanwhile, we are adjusting our viral content in order to achieve a more efficient in vivo conversion for SCI therapies. (This work was funded by a grant from the Craig H. Neilsen Foundation to LQ).

E77

Is the Eicosanoid Producing Enzyme 12-Lipoxygenase (ALOX12) a Tumor Suppressor?

G.F. Gerlach[1], P. Niethammer[1];
[1]Cell Biology, Memorial Sloan Kettering Cancer Center, New York, NY

Epithelial barrier disruption causes inflammation, which if unchecked over time can cause malignant transformation. The pathways that link chronic epithelial barrier disruption to transformation remain largely unknown. Emerging evidence suggests arachidonic acid (AA) metabolites, collectively termed eicosanoids, may play a central role in this process. Cyclooxygenases convert AA into proliferative and trophic eicosanoids, the prostaglandins, which are mediators of tumorigenesis. Lipoxygenases, on the other hand, convert AA into pro- and anti-inflammatory eicosanoids, such as leukotrienes and lipoxins. One of the least studied, yet most highly expressed epidermal lipoxygenases, ALOX12B, is essential for epithelial barrier integrity throughout phyla. We hypothesize that this enzyme acts as a tumor-suppressor in two ways: first, through its known function in epithelial barrier maintenance, preventing epithelial inflammation. Second, by shunting AA away from the pro-tumorigenic prostaglandin metabolism. Intriguingly, ALOX12B is co-deleted with p53 in many human tumors. Thus, understanding its cancer-related functions is highly relevant. Transparent zebrafish larvae are especially well suited to test the role of eicosanoid-mediated epithelial barrier maintenance, inflammatory, and malignant transformation by live imaging. Here we show loss, by CRISPR gene editing and morpholino mediated knockdown, of zebrafish alox12, the functional ortholog of ALOX12B, generates hyperplastic lesions in zebrafish epithelium. This phenotype is associated with leukocyte infiltration, and up regulation of inflammatory genes, indicative of a tumor-promoting tissue microenvironment. Through live imaging and lipidomic analysis in zebrafish and cell culture models, we are dissecting the fundamental

mechanisms of eicosanoid-induced epithelial barrier homeostasis, inflammation, and malignant transformation that may be exploited for therapeutic benefit in the future.

Microsymp 12: Mechanoregulation and Translational Studies of the Cytoskeleton

E78

Graded activation of ROCK and MLCK tunes regional stress fiber formation and mechanics via preferential myosin light chain phosphorylation.

E. Kassianidou[1,2], J.H. Hughes[1,2], S. Kumar[2,3];
[1]UC Berkeley - UCSF Graduate Program in Bioengineering, Berkeley, CA, [2]Bioengineering, University of California, Berkeley, Berkeley, CA, [3]Chemical and Biomolecular Engineering, University of Berkeley, Berkeley, CA

Stress fibers (SFs) are prominent force-generating cytoskeletal structures chiefly comprised of actin and non-muscle myosin II (NMMII). NMMII motor activity is strongly promoted by phosphorylation of the NMMII light chains (MLCs) by MLC kinase (MLCK) and Rho-associated kinase (ROCK). MLCs can be phosphorylated once (p-MLC) or twice (pp-MLC), with each phosphorylation state distinctly influencing myosin ATPase activity. Prior work suggests MLCK and ROCK regulate SF formation in the cell periphery and cell center respectively, and both MLC phosphorylation states have been observed throughout the cell. However, it is unclear how MLCK and ROCK differ in their regulation of MLC phosphorylation state, how these kinases regulate these two pools of SFs, and how these parameters influence SF mechanics. Here, we address these questions by combining inducible genetic expression of MLCK or ROCK with mechanical measurements of single SFs in live cells and phospho-specific immunoanalysis. Immunoblots reveal that constitutive activation (CA) of MLCK preferentially increases whole-cell levels of p-MLC while CA of ROCK increases pp-MLC. Quantitative immunofluorescence analysis demonstrates that MLCK preferentially increases recruitment of p-MLC to peripheral SFs while ROCK recruits pp-MLC to central SFs. To relate kinase activity to SF mechanical properties, we used ablation to sever single SFs in live cells and tracked the retraction of the severed ends as tension dissipated. We found MLCK increased the effective viscosity and total stored elastic energy in peripheral SFs but not central SFs, while ROCK preferentially increased these same parameters in central SFs. To directly link MLC phosphorylation state and stress fiber mechanics, we overexpressed phosphomimetic mutants of p-MLC and pp-MLC and found that phosphomimetic p-MLC influenced only peripheral SF viscoelastic parameters while phosphomimetic pp-MLC influenced only central SF viscoelastic parameters, phenocopying our ablation findings. Finally, by varying the concentration of the small-molecule inducer in the culture medium, we could establish gradations in kinase expression and concomitant p- and pp-MLC levels, thereby enabling quantification of these relationships. Application of this strategy revealed surprising and previously unappreciated nonlinearities between MLC phosphorylation state and SF viscoelastic properties. This work supports a model in which MLCK regulates peripheral stress fiber viscoelasticity via mono-phosphorylation of MLC while ROCK regulates central stress fiber viscoelasticity via di-phosphorylation of MLC.

E79

Profiling Mechanical Stress-Dependent Cytoskeleton Organization at Varying Curvatures Using Self-Induced Rolling Membrane (SIRM).

J. Kim[1], L. Vannozzi[2], Y. Chen[1,3];
[1]Mechanical Engineering , Johns Hopkins University, Baltimroe, MD, [2]The BioRobotics Institute, Sant'Anna School of Advanced Studies, Pisa, Italy, [3]Center for Cell Dynamics, Johns Hopkins University,

Baltiorme, MD

The geometry of the tissue microenvironment determines the local mechanical stress experienced by cells. It is known that mechanical stress can regulate mechanosignaling and subsequent gene expression. For example, it has been reported that the frequency of breast cancer occurrence is higher at the mammary ducts of higher curvatures. However, the detailed mechanism by which local geometry regulates cellular behaviors is yet to be elucidated. We have thus devised a self-induced rolling membrane (SIRM) platform, a rectangular thin elastic film rolled into a cylindrical roll, to study the effect of various curvatures on cytoskeleton organization in the epithelium. The SIRM is characterized by a continuously varying curvature ranging from a radius of 50 – 500um, allowing various curvature conditions to be investigated in one experiment. First, Mouse mammary epithelial cells (EPH4-EV) were seeded and cultured for 24 hours. The SIRM was then rolled and incubated for another 24 hours to induce the curvature effects on the epithelium. We then unrolled the SIRM for high-resolution microscopy and examined the actin organization of the cells cultured at different curvatures by phalloidin staining. It was observed under fluorescence microscopy that the ratio of cytosolic actin to cortical actin difference between high and low curvature is approximately a factor of 1.2, suggesting that the curvature at the scale 50-500um radius governs the differential organization of the cytoskeleton. In summary, the SIRM is a high-throughput platform compatible with high-resolution microscopy that makes it an ideal system to study curvature effects at tissue level.

E80

Morphological Changes of Epithelia Cells Induced by Viscous Conditions.

J. Flournoy[1], G. Sun[1], D. Maity[2], Y. Chen[1];
[1]Mechanical Engineering, Johns Hopkins University, Baltimore, MD, [2]Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore , MD

Cells are highly sensitive to their microenvironments. While many chemical effects on the microenvironment are known, most biophysical effects are yet to be studied. One example is the effect of viscosity on cells. Understanding the effects of viscosity is pertinent to cancer research as increased viscosity is a prominent mechanical effect observed in the tumor microenvironment. In this study, we used media containing a 2% thickening agent to examine the effects of viscosity on a variety of cell lines, including EPH4, 67NR, 4T1, NIH-3T3, and MDA-MB231. Using reflectance imaging, we observed that cells exhibit a significant morphological change when viscous conditions are introduced by replacing regular medium with medium containing a thickening agent. Parameters such as the percentage of filopodia positive cells, number of filopodia per cell, the length of the filopodia, the basal area of the cell, and the length of the cell were quantified before and after the viscosity change. We found the under viscous conditions all cell lines became 100% filopodia positive. All cells exhibited an increase in the average number of filopodia per cell. The greatest increase in the average number of filopodia occurred in 4T1 and MDA-MB231, which were 8.28 fold and 13.66 fold respectively. The average length of filopodia per cell also increased in all cells lines. EPH4 had the largest increase in average filopodia length, which was 2.78 fold. The average cell area increased in all cell lines except MDA-MB231 which decreased by 30%. The average aspect ratio increased significantly in EPH4, 67NR and MDA-MB231. Despite the changes in the basal area and the length of the cells, the volume of the cells remained constant. Furthermore, the cell motility of NIH-3T3 cells was compared between control and viscous conditions and no significant differences were observed. These results suggest a potentially distinct mechanism for cells navigating at high viscosity as under viscous conditions all cells exhibited significantly denser and longer filopodia. It is especially interesting that metastatic breast cancer cell lines, MDA-MB231 and 4T1, had the greatest increase in filopodia per cell, indicating that this may aid in the ability to navigate the tumor

microenvironment. Further investigation could lead to the mechanistic insight for how cancer cells adapt to the tumor microenvironment with higher success, as well as potentially novel treatment strategies for breast cancer.

E81

Myofibroblast Differentiation of Fetal Fibroblasts is Inhibited in Response to ECM Rigidity and TGF-b1.

R.J. Jerrell[1], M.J. Leih[1], A. Parekh[1,2,3,4];
[1]Otolaryngology, Vanderbilt University Medical Center, Nashville, TN, [2]Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, [3]Biomedical Engineering, Vanderbilt University, Nashville, TN, [4]Cancer Biology, Vanderbilt University, Nashville, TN

During dermal wound healing, extracellular matrix (ECM) rigidity and transforming growth factor-b1 (TGF-b1) induce fibroblasts to differentiate into myofibroblasts in the wound bed. Myofibroblasts generate large contractile forces using stress fibers rich in a-smooth muscle actin (a-SMA) that are transduced via focal adhesions to excessively contract and remodel the ECM leading to scarring and fibrosis. In contrast, injured fetal skin heals scarlessly without myofibroblast activity suggesting that fetal fibroblasts exert smaller contractile forces due to their unique wound environment. However, fetal wounds have less TGF-b1 and are more compliant than adult wounds. Therefore, it remains unclear whether the lack of myofibroblast differentiation is a result of inherent properties of fetal fibroblasts or biochemical and biomechanical differences in fetal wounds. In this study, we tested whether physiologic wound rigidities and TGF-b1 can mediate actomyosin contractility in fetal fibroblasts and promote myofibroblast differentiation. Using traction force microscopy and polyacrylamide gels (PAAs) that mimic the biomechanical stages of healing wounds, we found that traction forces and focal adhesion formation by fetal fibroblasts were impaired on rigid PAAs that mimic late stage granulation tissue when compared to their adult counterparts. On rigid PAAs with TGF-b1 stimulation, we found that fetal fibroblasts exhibited no differences in traction forces, focal adhesions, or a-SMA while all three increased for adult fibroblasts indicating myofibroblast differentiation. Overall, our data indicate that fetal fibroblasts exhibit a unique contractile phenotype that prevents myofibroblast differentiation due to altered mechanical responses to ECM rigidity and TGF-b1.

E82

Molecular force loading explains cell sensing of extracellular ligand density and distribution.

R. Oria[1,2], T. Wiegand[3,4], J. Escribano[5], A. Elosegui-Artola[1], J. Uriarte[2], C. Moreno-Pulido[1], I. Platzman[3], P. Delcanale[1], L. Albertazzi[1], D. Navajas[1,2], X. Trepat[1,2,6], J.M. García Aznar[5], .A. Cavalcanti-Adam[3,4], P. Roca-Cusachs[1,2];
[1]Institute for Bioengineering of Catalonia, Barcelona, Spain, [2]University of Barcelona, Barcelona, Spain, [3]Max-Planck-Institute for Medical Research, Heidelberg, Germany, [4]University of Heidelberg, Heidelberg, Germany, [5]Multiscale in Mechanical and Biological Engineering (M2BE), University of Zaragoza, Zaragoza, Spain, [6]Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain

A fundamental physical parameter driving cell function is ligand distribution in the extracellular matrix (ECM). Previous experiments using stiff substrates nanopatterned with adhesive ligands led to the hypothesis that cells directly measure ligand spacing[1-4] . In contrast, here we show that ligand spacing determines cell response by regulating molecular force loading. By using new substrates with tunable rigidity and integrin ligand nano-distribution, we report counterintuitive results showing that (i) increasing ligand spacing promotes -rather than inhibits- the growth of cell-ECM adhesions, and (ii)

increasing stiffness beyond a threshold leads to adhesion collapse -rather than growth. Furthermore, disordering ligand distribution drastically increases adhesion growth, but reduces the rigidity threshold for adhesion collapse. Measurements of cellular traction forces and actin flow speeds, combined with an expanded computational molecular clutch model, demonstrate that integrin ligand distribution drives adhesion growth and collapse, and YAP nuclear localization, by determining force loading of integrin-ECM bonds.

1 Arnold, M. et al. Activation of integrin function by nanopatterned adhesive interfaces. Chemphyschem : a European journal of chemical physics and physical chemistry 5, 383-388, doi:10.1002/cphc.200301014 (2004).
2 Cavalcanti-Adam, E. A. et al. Lateral spacing of integrin ligands influences cell spreading and focal adhesion assembly. European journal of cell biology 85, 219-224, doi:10.1016/j.ejcb.2005.09.011 (2006).
3 Altrock, E., Muth, C. A., Klein, G., Spatz, J. P. & Lee-Thedieck, C. The significance of integrin ligand nanopatterning on lipid raft clustering in hematopoietic stem cells. Biomaterials 33, 3107-3118, doi:10.1016/j.biomaterials.2012.01.002 (2012).
4 Amschler, K., Erpenbeck, L., Kruss, S. & Schon, M. P. Nanoscale integrin ligand patterns determine melanoma cell behavior. ACS nano 8, 9113-9125, doi:10.1021/nn502690b (2014).

E83

Laser-induced retraction of single actomyosin stress fibers reveals subtype-specific viscoelastic properties and contributions to tension generation.

S. Lee[1], S. Kumar[1,2,3];
[1]Graduate Program in Bioengineering, UC Berkeley-UCSF, Berkeley, CA, [2]Bioengineering, University of California, Berkeley, Berkeley, CA, [3]Chemical and Biomolecular Engineering, University of California, Berkeley, Berkeley, CA

Actomyosin stress fibers (SFs) are integral in defining cell shape, polarity, and migration by directing tension from the cytoskeleton to the extracellular matrix. Migrating cells display three SF subpopulations, dorsal SFs, transverse arcs, and ventral SFs, which differ in their location, connection to focal adhesions, and method of formation. Several models have been developed to explain how each of the subpopulations contribute to tensing and shaping the cell during migration. However, these models have not been rigorously tested by directly measuring the tension borne within single fibers in each of the subpopulations. Here, we infer the mechanics of SFs by examining their retraction kinetics upon incision with a femtosecond laser. We find that (1) dorsal SFs are not intrinsically tensed and bear less prestress than the other two subpopulations, (2) transverse arc retraction is dependent on the presence or absence of mechanically-coupled dorsal SFs, and (3) the degree of ventral SF retraction is partially dependent on SF length. In order to better understand the roles of entire subpopulations in generating tension in cells, we established cell lines in which either palladin or mDia2 were knocked down to deplete dorsal SFs or transverse arcs, respectively. We find that ventral SFs, at least some of which are formed from the fusion of dorsal and transverse arc precursor fibers, have altered retraction kinetics in these SF-depleted cells. The altered ventral SF retraction kinetics can be can be partially phenocopied in constrained cells that cannot form a canonical lamella and hence lack both transverse arc and dorsal SFs. Taken together, these results indicate that SF subpopulations each have distinct contributions to maintaining and/or generating tension, and confirm models that predict how dorsal SFs and transverse arcs are mechanically-coupled to flatten the lamella. Furthermore, dorsal SFs and transverse arcs may uniquely contribute to the viscoelastic properties of ventral SFs that subsequently form. These studies show that SF subpopulations form a dynamic and interdependent network to collectively shape and tense the cell.

E84

Actin-generated forces during mammalian endocytosis.

M. Akamatsu[1], D.G. Drubin[1];
[1]Molecular and Cellular Biology, University of California, Berkeley, Berkeley, CA

Cells use the polymerization of actin filaments to convert polymerization energy into mechanical work. In the case of endocytosis, actin polymerization generates force to bend and pull a small region of the plasma membrane into a round vesicle for internalizing transmembrane and extracellular cargo. Despite detailed knowledge of the biochemical function of individual actin cytoskeletal proteins, the mechanism by which they work collectively to bend and pull the plasma membrane in cells is not understood, particularly in metazoan cells. Recent advances in genome editing and fluorescence imaging now allow the design of quantitative and molecular mechanistic studies in live diploid mammalian cells.
We combined live-cell quantitative fluorescence microscopy in genome-edited human induced pluripotent stem (iPS) cells and mathematical modeling to investigate the mechanism by which branched actin networks generate force during endocytosis. Using GFP-tagged self-assembling protein nanocages of defined copy number (Hsia et al., 2016), we generated a calibration curve relating fluorescence intensity to molecule number in live cells. Using spinning-disk confocal microscopy and semi-automated image analysis software we counted the numbers of molecules over time of key endocytic proteins responsible for force generation and membrane scission at the endocytic site, including the Arp2/3 complex and dynamin2. We found that mammalian endocytic sites have fewer Arp2/3 complexes than in yeast, corresponding to less resistance from the plasma membrane. Active Arp2/3 complex is required for efficient endocytosis in mammalian cells.
We used these measurements to constrain a mathematical model of actin polymerization coupled to vesicle internalization using the filament-based modeling software Cytosim (Nédélec and Foethke, 2007). In the model, Arp2/3 complex on the plasma membrane generates branched actin filaments, and a nascent vesicle coated with actin-binding proteins elastically resists internalization into the cell. Actin filaments are stochastically capped and stall under load. Stochastic simulations of the model revealed that Arp2/3-nucleated actin networks self-organize into a dendritic cone focused against the attachment site (the nascent endocytic vesicle). Under a range of parameter values, the network produced ~5 pN of force and internalized the vesicle 100-200 nm in ~10 s. Thus, branched actin networks along the plasma membrane of mammalian cells are weak but sufficient to overcome physiological membrane tension and carry out endocytosis.
1) Hsia, Y. et al. (2016). Design of a hyperstable 60-subunit protein icosahedron. Nature *535,* 136–139.
2) Nédélec, F., and Foethke, D. (2007). Collective Langevin dynamics of flexible cytoskeletal fibers. New J. Phys. *9*, 427.

Tuesday, December 5

Louis-Jeantet Prize Lectures

A5

Circuits for Movement.

S. Arber[1,2];
[1]Biozentrum, University of Basel, Basel, Switzerland, [2]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Movement is the behavioral output of the nervous system. Animals carry out an enormous repertoire of distinct actions, spanning from seemingly simple repetitive tasks like walking to more complex movements such as forelimb manipulation tasks. This lecture will focus on recent work elucidating the organization and function of neuronal circuits at the core of regulating distinct motor behaviors. It will show that dedicated circuit modules within different brainstem nuclei and their interactions in the motor system play key roles in action diversification.

A6

Sensing infection and tissue damage.

C. Reis e Sousa[1];
[1]Immunobiology, The Francis Crick , London, United Kingdom

Innate and adaptive immunity work concertedly in vertebrates to restore homeostasis following pathogen invasion or other insults. Like all homeostatic circuits, immunity relies on an integrated system of sensors, transducers and effectors that can be analysed in cellular or molecular terms. At the cellular level, T and B lymphocytes act as an effector arm of immunity that is mobilised in response to signals transduced by innate immune cells that detect a given insult. These innate cells are spread around the body and include dendritic cells (DCs), the chief immune sensors of pathogen invasion and tumour growth. At the molecular level, DCs possess receptors that directly sense pathogen presence and tissue damage and that signal via transduction pathways to control antigen presentation or regulate a plethora of genes encoding effector proteins that regulate immunity. Notably, molecular circuits for pathogen detection are not confined to DCs or even to immune cells. All cells express sensors and transducers that monitor invasion by viruses and bacteria and elicit suitable effector barriers to pathogen propagation. I will discuss work from my laboratory that has contributed to our understanding of these issues over the years.

ASCB E.B. Wilson Medal Presentation and Address

A7

Protein Folding in the Cell: The Role of Molecular Chaperones.

U. Hartl[1];
[1]Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Martinsried, Germany

The past two decades have witnessed a paradigm shift in our understanding of protein folding. While the three-dimensional structures of functional proteins are determined by their amino acid sequences, as originally demonstrated by Anfinsen, we now know that in the crowded environment of cells many proteins depend on molecular chaperones to reach their folded states efficiently and at a biologically

relevant time scale. Assistance of protein folding is provided by different types of chaperone which cooperate to prevent misfolding and aggregation, often in an ATP-dependent mechanism. In the cytosol, nascent chain-binding chaperones, including Trigger factor and Hsp70, stabilize elongating polypeptide chains on ribosomes in a non-aggregated state. Folding is then achieved either on controlled chain release from these factors or following polypeptide transfer to downstream chaperones, such as the cylindrical chaperonins GroEL (Hsp60) and TRiC. The latter function by providing a cage-like nano-compartment for single protein molecules to fold in isolation, unimpaired by aggregation. The discovery of their fascinating mechanism was critical in developing the concept of chaperone-assisted protein folding.

Once folded, many proteins continue to require chaperones to retain their functional states, particularly under conditions of cell stress. Failure of the chaperone machinery to maintain the conformational integrity of the proteome may facilitate the manifestation of age-dependent diseases in which proteins misfold and are deposited as aggregates, such as Parkinson's and Huntington's disease. Motivated by the desire to find a cure for these debilitating diseases, researchers are now searching for drugs that can activate the chaperone system, thereby delaying the onset of aggregation diseases and prolonging the healthy human lifespan.

Hartl, F.U. (1996). Molecular chaperones in cellular protein folding. Nature 381, 571-580. Balchin, D., Hayer-Hartl, M., and Hartl, F.U. (2016). In vivo aspects of protein folding and quality control. Science 353(6294):aac4354.doi: 10.1126/science.aac4354.

A8

Chaperonin-mediated protein folding.

A.L. Horwich[1,2];
[1]Genetics, Yale University School of Medicine, New Haven, CT, [2]Howard Hughes Medical Institute, New Haven, CT

Chaperonins are remarkable molecular devices that mediate essential ATP-dependent kinetic assistance to de novo protein folding in the cell. This action was originally identified in collaborative work with Ming Cheng and Ulrich Hartl through the identification of a ts yeast mitochondrial import mutant in which proteins could be imported into mitochondria and cleaved to their mature forms, but then failed to reach native form. The double ring assembly, Hsp60, composed of two back-to-back seven member rings, was found to be the affected component. Hsp60 was an essential gene at all temperatures, indicating a fundamental role in de novo protein folding under all conditions. Sequence analysis made clear that related machines were present in the bacterial cytosol (GroEL), the chloroplast stroma (Rubisco binding protein), and the cytosol of archaebacteria (e.g. thermosome) and eukaryotic cells (TRiC/CCT).

Our subsequent work focused on the mechanism of action of the bacterial chaperonin, GroEL, and its cooperating cochaperonin "lid" structure GroES, involving a series of X-ray, EM, NMR, and biochemical studies in collaboration with Paul Sigler, Helen Saibil, and Kurt Wüthrich. These studies revealed that GroEL functions through two principal actions.

One action involves *binding* of non-native polypeptide in the open central cavity of a ring, via hydrophobic interactions between exposed surfaces in the substrate protein (buried to the interior in the native state) and a continuous surrounding hydrophobic surface formed by the terminal apical domains of the chaperonin. This prevents the substrate surfaces from forming multimolecular aggregates with other proteins. Multivalent binding by the surrounding apical domains can also exert an unfolding action that pulls apart a non-native protein to effectively place it at the "top of the energy landscape" to give it a fresh chance, upon release, to find its way to the native state. Indeed, bound polypeptides were found to exhibit no stable secondary or tertiary structure in NMR studies.

The second action is the step of *folding* to native form and involves the binding of ATP to the polypeptide-containing ring, mobilizing the apical domains, enabling GroES to collide with them and encapsulate the bound protein. This is followed by large rigid body movements of the apical domains that release polypeptide into what is now a hydrophilic chamber in which folding proceeds in solitary confinement, where aggregation cannot occur. Subsequent ATP hydrolysis in the folding-active ring leads to release of the substrate protein, whether native or not, from the chamber. Multiple rounds of binding and folding action by GroEL/GroES are required for efficient folding of many "stringent" substrates.

Symp 5: DNA/RNA Biology

S11

Folding, unfolding and refolding of genomes.

J. Dekker[1];
[1]Program in Systems Biology, HHMI; University of Massachusetts Medical School, Worcester, MA

In order to understand how the genome operates, we need to understand not only the linear encoding of information along chromosomes, but also its 3-dimensional organization. The 3D organization of the genome is critical for gene regulation, genome stability and faithful transmission of chromosomes to daughter cells. We invented Chromosome Conformation Capture-based technologies to determine how cells fold their chromosomes, to discover the processes that drive the spatial organization of genomes and to identify the mechanisms by which this organization contributes to genome regulation and activity. We discovered that at the nuclear level, chromosomes are compartmentalized into large multi-Mb domains that are either active and open or inactive and closed. These compartments themselves are composed of smaller sub-Mb Topologically Associating domains (TADs) (Dixon et al. 2012, Nora et al. 2012). Finally, long-range gene regulation occurs within TADs through long-range looping interactions between genes and regulatory elements. In mitotic cells we found a completely different chromosome organization: inside compact metaphase chromosomes the genome folds as longitudinally compressed randomly positioned loop arrays, consistent with classical models proposed by the Laemmli lab. These observations show that during the cell cycle genome folding is dramatically altered, and that the interphase and mitotic state we have described must be interconverted through elaborate folding and refolding pathways. Indeed, detailed timecourse experiments using highly synchronized cells allowed us to observe how the interphase state is converted into the mitotic state in real time through new folding intermediates. Further, we discovered key roles for several chromosome architectural proteins in this folding pathway. Combined our work is starting to reveal how the genome folds, unfolds and refolds to facilitate gene regulation and chromosome transmission.

S12

Cell autonomous and cell non-autonomous effects of aneuploidy.

A. Amon[1], S. Santaguida[1], L. Zasadil[1], B. Chris[1], M. Trakala[1];
[1]Department of Biology, MIT/HHMI, Cambridge, MA

Aneuploidy, a karyotype that is not a multiple of the haploid complement, is a hallmark of cancer. 90 percent of all solid human tumors harbor an incorrect karyotype. Thus, determining how aneuploidy arises and how it impacts cellular behavior is critical for our understanding of tumorigenesis. We developed yeast and mouse models to study the effects of aneuploidy on cell physiology. Our analyses revealed that the condition causes chromosome-specific phenotypes, and, remarkably, phenotypes

shared by many different aneuploid yeast and mouse cells, which we collectively call the aneuploidy-associated stresses. Among them, proteotoxic stress, caused by aneuploidy-induced proteomic imbalances, makes aneuploid cells especially vulnerable.
Despite the adverse effects of chromosome gains and losses on cell physiology, aneuploidy has been shown to drive tumorigenesis. We thus searched for properties of aneuploid cells that could promote tumorigenesis despite aneuploidy's anti-proliferative effects. We discovered that aneuploidy causes multiple forms of genome instability, potentially explaining why cancer cells are aneuploid. The condition could facilitate the evolution of genetic alterations that drive malignant growth.
Given that aneuploid cells are genomically unstable and hence potential to become cancerous, a key question is whether pathways exist that limit the prevalence of such cells in organisms. We found that cells with highly aneuploid karyotypes induce an inflammatory gene expression signature and are eliminated by natural killer (NK) cells in vitro. In vivo analyses confirmed that mechanism indeed exist that limit the prevalence of aneuploid cells in organisms. These findings suggest that cells with highly aberrant karyotypes are recognized and eliminated by the innate immune system. We propose that cells with abnormal karyotypes generate a signal for their own elimination that may serve as a means for cancer cell immunesurveillance.

S13

Division of Labor Among the Subunits of a Highly Coordinated Ring ATPase.

C. Bustamante[1];
[1]Physics, Molecular Cell Biology and Chemistry, University of California, Berkeley, Berkeley, CA

Many transport processes in the cell are performed by a diverse but structurally and functionally related family of proteins. These proteins, which belong to the ASCE (Additional Strand, Conserved E) superfamily of ATPases, often form mutimeric rings. Despite their importance, a number of fundamental questions remain as to the coordination of the various subunits in these rings. Bacteriophage phi29 packages its 6.6 mm long double-stranded DNA using a pentameric ring nano motor This portal motor is ideal to investigate these questions and is a remarkable machine that must overcome entropic, electrostatic, and DNA bending energies to package its genome to near-crystalline density inside the capsid. Using optical tweezers, we find that this motor can work against loads of up to ~55 picoNewtons on average, making it one of the strongest molecular motors ever reported. We establish the force-velocity relationship of the motor. Interestingly, the packaging rate decreases as the prohead fills, indicating that an internal pressure builds up due to DNA compression attaining the value of ~3 MegaPascals at the end of packaging. This pressure, we show, is used as part of the mechanism of DNA injection in the next infection cycle. We have used high-resolution optical tweezers to characterize the steps and intersubunit coordination of the pentameric ring ATPase responsible for DNA packaging in bacteriophage Phi29. By using non-hydrolyzable ATP analogs and stabilizers of the ADP bound to the motor, we establish where DNA binding, hydrolysis, and phosphate and ADP release occur relative to translocation. Surprisingly, there exists a division of labor among the subunits: while only 4 of the subunits translocate DNA, all 5 bind and hydrolyze ATP, suggesting that the fifth subunit fulfills a regulatory function. Finally, we show that the motor not only can generate force but also torque. We characterize the role played by the special subunit in this process and identify the symmetry-breaking mechanism in the motor. These results represent the most complete studies done to date on these widely distribute class of ring nano motors.

Minisymposium 14: Autophagy

M139

Interplay between ubiquitin and cargo receptors in selective autophagy.

S. Martens[1];
[1]Max F Perutz Laboratories, University of Vienna, Vienna, Austria

The removal of misfolded, ubiquitinated proteins is an essential part of the cellular quality control system and its malfunction results in a plethora of diseases including neurodegeneration. Misfolded proteins are primarily degraded by the ubiquitin-proteasome system (UPS), while autophagy functions as an essential backup system when the capacity of the UPS is exceeded. How the UPS and selective autophagy are coordinated is largely unclear. During the selective autophagy of ubiquitinated proteins these are clustered into larger structures in a p62 dependent manner. Here we define in a fully reconstituted system that p62 is sufficient for the specific clustering of ubiquitinated proteins. This activity depends on ubiquitin binding and oligomerization. We further define the preferred substrates of p62 and dissect the role of the ubiquitin chain linkage type on cluster formation. The clusters behave as solid aggregates stably sequestering the ubiquitinated substrates and represent filamentous p62 oligomers likely cross-linked by the ubiquitin-positives substrates. The p62 clusters recruit the ubiquitin-like LC3B protein and addition of LC3B slows down the clustering reaction suggesting a regulatory cross-talk between autophagosome formation and aggregation.

M140

The regulation of energy deprivaiton induced autophagy.

L. Yu[1], Y. Wang[1];
[1]School of Life Sciences, Tsinghua University, Beijing, China

Autophagy is essential for maintaining glucose homeostasis, but the mechanism by which energy deprivation activates autophagy is not fully understood. Recently, we found energy deprivaiton induce autophagy through a mitochondrial respiraiton dependent pathway, enrgy deprivaiton cause translocation of Snf11, Mec1 and Atg1 to mitochondrion, and formation of a Snf1-Mec1-Atg1 module on mitochondria governs energy deprivation-induced autophagy by regulating mitochondrial respiration. In this talk, I will discuss our recent progress on how Snf1-Mec1-Atg1 module regulates mitochodrial respiraiton and autophagy.

M141

Autophagosomal closure is mediated by the ESCRT machinery.

K. Morita[1], I. Koyama-Honda[1], Y. Yamashita[2], T. Ueno[2], E. Morita[3], H. Mano[2], N. Mizushima[1];
[1]Dept. of Mol. Biol, Grad. Sch. of Med, Univ. of Tokyo, Tokyo, Japan, [2]Dept. of Cell. Signal., Grad. Sch. of Med, Univ. of Tokyo, Tokyo, Japan, [3]Dept. of Biochem. and Mol. Biol., Fac. of Agric. and Life Sci., Hirosaki Univ., Hirosaki, Japan

Autophagy is an intracellular degradation process that delivers cytoplasmic contents to the lysosome. During the autophagic process, a single-membraned sac termed the isolation membrane elongates, bend, and engulfs a part of the cytoplasm. Finally, the edge of the isolation membrane is closed to form a double-membraned structure, the autophagosome. The autophagosome fuses with lysosomes to degrade its contents. Although many molecules such as ATG proteins are involved in this process, the molecular mechanism of the closure of the autophagosomal edge remains to be elucidated.

In this study, in order to identify novel autophagy-related factors, a genome-wide screen was performed using the CRISPR-Cas9 system and the novel autophagic flux probe GFP-LC3-RFP. As a result, in addition to many conventional ATGs, VPS37A was identified as one of the positive regulators of autophagy. VPS37A is a member of the ESCRT (endosomal sorting complex required for transport) machinery, which is involved in membrane scission processes such as the formation of multivesicular bodies, viral budding, and cytokinesis. Because the autophagosomal closure is also a scission process, we hypothesized that it is mediated by the ESCRT machinery. Our protease protection assays suggested that autophagosomes are not closed when VPS37A as well as other ESCRT proteins were depleted. The endocytosis and lysosomal function may be partially affected but almost normal in VPS37A knockout cells. Furthermore, live-cell imaging showed that ESCRT proteins were recruited to the edge of autophagosomes immediately before spherical change, which is likely the timing of the closure of the autophagosome. In conclusion, we have identified VPS37A as a novel autophagy-related factor by a non-biased forward genetic screen. Our further analysis suggests that the autophagosomal edge is closed by the ESCRT-mediated membrane fission.

M142

The Autophagy Conjugation Machinery Specifies The Loading of RNA-Binding Proteins Into Extracellular Microvesicles.

A.M. Leidal[1], H.H. Huang[2], T. Solvik[1], J. Ye[1], T. Marsh[1], F. Kai[3], J. Goldsmith[1], J.Y. Liu[1], A.P. Wiita[2], J. Debnath[1];
[1]Pathology, University of California, San Francisco, San Francisco, CA, [2]Laboratory Medicine, University of California, San Francisco, San Francisco, CA, [3]Surgery, University of California, San Francisco, San Francisco, CA

Autophagy is an auto-degradative pathway in which cytoplasmic material is sequestered into double-membrane vesicles and delivered to the lysosome for breakdown. Predominantly viewed as a catabolic mechanism, we are beginning to appreciate that the autophagy pathway also promotes unconventional secretion of proteins lacking N-terminal signal sequences. However, the underlying mechanisms and full repertoire of proteins released via autophagy-dependent secretion remains unknown. To further address the role of autophagy in secretion, we developed a novel strategy using proximity-specific biotinylation to label proteins that engage the autophagy regulator MAP1LC3B (LC3), and subsequently, are secreted outside of the cell. Quantitative proteomic analysis of the LC3-labelled secretome revealed a highly interconnected network enriched in RNA-binding proteins (RBPs) and the cargoes of extracellular microvesicles (EMVs, exosomes), indicating that specific proteins are loaded into EMVs via secretory autophagy. Focusing on a number of RBP candidates for further mechanistic study, including heterogeneous nuclear ribonucleoprotein K (HNRNPK) and scaffold-attachment factor B (SAFB), we demonstrate these proteins biochemically interact with LC3 and are secreted within EMVs highly enriched with lipidated LC3 (LC3-II). Secretion of LC3-II and LC3-binding RBPs requires essential components of the autophagy conjugation machinery and neutral sphingomyelinase 2 (nSMase2)-mediated ceramide production. Collectively, our data demonstrates a new role for the autophagy pathway in specifying RBPs that are packaged into EMVs and highlights a novel mechanism by which autophagy controls the secretion of RBPs and RNAs outside of the cell.

M143

Yeast *FIT2* homologs mediate the crosstalk between lipid droplet biogenesis, the unfolded protein response and cytoplasmic proteostasis.

P.T. Shyu[1], W. Gien[1], G. Thibault[1,2];
[1]School of Biological Sciences, Nanyang Technological University, Singapore, Singapore, [2]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore

Lipid droplets (LDs) have long been regarded as inert cytoplasmic organelles with the primary function of housing excess intracellular lipids. More recently, LDs have been strongly implicated in conditions of lipid and protein dysregulation. Moreover, these conditions are major contributors to the pathophysiology of metabolic diseases and concomitantly activate cellular stress response pathways, namely the unfolded protein response (UPR) and heat shock response (HSR). However, despite the increasing support for the involvement of LDs in other cellular processes, mechanistic insight into the fundamental process of LD biogenesis and its direct physiological relevance to the cell remains rudimentary. The fat storage inducing transmembrane (FIT) family of proteins comprises of evolutionarily conserved endoplasmic reticulum (ER)-resident proteins that have been reported to induce LD formation. Using *Saccharomyces cerevisiae* as a model, this study aims to dissect the role of LDs in cellular lipid and protein homeostasis through the yeast *FIT* homologs (*ScFITs*), *SCS3* and *YFT2* . While LD biogenesis and basal UPR activation remain unaltered in *ScFIT* mutants, *SCS3* was found to be essential in the absence of the sole yeast UPR sensor *IRE1* . Devoid of a functional UPR, Δ*scs3* mutants exhibited increased microsomal triacylglycerol levels, indicative of impaired ER-LD lipid partitioning and suggesting a UPR-dependent compensatory mechanism for LD biogenesis. Surprisingly, the absence of ScFIT results in the downregulation of the closely-related HSR pathway. In line with this observation, the turnover of cytoplasmic misfolded proteins is impaired in Δ*ScFIT* cells, while a screen for interacting partners of Scs3 identifies components of the proteostatic machinery as putative targets. Taken together, these suggest that ScFITs may modulate proteostasis and stress response pathways with LD biogenesis at the interface between the two cellular processes.

M144

Compartment-specific regulation of neuronal autophagy during homeostasis and stress.

A. Dong[1], A. Kulkarni[1], V. Kulkarni[1], J. Chen[1], S. Maday[1];
[1]Neuroscience, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

Autophagy is an essential degradative pathway that maintains neuronal homeostasis and prevents axon degeneration, but neuron-specific mechanisms are poorly understood. Here, we use live-cell imaging in mouse hippocampal neurons to establish the compartment-specific mechanisms of autophagy under basal conditions, and during stress induced by nutrient deprivation. We find that at steady state, axonal autophagy is a vectorial process that delivers cargo from the distal axon to the soma. The soma, however, contains multiple populations of autophagosomes at different maturation states, including input received from the axon combined with locally generated autophagosomes. Once in the soma, autophagosomes are confined within the somatodendritic domain, which likely facilitates cargo degradation by promoting fusion with proteolytically-active lysosomes that are enriched in this region. Surprisingly, canonical autophagy inducers such as starvation or mTOR-inhibition that robustly activate autophagy in other cell types (e.g. hepatocytes and HeLa cells), do not markedly upregulate autophagy in neurons, in either the axonal or somatodendritic compartments. Interestingly, we find that autophagy in glial cells is regulated differently as compared with neurons, suggesting alternative mechanisms of coordinating autophagy in the brain. Together, these observations suggest that the

primary physiological function of autophagy in neurons, unlike in other cell types, may not be to mobilize amino acids and other biosynthetic building blocks in response to nutrient deprivation. Rather, constitutive autophagy in neurons may function to maintain cellular homeostasis and regulate the quality of the neuronal proteome by balancing synthesis and degradation, especially within distal axonal processes far removed from the soma.

M145

The RNA binding protein Zfp106 protects against neurotoxicity caused by *C9orf72* GGGGCC repeats.

B. Celona[1], J. Von Dollen[2], S.C. Vatsavayai [3], R. Kashima[1], J. Johnson[2], A.A. Tang[4], A. Hata[1], B.L. Miller[3], E.J. Huang[4], N. Krogan[2], W.W. Seeley[3,4], B.L. Black[1,5];
[1]Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA, [2]Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, CA, [3]Neurology, University of California, San Francisco, San Francisco, CA, [4]Pathology, University of California, San Francisco, San Francisco, CA, [5]Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA

Expanded GGGGCC repeats in the first intron of the *C9orf72* gene represent the most common cause of familial ALS, but the mechanisms underlying repeat-induced disease remain incompletely resolved. One proposed gain-of-function mechanism is that repeat-containing RNA forms aggregates that sequester RNA binding proteins, leading to altered RNA metabolism in motor neurons.
To identify new proteins associated with GGGGCC repeats, we performed RNA pulldown assays, followed by mass spectrometry and identified Zfp106 as a specific GGGGCC RNA repeat-binding protein. We found that Zfp106 binds directly to GGGGCC RNA repeats, and functionally interacts with the repeats in cultured neuronal cells. To gain additional insight into the molecular function of Zfp106, we used mass spectrometry to identify protein interactors of Zfp106. Remarkably, we found that Zfp106 interacts with multiple other RNA binding proteins, including the ALS-associated factors TDP-43 and FUS. Zfp106 is highly expressed in skeletal muscle and motor neurons, and its human ortholog *ZNF106* is located at chromosome 15q15.1, a locus associated with a familial recessive form of ALS. Therefore, we genetically inactivated the *Zfp106* gene in mice. *Zfp106* knockout mice develop severe motor neuron degeneration, which can be suppressed by transgenic restoration of Zfp106 specifically in motor neurons.
Finally, we used *Drosophila* as an *in vivo* gain-of-function model of *C9orf72* neurodegeneration to test if the interaction between Zfp106 and GGGGCC repeats had a functional consequence on neurotoxicity. Expression of Zfp106 in glutamatergic neurons suppressed the loss of larval active zones at NMJs and pupal lethality caused by expression of 30 copies of GGGGCC. Moreover, Zfp106 co-expression partially suppressed the adult locomotor defect caused by expression of *C9orf72* repeats. Therefore, Zfp106 is a potent suppressor of GGGGCC repeat-mediated neurotoxicity in a *Drosophila* model of *C9orf72* ALS.

M146

Ribosome profiling reveals that autophagy impacts DNA damage repair, cell cycle progression and centrosome maintenance through protein translation regulation.

J. Goldsmith[1], S. Asthana[2], T. Marsh[1], D. Suresh[1], A. Olshen[2], J. Debnath[1];
[1]Pathology, University of California, San Franscisco, San Francisco, CA, [2]Department of Epidemiology and Biostatistics, University of California, San Franscisco, San Francisco, CA

Autophagy is a cellular recycling system that degrades large proteins and organelles by delivery to the lysosome. Protein translation is tightly tuned to the metabolic state of the cell by sensing lysosomal amino acid levels and signaling through mTORC1. During metabolic stress, protein translation is abrogated while autophagy is concurrently induced. Accordingly, autophagy is proposed as a principal regulator of the protein translational landscape, especially during starvation, yet to date evidence supporting this hypothesis is lacking. Here, we utilize ribosome profiling to dissect how the autophagy pathway impacts translation, both at baseline and in response to starvation.
Although the prevailing viewpoint is that the recycling functions associated with autophagy sustain de novo protein translation, we discovered that in both nutrient replete conditions and in nutrient starvation, global translation rates as well as intracellular levels of most amino acids were intact in autophagy deficient cells. Instead, we observed that specific proteins were translated in an autophagy dependent manner. To identify these products of autophagy-dependent translation, we employed ribosome profiling in autophagy competent vs. deficient fibroblasts, achieved via genetic deletion of the key autophagy regulator ATG12, in both fed and starved conditions. Using biochemical approaches, we validated that autophagy controls the translation of multiple targets. Most strikingly, we found that basal autophagy is important for the translation of a group of targets involved in centrosome clustering, DNA repair and cell cycle control, including BRCA2, both in vitro and in vivo. While increased DNA damage in autophagy deficient cells has been largely attributed to an increase in defective mitochondria and production of reactive oxygen species, our results reveal translational control as another critical pathway through which autophagy regulates DNA damage levels. We are working to identify how autophagy controls this program of protein translation, hypothesizing that it acts through targeted degradation or sequestration of key RNA binding proteins.
The increase in baseline DNA damage and decreased BRCA2 levels means that the autophagy impaired cells have increased sensitivity to various cytotoxic reagents, including PARP inhibitors. These data broach the hypothesis that tumors with high rates of chromosome instability may rely more heavily on autophagy-dependent translation of proteins. Overall, these results expand the functions of autophagy beyond its traditional role as an intracellular degradation pathway, and reveal new roles for autophagy an anabolic pathway that instructs that translation of specific proteins in cell cycle control.

M147

Post-transcriptional regulation of autophagy.

E. Delorme-Axford[1], D.J. Klionsky[1];
[1]Life Sciences Institute, University of Michigan, Ann Arbor, MI

The goal of this study is to identify and characterize novel post-transcriptional regulators of autophagy in the yeast *Saccharomyces cerevisiae*. Autophagy is a dynamic, highly conserved mechanism of cellular self-eating that is essential for survival during stress conditions. Basal cellular autophagy is low but is markedly upregulated by starvation, various other forms of stress and pathogen infection. Nonselective autophagy targets bulk cytoplasm; whereas selective forms of autophagy target specific cargo (such as damaged or superfluous organelles) by receptor-mediated interactions for uptake into the phagophore,

the autophagosome precursor. The hallmark morphological feature of autophagy is the double-membrane vesicle known as the autophagosome. A late step in autophagy is the fusion of the autophagosome with the vacuole (in yeast) or the lysosome (in mammalian cells). Fusion between the autophagosome and the degradative compartment results in breakdown of the autophagic cargo, followed by efflux of the resulting macromolecules. Notably, aberrant autophagy is associated with diverse human pathologies such as cancer, neurodegenerative diseases and lysosomal storage disorders. Therefore, the cell must strictly modulate autophagy activity at multiple levels (transcriptional, post-transcriptional, translational, and post-translational) to prevent deregulation of this critical process. However, little is known about the post-transcriptional regulation of autophagy. One mechanism whereby cells exert post-transcriptional control over gene expression is through RNA decay pathways. Here, we utilize *Saccharomyces cerevisiae* to identify and characterize a novel post-transcriptional regulator of autophagy through a targeted genetic and biochemical screen. Our studies have demonstrated through multiple assays that the exoribonuclease Xrn1 functions as a negative regulator of autophagy. By transmission electron microscopy (TEM) analysis, we have found that Xrn1 controls the frequency of autophagosome formation during starvation. Loss of Xrn1 results in the upregulation of select autophagy-related (*ATG*) transcripts under nutrient-replete conditions, and this effect is dependent on the ribonuclease activity of Xrn1 as demonstrated by real-time quantitative PCR (RT-qPCR). The yeast transcription factor Ash1 regulates Xrn1 expression (at least in part) under fed conditions. Xrn1 expression decreases during starvation, further supporting its role as a negative autophagy regulator. This work provides insight into how cells regulate autophagy post-transcriptionally through the mRNA decay factor Xrn1. Our findings support a model in which cells produce an excess of *ATG* transcripts during fed conditions, which function to “jumpstart” autophagy once the cell is starved.

M148

Microvillar sensation of shear stress induces autophagic flux in the intestinal epithelium.

S. Kim[1,2], J. Ehrman[3], M. Ahn[4], J. Kondo[5], S.W. Crawley[6], Y. Oh[7], A. Lopez[8], J.R. Goldenring[9,10], M.J. Tyska[1,2], E. Rericha[3], K. Lau[1,2];
[1]Department of Cell and Develomental Biology, Vanderbilt University, Nashville, TN, [2]Epithelial Biology Center, Vanderbilt University, Nashville, TN, [3]Department of Physics and Astronomy, Vanderbilt University, Nashville, TN, [4]Department of Food Science and Nutrition, Dong-A University, Busan, Korea, South, [5]Department of Biochemistry, Osaka International Cancer Institute, Osaka, Japan, [6]Department of Biological Sciences, University of Toledo, Toledo, OH, [7]Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN, [8]Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN, [9]Department of Surgery, Vanderbilt University, Nashville, TN, [10]Nashville VA Medical Center, Nashville, TN

The intestinal epithelial cells lining the lumen of the neonatal gut are exposed to the flow of fluid from a liquid diet. Mechanosensors, such as the primary cilia of the kidney epithelium or stereocilia of the inner hair cell of the ear, have been identified to respond to shear forces. However, whether intestinal epithelial cells can respond to fluid shear stress is unknown.
To study the effect of shear on intestinal epithelial cells, we applied fluid shear stress on the intestinal epithelial cell line, Caco-2 BBE, through the use of a microfluidics design. Interestingly, we observed that exposure to fluid shear stress causes the formation of large vacuoles within intestinal epithelial cells when they are organized as monolayers. These observations raised two questions: first, what structural elements allow intestinal epithelial cells to transduce shear stress, and second, what mechanisms support the formation of large vacuoles that can exceed 80% of cell volume. As a result, we found that structurally, intestinal epithelial microvilli play a role in sensing extracellular shear stress, since shear-induced vacuole formation was significantly diminished when the intestinal microvilli were

experimentally reduced. In addition, we identified that autophagic flux, and not endocytosis, to be the major pathway connecting mechanical sensation to vacuole formation. Loss-of-function studies by pharmaceutical and genetic inhibition of autophagy pathway components support our observations that shear stress-induced vacuole formation is mediated by the autophagic machinery.
In conclusion, our results revealed a novel link between the intestinal microvilli, the macroscopic transport of fluids across cells, and the autophagy pathway in organized epithelial monolayers. Our study contributes to the understanding of how physical stress affects cellular response in both intestinal physiological and pathological contexts.

Minisymposium 15: Axonal and Synaptic Cell Biology

M149

The cell biology of astrocyte-synapse interactions.

C. Eroglu[1];
[1]Cell Biology, Duke University Medical Center, Durham, NC

Astrocytes are highly morphologically complex glial cells of the brain that interact with neurons, vasculature and other glia. Through these cellular associations, astrocytes are positioned to control a number of critical brain functions including establishment of the blood-brain-barrier, synapse formation and function, and provide trophic support to neurons. Important association partners of astrocytes are synapses. Fine astrocyte processes ensheathe pre and postsynaptic structures in order to provide a suitable environment for synaptic transmission. A single astrocyte can encompass and ensheathe more than 100,000 synapses in the mouse brain cortex due to its extensive branching complexity. However, we know very little of the molecular mechanisms that control astrocyte morphological complexity and astrocyte-synapse interactions. Here we will discuss our recent findings showing that astrocyte morphogenesis is regulated by direct neuronal contact and is altered by experience-dependent changes in synaptic circuits. With this basis, we reasoned and screened for astrocytic cell adhesion molecules (CAMs) that regulate both astrocyte architectural complexity and synaptic association. This screen identified a family of CAMs that control different developmental stages of astrocyte morphogenesis and facilitate the formation of synapses. This work reveals a novel mechanism whereby astrocytes communicate with synaptic circuitry through a cell adhesion complex that controls both astrocyte morphogenesis and synaptic connectivity. Disruptions in this contact-dependent astrocyte-synapse interaction may underlie some of the pathologies seen in neurodevelopmental disorders.

M150

Multicluster Pcdh diversity is required for mouse olfactory neural circuit assembly.

G. Mountoufaris[1], W. Chen[1], Y. Hirabayashi[1], S. O'Keeffe[1], M. Chevee[1], C. Nwakeze[1], F. Polleux[1], T. Maniatis[1];
[1]Biochemistry, Columbia University, NEW YORK, NY

The vertebrate clustered protocadherin (Pcdh) cell surface proteins are encoded by three closely linked gene clusters (Pcdhα, Pcdhβ, and Pcdhγ). Here, we show that all three gene clusters functionally cooperate to provide individual mouse olfactory sensory neurons (OSNs) with the cell surface diversity required for their assembly into distinct glomeruli in the olfactory bulb. Although deletion of individual Pcdh clusters had subtle phenotypic consequences, the loss of all three clusters (tricluster deletion) led to a severe axonal arborization defect and loss of self-avoidance. By contrast, when endogenous Pcdh diversity is overridden by the expression of a single–tricluster gene repertoire (α and β and γ), OSN

axons fail to converge to form glomeruli, likely owing to contact-mediated repulsion between axons expressing identical combinations of Pcdh isoforms.

M151

Super-resolution imaging of perineuronal net and synaptic maturation of parvalbumin interneurons during postnatal development in visual cortex.

Y. Sigal[1,2], H. Bae[3], L.J. Bogart[3], T. Hensch[3,4], X. Zhuang[1,2,5];
[1]Howard Hughes Medical Institute, Cambridge, MA, [2]Chemistry and Chemical Biology, Harvard University, Cambridge, MA, [3]Molecular and Cellular Biology, Harvard University, Cambridge, MA, [4]Neurology, Boston Children's Hospital, Harvard Medical School, Boston, MA, [5]Physics, Harvard University, Cambridge, MA

Critical periods are transient windows of development when particular brain regions become malleable in response to environmental stimuli. These plastic windows are triggered by the maturation of fast-spiking inhibitory interneurons expressing the calcium-binding protein, parvalbumin (PV). As synaptic input onto PV+ cell somata and proximal neurites develop, they become enwrapped in a specialized form of the extracellular matrix enriched in chondroitin sulfate proteoglycans termed the perineuronal net (PNN). Weak or disrupted PNNs have been shown to extend critical period plasticity. The challenge of imaging the nano-scale organization of synaptic structures along with the complexity of mammalian brain tissue has hindered the understanding of this important biological structure. Here, we developed a fast serial-section superresolution resolution microscopy method to quantify the development of PV+ cells during and after the visual critical period (postnatal days 30 and 90, respectively). Reconstructed PNN-ensheathed somata and proximal neurites revealed a range of net structures and a stereotypic maturational profile of the PNN across ages. We then continued to examine the nanoscale re-organization of PNNs and synaptic molecules that accompany typical brain development following environmental or genetic perturbations. We found that visual sensory experience was not required for many aspects of this progression as animals raised in the dark showed only a slower progression of synaptic and PNN development without gross morphological differences from light-reared controls. In contrast, the Mecp2-deficient mouse model of Rett syndrome displayed a hyper-mature PNN profile at P30. Moreover, we discovered a striking defect in reciprocal connectivity between PV+ neurons at this stage prior to the regressive loss of visual acuity in these animals: Mecp2-deficient PV+ cells made smaller synaptic contacts onto each other accompanied by a redistribution of the calcium-binding protein Synaptotagmin-2. These findings, derived from cutting-edge volumetric super-resolution imaging, provided new insight into an important developmental process of the brain at the cellular and molecular level, identified early mis-wiring in a mouse model of an autism-like disorder, and demonstrated the power of this method to visualize and understand molecular organization within a complex tissue environment.

M152

A pro-synaptogenic function of the Frizzled receptor, working in conjunction with Neurexin, is inhibited by WNT binding.

P. Kurshan[1], K. Shen[1];
[1]Biology, Stanford University, Stanford, CA

The molecular mechanisms of synapse assembly, while long a focus of study, are still largely opaque. Both pro- and anti-synaptogenic roles for WNT signaling have been reported, leaving the precise function of this pathway unclear. The synaptic cell adhesion molecule Neurexin has well-established

roles in presynaptic development and function and has been implicated in diseases such as autism and schizophrenia. However, precisely how this protein organizes presynaptic specializations is still unknown. Here we report that C. elegans Frizzled (LIN-17) promotes presynaptic assembly in vivo in concert with Neurexin. In the DA9 motor neuron, whose posterior boundary is defined by WNT-mediated synapse elimination, nrx-1;lin-17 double mutants exhibit an almost complete loss of presynaptic specializations, suggesting that these two parallel pathways are the main drivers of presynaptic assembly in these neurons. This effect is not mediated through either protein's canonical binding partner, as neither Neuroligin nor WNT mutants share this phenotype. The effect can be rescued cell-autonomously by motor neuron expression of either Frizzled or Neurexin, including by a recently discovered conserved short isoform of Neurexin lacking all the extracellular LNS/EGF domains, gamma-Neurexin. Our data suggest that WNT may function to eliminate synapses via endocytosis of both Frizzled and Neurexin, which in the absence of WNT act in parallel to drive presynaptic assembly. Together these data suggest novel interactions and roles remain to be discovered even for very well studied proteins.

M153

Cell biology of functional axon regeneration.

C. Ding[1,2], M. Hammarlund[1,2];
[1]Genetics, Yale School of Medicine, New Haven, CT, [2]Neuroscience, Yale School of Medicine, New Haven, CT

Functional axon regeneration requires a regenerating neuron to form new synapses onto relevant targets, thus rebuilding an effective circuit. To model this process *in vivo*, we developed an assay in *C. elegans* that links regeneration of a single, specific axon (DA9) with regained activation of its post-synaptic targets and recovery of relevant behavior. By imaging DA9 axon morphology and synaptic components during functional axon regeneration, we can analyze the process of rebuilding circuits in molecular detail. DA9 normally has synapses only in a specific region of its axon in the dorsal nerve cord. Surprisingly, we find that axon injury initially results in the formation of transient synaptic vesicle clusters in the DA9 dendrite. These clusters form independently of the injury-sensing DLK pathway. DA9 microtubule polarity is not altered by axotomy, suggesting that injury may trigger minus end-directed transport of vesicle clusters. Optogenetic stimulation of DA9 during this early phase of regeneration results in dendritic vesicle release, activation of ventral cells, and aberrant behavioral response. Later, as the DA9 axon regenerates into its former dorsal synaptic area, new synapses are formed in the correct dorsal region. This remodeling enables activation of dorsal cells when DA9 is stimulated, restoring largely normal circuit function and behavior. Together, these data reveal a surprising amount of circuit plasticity during functional axon regeneration, and establish an *in vivo* system to investigate its mechanisms.

M154

Distinct effects of tubulin isotype mutations on microtubule stability and neurite growth in *Caenorhabditis elegans*.

C. Zheng[1], M. Diaz-Cuadros[1], S.L. Jao[1], E.G. Atlas[1], K.C. Nguyen[2], D.H. Hall [2], M. Chalfie[1];
[1]Biological Science, Columbia University, New York, NY, [2]Neuroscience, Albert Einstein College of Medicine, Bronx, NY

Tubulins, the building block of microtubules (MTs), play a critical role in both supporting and regulating neurite growth. Eukaryotic genomes contain multiple alpha and beta tubulin isotypes, and their

dominant missense mutations cause a range of neurodevelopmental defects in humans. Thus, identifying the functions of each tubulin isotype and evaluating how particular mutations would change those functions during neuronal morphogenesis is important.
Using the *C. elegans* touch receptor neurons, we analyzed the effects of 67 missense mutations in tubulin genes, *mec-7*/beta-tubulin and *mec-12*/alpha-tubulin, on neurite growth. Three types of mutations emerged: 1) loss-of-function (*lf*) mutations, which are found throughout the molecule and caused mild defects in neurite growth; 2) antimorphic (*anti*) mutations, which map to the GTP binding site and intradimer and interdimer interfaces and significantly reduced MT stability, causing severe neurite growth defects; 3) neomorphic (*neo*) mutations, which map to the exterior surface and increased MT stability, causing ectopic neurite growth. Such structure-function analysis revealed a causal relationship between tubulin structure and MT stability, which in turn affects neuronal morphogenesis. Importantly, we engineered several disease-associated human tubulin mutations into *C. elegans* genes and found their phenotype on neurite growth also fell into the three categories; those results provided a proof-of-concept for using the TRNs as a platform to study the impact of human tubulin mutations on neuronal development at a cellular level.
Searching for the suppressors of the tubulin *neo* mutations, we found that an F-box protein MEC-15 and the ubiquitination pathway are needed not only for the normal neuronal morphogenesis but also for the excessive neurite outgrowth in the *neo* mutants. Thus, protein homeostasis appears to control the level of negative regulators of MT stability during neurite growth.
We also discovered a MT-destabilizing alpha-tubulin isotype TBA-7, whose loss led to the formation of hyperstable MTs and the generation of ectopic neurites; the lack of potential sites for polyamination and polyglutamination on TBA-7 may be responsible for this destabilization. These results support the "multi-tubulin" hypothesis that distinct tubulin isotypes have specific functions and posttranslational modification is a key for the functional difference.

M155

Axon initial segment assembly requires phosphorylation of 480 kDa ankyrin-G.

R. Yang[1], K. Walder[1], V. Bennett[1];
[1]HHMI/Department of Biochemistry, Duke University Medical Center, Durham, NC

Axon initial segments (AIS) are specialized plasma membrane domains in vertebrate neurons that are enriched in voltage-gated Na channels and integrate dendritic signals to generate action potentials. AIS structural plasticity occurs during postnatal development and in response to neuronal activity. We report here that 480 kDa ankyrin-G (ankG), which is the master organizer of the AIS, requires phosphorylation within its neuro-specific giant exon in order to recruit beta-4 spectrin as well as voltage-gated Na channels and the L1CAM, 186 kDa neurofascin. Moreover, we find that the AIS-specific sigma 6 isoform of beta-4 spectrin, which recruits CAMK2 to the AIS, also is differentially phosphorylated in brain tissue. Using mass spectrometry analysis of 480 kDa ank-G isolated from mouse brain, we identified multiple high stoichiometry phosphorylation sites (0.1 mol/mol and greater) that were located within its neuro-specific giant exon. We evaluated the function of these phosphorylation sites in AIS assembly by reconstituting ank-G null neurons lacking an AIS with 480 kDa ank-G that was either WT or bearing alanine mutations that prevent phosphorylation. One site located at S2619 is required for 480 kDa ank-G to target to the AIS and to recruit beta-4 spectrin as well as voltage-gated Na channels and 186 kDa neurofascin. S2619A 480 kDa ank-G also lost ability to bind to 186 kDa neurofascin. S2619 thus functions as a master switch regulating multiple functions of 480 kDa ank-G. Another site (S2406) is required to recruit beta-4 spectrin to the AIS, although 480 kDa ank-G itself targets normally. qv3j mutant mice have a beta-4 spectrin mutation resulting in truncation that eliminates a binding motif for CAMK2 and prevents recruitment of CAMK2 to the AIS (Hund et al. J Clin

Invest. 2010;120(10):3508–3519). We found that 480 kDa ank-G isolated from qv3j mouse brain had a marked reduction in phosphorylation at S2406. In addition, although 480 kDa ankG in the qv3J mice still targets to the AIS, the AIS extends much further into the distal axon. Together these findings suggest that 480 kDa ankG is locally activated at the AIS by phosphorylation, and that one of the protein kinases is a member of the CAMK2 family. Moreover, 480 kDa ankG, beta-4 spectrin, and CAMK2 engage in a positive feedback loop where 480 kDa ankG recruits beta-4 spectrin, beta-4 spectrin recruits CAMK2, and CAMK2 further activates 480 kDa ankG to recruit more beta-4 spectrin.

M156

Slow axonal transport of clathrin.

S. Roy[1], A. Ganguly[2], C. Leterrier[3];
[1]Pathology, University of Wisconsin-Madison, Madison, WI, [2]Pathology, UCSD, San Diego, CA, [3]Cell Biology, Aix Marseille University, Marseille, France

Clathrin is a critical protein in neurons with established roles in endocytosis at synapses. Previous pulse-chase radiolabeling studies have shown that the vast majority of clathrin is synthesized in the neuronal soma and conveyed into the axon via slow axonal transport. However, underlying mechanisms are unknown. We used endogenous labeling, live imaging, proteomics, super-resolution (STORM) and 3-D EM tomography to study the organization and dynamics of clathrin along axon-shafts. We found that clathrin and its accessory proteins are conveyed in axons as a unique structure that we call a clathrin transport carrier (CTC). Individual CTC's move rapidly but infrequently in axons, with an overall slow anterograde bias. This movement is independent of endocytosis, and is very different from the behavior of clathrin in dendrites, where typical on/off blinking is seen – consistent with clathrin-dependent endocytosis. EM tomography using Apex-labeled clathrin, as well as STORM show that CTCs are unique in organization; different from clathrin cages and other known clathrin structures in cells. Specifically, CTCs appear as small clathrin-coated organelles that about 50 to 60 nm in diameter, but with peculiar 5-10 nm radiating spokes on the surface. Axonal CTC's are not associated with the plasma membrane but appear to be in close apposition with microtubules. Motile axonal CTC's often stop and accumulate at presynaptic boutons, where integer quantities of these particles accumulate; suggesting a mechanism by which CTC's synthesized in the soma are targeted en-masse to synapses – where they are ultimately functional. Collectively, the data offer mechanistic insight into the slow axonal transport of clathrin, and identifies a unique clathrin transport organelle in neurons.

M157

Determining the molecular basis of ultrafast endocytosis.

Y. Imoto[1], Q. Gan[1], L. Mamer[2], S. Markert[3], I. Milosevic[4], P. De Camilli[5], C. Rosenmund[2], E. Jorgensen[6], S. Watanabe[1];
[1]Department of Cell Biology, John Hopkins University School of Medicine, Baltimore, MD, [2]Institute of Neurophysiology, Charité Universitätsmedizin Berlin, Berlin, Germany, [3]Division of Electron Microscopy, University of Würzburg, Würzburg, Germany, [4]European Neuroscience Institute, Goettingen, Germany, [5]Departments of Neuroscience and Cell Biology, Program in Cellular Neuroscience, Neurodegeneration and Repair, Kavli Institute for Neuroscience, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT, [6]Department of Biology, University of Utah, Salt Lake City, UT

Ultrafast endocytosis is a novel clathrin-independent endocytic pathway that recycles synaptic vesicles under physiological conditions. In mouse hippocampal neurons, vesicle membrane is retrieved within 100 ms after fusion via this endocytic pathway. However, molecular mechanism underlying ultrafast

endocytosis remains elusive. To reveal molecular mechanisms, we coupled molecular perturbation with flash-and-freeze approach that visualizes membrane dynamics in electron micrographs with millisecond temporal resolution. Our data have shown that actin polymerization is required for the initial membrane curvature. The transition from shallow to deep pits is mediated by synaptojanin, phosphatidylinositide phosphatase. This transition requires the conversion of plasma membrane PI4,5P2 to PI4P. The steep negative curvature at the base of the endocytic pit is then stabilized and further constricted by endophilin. Finally, Dynamin-1 severs the vesicle from the plasma membrane. Furthermore, pharmacological inhibition of lipid binding of Dynamin-1 does not abolish ultrafast endocytosis, suggesting that the PH domain may be dispensable for ultrafast endocytosis. Thus, despite its speed, ultrafast endocytosis is mediated through coordinated actions of multiple proteins.

M158

Synaptic vesicle clusters in nerve terminals: an example of liquid-liquid phase separation.

D. Milovanovic[1], P. De Camilli[1];
[1]Departments of Neuroscience and Cell Biology , Yale University School of Medicine, New Haven, CT

Neurotransmitter containing synaptic vesicles (SVs) form tight clusters at chemical synapses. These clusters act as a reservoir from which SVs are drawn for exocytosis during strong and prolonged activity. Several components associated with synaptic vesicles and likely form such clusters, including synapsin 1, have been identified. However, it remains unclear how SV clustering is compatible with their motility, so that release sites can be rapidly replenished after vesicle fusion events. Recently, liquid-liquid phase separation was shown to be a mechanism through which components of the cytoplasm (protein and RNA) can assemble themselves into distinct compartments without a limiting membrane. A key feature of proteins that can undergo liquid-liquid phase separation is their ability for engaging in multivalent interactions through protein-protein interacting domains and/or low complexity amino acid regions. We are exploring the possibility that SV clusters may represent an example of liquid-liquid phase separation in which one component of the phase are SVs and the other component is represented by proteins of the intervening matrix, synapsin 1 in particular. Synapsin 1 is highly abundant proteins at the nerve terminals, it binds membranes, and it contains an extended low complexity region: all of these properties make synapsin 1 an ideal candidate for mediating the phase separation.

Minisymposium 16: Mechanical Coupling from Nucleus to Extracellular Matrix

M160

YAP-independent mechanotransduction drives breast cancer invasion.

J.Y. Lee[1], J. Chang[2], S. Nam[1], H. Lee[1], A. Dominguez[3,4,5], S. Varma[6], L.S. Qi[3,4,5], R. West[6], O. Chaudhuri[1];
[1]Mechanical Engineering, Stanford University, Stanford, CA, [2]Genetics, Stanford University, Stanford, CA, [3]Bioengineering, Stanford University, Stanford, CA, [4]Chemical and Systems Biology, Stanford University, Stanford, CA, [5]ChEM-H, Stanford University, Stanford, CA, [6]Pathology, Stanford University, Stanford, CA

Ductal carcinoma in situ (DCIS) progresses to invasive ductal carcinoma (IDC) when carcinoma cells break through the laminin-rich basement membrane (BM) into the type-1 collagen (col-1) rich stroma, a key first step towards metastasis. The drivers of progression remain unclear, with no established biomarkers. However, increased tissue stiffness correlates with invasion and the transcriptional regulator YAP has been implicated as a mechanotransducer, largely based on 2D culture studies. To identify drivers of DCIS progression, we encapsulated mammary epithelial cells (MECs) in 3D hydrogels with a range of stiffnesses and that presented either BM-ligands or col-1 containing microenvironments

to the cells. Surprisingly, enhanced stiffness induced invasion in MECs independently of YAP activation in both BM-like and col-1 rich 3D hydrogels. To provide mechanistic insight into the unexpected lack of YAP activity during MEC mechanotransduction in 3D culture, we examined YAP activation in 2D culture as a point of comparison. Interestingly, confocal microscopy and mass spectrometry showed that YAP colocalized and immunoprecipitated with actin, respectively, and identified distinct YAP binding partners depending on cell context. As actin network architecture is dramatically altered in 3D culture, this may explain differences in YAP activation in 2D versus 3D culture. Further, RNA-seq identified the global gene expression changes induced by increased 3D stiffness, and bioinformatic analysis combined with inhibition studies identified transcriptional regulators p300 and STAT3 as mediators of stiffness-induced invasion. RNA-seq also showed that col-1 exposure in stiff gels promotes alterations in carcinoma phenotype, inducing tumor cell expression of genes whose protein products remodel the col-1 network including FN1 and LOX. 3SEQ analysis of breast cancer patient samples revealed that genes identified by 3D culturing in hydrogels were upregulated in breast cancer patients, suggesting expression of S100A7 as a potential biomarker of cancer progression. While genetic mutations initiate transformation, these results reveal the molecular events underlying breast cancer invasion initiated by physical properties of the microenvironment, as well as alteration of the carcinoma phenotype following invasion through the BM into the col-1 stroma.

M161

Force-activated vinculin dynamics regulate sub-cellular processes critical in directed cell migration.

K.E. Rothenberg[1], D.W. Scott[2], B.D. Hoffman[1];
[1]Biomedical Engineering, Duke University, Durham, NC, [2]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, Chapel Hill, NC

Efficient cell migration requires coordination of many subcellular processes that depend on the concurrent control of both cytoskeletal force generation and adhesion turnover. In this study, we aimed to examine the force-dependent regulation of vinculin dynamics and the impact of these force-activated dynamics on the coordination of adhesion and protrusion that result in effective cell migration. We used a Forster Resonance Energy Transfer (FRET)-based vinculin tension sensor (VinTS) expressed in vinculin null fibroblasts to measure vinculin loading at focal adhesions (FAs), while simultaneously using Fluorescence Recovery After Photobleaching (FRAP) of the bleached acceptor signal to measure vinculin dynamics. We used point mutations to disrupt vinculin binding to talin (A50I) and to actin (I997A), as well as the ROCK inhibitor Y-27632 to disrupt cytoskeletal contractility to investigate the role of these factors in maintaining the force-dynamics relationship of vinculin. Adhesion and protrusion dynamics were assayed, and measurements of random 2D and directed migration were made. Our novel technique of comparing the FRET efficiency and half-time of FRAP recovery of single FAs, which we term FRET-FRAP, informs about the force-activated vinculin dynamics in living cells. Our data show that WT VinTS acts as an “effective” catch bond, where increased load across vinculin corresponds with decreased turnover. Introduction of the A50I mutation or treatment with Y-27632 results in reversal of this behavior to an “effective” slip bond, where increased load across vinculin corresponds with increased turnover. Finally, introduction of the I997A mutation resulted in force-insensitive vinculin dynamics. The catch bond-like behavior, unique to unperturbed cells expressing WT VinTS, is important in stabilizing adhesion dynamics and enabling persistent protrusions, as well as in allowing cells to migrate effectively in response to a haptotactic fibronectin cue. These results indicate that there is a relationship between vinculin load and vinculin turnover in individual FAs that depends on vinculin binding to talin and actin, as well as cytoskeletal contractility. The self-stabilizing “effective” catch bond

formed by vinculin appears to be critical in enabling vinculin's role as part of the molecular clutch, and thus in mediating the coordination of several sub-cellular processes important for efficient cell migration. Overall, this work demonstrates the usefulness of FRET-FRAP for probing force-activated proteins dynamics in living cells and suggests that this technique will be useful for studying diverse aspects of force-activated protein dynamics in mechanosensitive cellular processes.

M162

Human brain organoids on a chip to model normal development and disease.

O. Reiner[1], E. Karzbrun[1], S. Cohen[1];
[1]Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

The origin of human brain wrinkling remains an open fundamental problem, with implications to neurodevelopmental disorders. Physical studies in polymer gel models suggest wrinkling emerge spontaneously due to development of compression forces during differential growth of cell layers, however biological evidence is limited. Here, we report the emergence of surface wrinkles during in vitro development of human brain organoids, in a micro-fabricated compartment, which supports in situ imaging at subcellular resolution over weeks. The folding dynamics and morphology exhibit similarity to folding in vivo. By studying the cellular dynamics, we observe nuclei compression during development, and the emergence of convolutions at a critical cell density. We identify two opposing forces which contribute to differential growth: cytoskeleton contraction at the organoid core, and nuclear expansion during cell-cycle at the organoid perimeter. The wrinkling wavelength exhibits linear scaling with tissue thickness, consistent with polymer physics. Remarkably, lissencephalic (smooth brain) organoids exhibited reduced convulsions, linear scaling with an increased prefactor, and reduced elastic modulus. Our results support a physical mechanism for lissencephaly, in which change in tissue elasticity results in reduced folding.

M163

Cellular force propagation through monolayers investigated by tracking sub-nuclear sensors.

T.J. Armiger[1], M. Lampi[2], C. Reinhart-King[3], K.N. Dahl[1];
[1]Chemical Engineering, Carnegie Mellon University, Pittsburgh, PA, [2]Biomedical Engineering, Cornell University, Ithaca, NY, [3]Biomedical Engineering, Vanderbilt University, Nashville, TN

Proper regulation of tissue function relies on cell's ability to sense their physical extracellular environment by transmitting mechanical cues from outside the cell, through the cytoskeleton, and ultimately to the cell nucleus. This mechanosensitivity is particularly important for maintaining cell functions in the areas of wound healing, collective migration, tissue development, and monolayer maintenance. Here we aim to determine the key factors that affect force transmission through cells, primarily within monolayers, by using a novel technique termed sensors from intranuclear kinetics (SINK), which involves tracking the motion of DNA-bound fluorescent proteins within live cell nuclei. This tracking involves image processing to remove rigid body nuclear motion in order to quantify only the intranuclear motion of the DNA bound probe. We show that motion within the cell nucleus is responsive to actomyosin cytoskeletal contraction through both chemical stimulation and through physical sensing of the stiffness of the extracellular environment. We demonstrate the importance of cytoskeletal changes as cells transition from isolated cells to cell monolayers. These cytoskeletal changes lead to changes in force propagation through cells and ultimately regulate monolayer function. Finally, we demonstrate the importance of the linker of the nucleoskeleton and cytoskeleton (LINC) complex within a given cell and the extent that this connectivity affects surrounding cells in a monolayer. Mainly, we

determine that a mechanically compromised cell within a monolayer can affect surrounding cells over 45 µm away. This demonstrates the power of our SINK technique for investigating disease progression such as cancer, which often begins as a point defect in a relatively homogeneous system. A highlight of this work is the use of the cell nucleus as a relative force sensor in order to get a more complete understanding of the extent that cytoskeletal forces propagate through cells and the factors which regulate this propagation. Future work using this technique could be extended to three-dimensional or in vivo systems where current force measurement techniques are difficult to implement.

M164

Force triggers YAP nuclear entry by mechanically regulating transport across nuclear pores.

A. Elosegui-Artola[1], I. Andreu[2,3], A. Beedle[4], A. Lezamiz[4], M. Uroz[1], A. Kosmalska[1,5], R. Oria[1,5], J.Z. Kechagia[1], P. Rico-Lastres[4,6], A. Le Roux[1], C.M. Shanahan[4], X. Trepat[1,5,7], D. Navajas[1,5], S. Garcia-Manyes[4,6], P. Roca-Cusachs[1,5];
[1]Institute for Bioengineering of Catalonia, Barcelona , Spain, [2]Mondragon University, Arrasate, Spain, [3]CEIT and TECNUN (University of Navarra), San Sebastian, Spain, [4]Randall Division of Cell and Molecular Biophysics, King's College London, London, United Kingdom, [5]University of Barcelona, Barcelona, Spain, [6]King's College London, King's College London, London, United Kingdom, [7]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona , Spain

YAP is a mechanosensitive transcriptional activator with a critical role in cancer, regeneration, and organ size control. Here we show that force applied to the nucleus directly drives YAP nuclear translocation by decreasing the mechanical restriction of nuclear pores to molecular transport. We demonstrate that the nucleus only connects mechanically to the cytoskeleton above a threshold in substrate rigidity, allowing forces exerted through focal adhesions to reach the nucleus. This leads to nuclear flattening, which increases YAP nuclear import by decreasing the mechanical restriction of nuclear pores to molecular transport. This restriction is further regulated by the mechanical stability of the transported protein. Control of YAP translocation by nuclear force is independent of focal adhesions, the actin cytoskeleton, substrate rigidity, cell-cell adhesion, and the Hippo pathway. Our results unveil a mechanosensing mechanism mediated directly by nuclear pores, demonstrated for YAP but with potential general applicability in transcriptional regulation.

M165

Forces and dynamics in three-dimensional epithelia of controlled size and shape.

E. Latorre[1,2], L. Casares[2], S. Kale[1], M. Gomez-Gonzalez[2], M. Uroz[2], L. Valon[2], M. Arroyo[1,2], X. Trepat[2,3,4,5];
[1]Universitat Politècnica de Catalunya-BarcelonaTech, Barcelona, Spain, [2]Institute for Bioengineering of Catalonia, Barcelona, Spain, [3]University of Barcelona, Barcelona, Spain, [4]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain, [5]Centro de Investigación Biomédica en Red en Bioingeniería, Biomat. y Nanomed., Madrid, Spain

Mechanobiology of epithelial tissues plays a central role in morphogenesis, tissue compartmentalization, protection against pathogens, wound healing, and tumor progression. Current understanding of epithelial mechanobiology relies on measurements of forces and deformations obtained in flat epithelial layers. However, most epithelia in vivo exhibit a curved three-dimensional shape that encloses a pressurized lumen. Here we provide direct measurements of epithelial mechanics in three-dimensional cell sheets. Using soft micropatterned substrates we produce massive parallel arrays of epithelial domes with controlled size and basal shape. By measuring 3D deformations of the substrate we obtain a direct measurement of epithelial tractions and luminal pressure. The local tension

in the freestanding epithelium is then mapped by combining measured luminal pressure and local curvature. We show that tension and dome curvature remain isotropic and uniform during perturbations of the contractile machinery, osmotic shocks, and spontaneous fluctuations of dome volume. By examining dome tension over time-scales of hours and for nominal strains reaching 300%, we establish that epithelial sheets exhibit superelastic behavior. Despite the fact that the dome is subjected to uniform tension, the areal strain of each individual cell can differ by more than one order of magnitude. This remarkable heterogeneity is suggestive of a mechanical instability caused by limited availability of structural components of the actin cortex. We develop a 3D vertex model that captures both the global tension/strain relationship in the dome and the strain heterogeneity. Our study provides the first direct measurement of pressure, tension and strain in three-dimensional epithelia and shows that epithelial cells can sustain extreme deformations under constant tension.

M166

Tissue explant imaging reveals spatially coordinated migration patterns in the tumor core.

R. Staneva[1], F. El Marjou[1], A.G. Clark[1], D. Matic Vignjevic[1];
[1]Institut Curie, Paris, France

Invasion of cancer cells into the stroma is a critical step in the malignant progression of carcinoma and can lead to metastasis. Cancer cell invasion is associated with an increased migratory capacity of cells leading to their dissemination to distant tissues. Until now, cancer cell migration has been described at the so-called “invasive front”, the region where cancer cells reach stromal tissue. The core of tumors has been considered as a relatively immobile tissue. However, due to the lack of technology required to image the tumor core in real time, this has never been directly addressed. In order to investigate cancer cell dynamics in a native tumor core, we use a mouse model which gives rise to spontaneous aggressive intestinal carcinoma. We explore cell migration phenotypes in tumor explants using ex vivo long-term 3D imaging by two-photon microscopy. We find that cancer cells in the tumor core are not stationary, as previously thought, but are rather remarkably dynamic and migrate with a range of cell speeds and persistence. Moreover, we observe a correlation between migration direction and distance between cells, where cells close together have a correlated direction of migration and move in local "currents". Such collective behaviors of neighboring cells give rise to large-scale tissue dynamics, such as collective streaming and vortex-like migration features. Although cells exhibit stop-and-start migration patterns with intermittent pauses, we do not observe any substantial cell pausing during division. This suggests that dividing cells in cancer tissues can still flow with their neighbors during division. This study represents the first investigation of the live dynamics of cells in the tumor core of spontaneously developing tumors. Our work will help understand collective behaviors of cells in the tumor core and sheds light on cancer migration as well as other dynamics systems, such as developing organisms.

M167

Conservation of organo-tropic metastasis of human tumor cells in a zebrafish xenograft model.

K. Tanner[1], C.D. Paul[1];
[1]LCB, NCI/NIH, BETHESDA, MD

Metastasis, the process by which cancer cells travel from a primary tumor to establish lesions in distant organs, is the cause of most cancer-related deaths. Clinical patterns of metastasis are non-random, with certain types of cancers preferentially metastasizing to certain secondary organs. However, the lack of models to simultaneously visualize the early stages of metastasis in multiple organs within the same animal have made it difficult to delineate the rate-limiting steps in cancer organotropism. Here, we used

bone- and brain-tropic subclones of MDA-MB-231 breast adenocarcinoma cells (231BO and 231BR, respectively) injected into the circulation of embryonic zebrafish as a model xenograft system of metastatic dissemination. The zebrafish contains vascular vessels on the scale of human capillaries, a brain with structural and cellular similarity to the mammalian brain, and hematopoietic tissue (HT) in the tail analogous to mammalian bone marrow. Remarkably, the organ-targeting characteristics of these cell types was conserved in the zebrafish, with 231BR cells more likely to be found in the zebrafish brain five days following injection than 231BO cells, which preferentially extravasated in the zebrafish tail. Similar results were observed with a brain-targeting subclone of Bt474-m1 breast ductal carcinoma cells. Timelapse microscopy revealed that the early dynamics of dissemination were similar, with both cell types arresting for longer periods of time in the sinusoidal vessels surrounding the HT during the first 12 h following injection into the circulatory system. Organ selectivity was an emergent phenomenon following cell arrest. with differential extravasation patterns emerging at later time periods. By 120 h post-injection, approximately 60% of the remaining 231BR cells had extravasated in the brain, while a similar percentage of 231BO cells had extravasated in the CVP. Little extravasation of 231BR cells in the tail was observed. Overall, these data suggest that patterns of metastatic spread are driven, at least in part, by different patterns of cell extravasation during early metastatic dissemination. To our knowledge, this is the first simultaneous observation of the steps in early metastatic dissemination in multiple organs in vivo

M168

Cell-free reconstitution of cytoskeletal dynamics, mechanics and motility.

G.H. Koenderink[1];
[1]Systems Biophysics, AMOLF, Amsterdam, Netherlands

To understand how the cytoskeleton determines the dynamics, mechanics and motility of animal cells, we reconstitute cell-free model systems that mimic certain functions of cells from purified cellular components. We currently focus on the interplay of actin filaments, microtubules, and intermediate filaments. These three types of cytoskeletal polymers have rather different structural and physical properties, enabling specific cellular functions. However, there is growing evidence that they also exhibit strongly coupled functions necessary for polarization, cell migration, and mechanoresponse. To identify the physical principles that underlie these coupled functions, we reconstitute hybrid networks of actin, microtubule, and vimentin filaments together with crosslinking and motor proteins. Using microscopy, we find that steric interactions and the activity of crosslinking molecules are sufficient to enable organizational feedback between different cytoskeletal systems. Using rheology, we find that cytoskeletal crosstalk leads to synergistic mechanical properties such as enhanced strength and resilience. Finally, I will highlight recent experiments where we reconstitute the interaction of cytoskeletal networks with the plasma membrane.

Minisymposium 17: Mechanics of Cell Division and Cytokinesis

M169

A myosin-10-wee1 interaction links spindle dynamics to mitotic exit in vertebrate epithelial cells.

J.C. Sandquist[1], M.E. Larson[2,3], S.E. Woolner[4], Z. Ding[1], W.M. Bement[2,3,5];
[1]Biology, Grinnell College, Grinnell, IA, [2]Cellular and Molecular Biology, University of Wisconsin, Madison, WI, [3]Laboratory of Cell and Molecular Biology, University of Wisconsin, Madison, WI, [4]Division of Developmental Biolgoy and Medicine, University of Manchester, Manchester, United Kingdom, [5]Zoology, University of Wisconsin, Madison, WI

The mitotic spindle is the cellular apparatus charged with the task of accurately and efficiently separating the cell's duplicated chromosomes during cell division. While many conditions must be met for cell division to be successful, spindle positioning is one of the most consequential factors. Since the spindle directs where the cytokinetic cleavage furrow forms, improper spindle positioning will result in daughter cells of incorrect size or ones that form in the wrong extracellular environment. The spatial constraints of cell division are particularly apparent during metazoan development, as misoriented divisions perturb normal tissue morphogenesis and are associated with disease. While the mechanisms controlling spindle positioning are relatively well-understood, little is known about how the cell ensures that proper spindle position is achieved prior to anaphase onset. Here we identify a novel interaction between myosin-10, an actin-based motor protein that contributes to proper spindle dynamics, and Wee1, a cell cycle regulatory kinase. We show that perturbing myosin-10 function via expression of a dominant negative tail fragment induces a delay in anaphase onset that correlates with decreases in pre-anaphase spindle dynamics and is dependent on Wee1 kinase activity. Small molecule inhibition of Wee1 rescues the delay induced by myosin-10 perturbation, and inhibiting Wee1 in wild-type embryos also perturbs spindle dynamics. Further, we make the novel observation that Wee1-mediated phosphorylation of Cdk1 is enriched at cell-cell junctions in intact epithelial cells, and that an increase in this inhibitory phosphorylation of Cdk1 at junctions correlates strongly with the anaphase delay. Based on these and prior results, we propose that the myosin-10-Wee1 interaction plays a part in a broader system that coordinates spindle dynamics with mitotic exit, thus facilitating proper spindle orientation during cell division.

M170

The role of mitotic cell-substrate adhesion remodelling in animal cell division.

C.L. Dix[1], H.K. Matthews[1], S. McLaren[1], L. Wolf[2], P. Almada[1], R. Henriques[1], M. Boutros[2], B. Baum[1];
[1]Medical Research Council Laboratory for Molecular Cell Biology, University College London, London, United Kingdom, [2]Division of Signaling and Functional Genomics, German Cancer Research Center (DKFZ), and Department for Cell and Molecular Biology, Medical Faculty Mannheim, Heidelberg University, Heidelberg, Germany

Animal cells undergo a dramatic series of shape changes as they divide which depend on remodelling of the contractile actomyosin cortex and on release of cell- substrate adhesions. Here, we show that while focal adhesion complexes are disassembled during mitotic rounding integrins remain in place. These integrin-rich contacts connect mitotic cells to the underlying substrate throughout mitosis, guide polar cell migration following mitotic exit, and are functionally important, since adherent cells undergo division failure when removed from the substrate. Further, the ability of cells to re-spread along pre-existing adhesive contacts is essential for division in cells compromised in their ability to construct an

actomyosin ring. Thus, Ect2 siRNA cells fail to divide on small adhesive islands, but successfully divide on larger patterns, as the connection between daughter cells narrows and severs as they migrate away from one another. Together these results reveal the importance of coupling adhesion remodelling to mitotic progression.

M171

Spindle morphology tailoring through time: Interplay between spindle architecture and morphogenesis of the mammalian brain.

D.C. Vargas-Hurtado[1], V. Marthiens[1], C. Pennetier[1], R. Basto[1];
[1]UMR144, Subcellular structure and cellular dynamics, Institut Curie, Paris, France

Accurate chromosome segregation is essential for genome stability. Chromosome number deviations (or aneuploidy) influence cellular fitness and performance. During mitosis, a highly dynamic microtubule-based structure, the mitotic spindle, is assembled to connect sister chromatids to the spindle poles providing the forces to segregate the chromosomes. Centrosomes, the main microtubule-organizing centres (MTOCs) of animal cells, organize the spindle poles contributing to spindle orientation and bipolarity. Interplay between centrosomes, spindle microtubules and associated proteins influence the fidelity of each cell division.
From all the organs in the human body the brain is particularly vulnerable to centrosome defects. Aneuploidy generated by centrosome dysfunction impairs embryonic neural progenitor survival, which culminates in severe brain size reduction at birth, a pathological condition known as microcephaly. Intriguingly, aneuploidy has also been reported in physiological conditions. Thus, there seems to be an intrinsic susceptibility of embryonic neural progenitors to mitotic errors. The origin of this susceptibility is not known. In order to unravel the mechanisms of error-prone mitosis in the neuroepithelium, we characterized mitotic spindle assembly during mouse neurogenesis in the WT brains.
Strikingly, we found that the morphology of the spindle changes during neurogenesis. While at early stages, the spindles of neural progenitors contain longer astral microtubules (MTs) that interact with the cell cortex, spindles at later stages gain in central spindle robustness at the expense of astral microtubule abundance. This change is followed by significant differences in the levels/distribution of key spindle associated factors along the spindle. Moreover, we identified Tpx2, a microtubule nucleating, organizing and stabilizing factor, as one of the key factors involved in the switch of spindle morphology.
Overall, our results indicate unexpected modifications in the pathways used by neural progenitors to build a bipolar spindle during neurogenesis, which confer a different chromosome segregation capacity. Indeed, by challenging the spindle formation with the presence of extra centrosomes, we observed an improvement of mitotic accuracy during the course of neurogenesis. We thus propose that during mammalian neurogenesis not all the progenitors are equally competent to segregate chromosomes correctly.

M172

Stem Cell Cytokinesis is Disrupted with Age Due to Diminished Jak/STAT Activity.

K. Lenhart[1], B. Capozzoli[1], S. DiNardo[1];
[1]Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA

Many aspects of stem cell function deteriorate with age. Defects in niche signaling and architecture, as well as changes in cytoskeletal dynamics, have been implicated in promoting age-related decline in

many tissues, yet the proximate causes of early-onset aging defects are often unknown. The Drosophila testis has emerged as an ideal system in which to study stem cell behavior and its regulation with age. We previously discovered a striking modification to cytokinesis in the germline stem cells (GSCs). This program is imposed by the niche in a stem cell-specific manner and serves to block abscission for a substantial period. The cytokinesis block is achieved through polymerization of an F-actin ring at the intercellular bridge between GSC-daughter pairs following contractile ring disassembly. Only once this ring has been disassembled can abscission occur and a GSC daughter be released from the niche to differentiate. Here we show that the earliest age-induced defect in this stem cell system targets the modified GSC cytokinesis program.
While young GSCs complete cytokinesis and consistently separate from their daughter cells, there is a significant rate of abscission failure in GSCs after only one week of adult life. This defect is progressive, with 30% of all GSC divisions resulting in failed abscission in flies reaching one-third of their lifespan. Abscission failure has severe consequences to the tissue, as GSC daughter cells are no longer released in a consistent manner to differentiate and form sperm. Genetic analyses identify diminished Jak/STAT responsiveness in GSCs as the cause of age-dependent defects in cytokinesis. We find that increasing Jak/ STAT activity specifically within GSCs significantly rescues the age-related abscission defect. Additionally, depletion of STAT from aged GSCs leads to a near complete block to abscission. Extensive live imaging reveals that this pathway regulates F-actin disassembly at the intercellular bridge between GSC-daughter pairs. Aged GSCs retain the F-actin ring and block abscission significantly longer than do young stem cells. This timing defect is rescued through increased STAT and exacerbated by decreased STAT activity in aged GSCs. Interestingly, loss of even a single functional copy of STAT results in substantial defects in the GSC cytokinesis program. Flies heterozygous for a STAT mutation or a deficiency that includes the STAT locus exhibit abscission defects at a young age that are indistinguishable from those observed in genotypically normal aged flies. Thus, diminished STAT is sufficient to precociously age stem cells. Taken together, this work has identified a novel role for an established niche signaling pathway in controlling stem cell cytokinesis and regulating stem cell behavior with age.

M173

The Aurora kinase A activator TPXL-1 mediates aster-based clearing of contractile ring proteins from the cell poles during cytokinesis.

S. Mangal[1], T. Kim[2], K. Oegema[2], E. Zanin[1];
[1]Department Biology II, Ludwig-Maximilians University, Planegg-Martinsried, Germany, [2]Department of Cellular and Molecular Medicine, Ludwig Institute for Cancer Research, La Jolla, CA

At the end of cell division the mother cell splits into two daughter cells in a process called cytokinesis. During cytokinesis a contractile actin-myosin ring assembles between the two segregating chromosome masses beneath the plasma membrane. The position of the contractile ring is determined by signals from the mitotic spindle and thereby chromosome segregation is tightly coupled to cleavage furrow formation. A stimulatory signal from the mitotic spindle promotes cortical contractility at the cell equator. In parallel, an inhibitory signal from the centrosomal microtubule asters prevents the accumulation of contractile ring components at the cell poles. Attempts to reveal the molecular basis of the inhibitory signal have been hindered by the absence of a robust assay. To identify the inhibitory signal from the centrosomal asters, we established a microscopy assay in the one-cell *C. elegans* embryo. Using this assay, we identified TPXL-1, the homologue of human TPX2, to be required for clearing the contractile ring component anillin from the cell poles. TPXL-1 is an established activator of aurora A kinase and localizes to the centrosome and the astral microtubules. In *tpxl-1* mutant embryos

the kinetochore microtubules are extremely short resulting in a small mitotic spindle at anaphase onset. To determine whether the short mitotic spindle or TPXL-1 depletion itself causes defects in aster-based suppression, we increased spindle length in *tpxl-1* mutants by depleting the kinetochore component *hcp-4*. Rescuing spindle length in *tpxl-1* mutants did not rescue the defects in aster-based suppression, suggesting that TPXL-1 has a direct role in this process. Next we tested whether aster-based clearing of anillin depends on the ability of TPXL-1 to activate aurora A kinase. To this end we generated wild type (TPXL-1^{WT}) and Aurora A binding-defective (TPXL-1^{FD}) RNAi-resistant TPXL-1 transgenes. We find that in the absence of endogenous TPXL-1, TPXL-1^{WT} but not TPXL-1^{FD} supports clearing of anillin from the cell poles. Our findings suggest that aurora A kinase activation by TPXL-1 is essential for the removal of contractile ring components from the cell poles. In summary, we identified TPXL-1 and aurora A kinase as the first molecular components of the aster-based mechanism that inhibits the accumulation of contractile ring proteins at the cell poles during cytokinesis.

M174

Polo-like kinase-1 and Aurora B act in redundant signaling pathways that drive cytokinesis initiation.

I.E. Adriaans[1], A. Basant[2], B. Ponsioen[1], M. Glotzer[2], S.M. Lens[1];
[1]Center for Molecular Medicine, Molecular Cancer Research, University Medical Center Utrecht, Utrecht, Netherlands, [2]Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL

Cytokinesis drives the physical separation of the daughter cells at the end of mitosis. It starts in anaphase with the formation of an actomyosin-based contractile ring at the equatorial cortex. Assembly and contractility of the contractile ring is governed by the local activation of the small GTPase RhoA, and hence strict spatial and temporal regulation of RhoA activity is necessary to coordinate cytokinesis with nuclear division. Despite extensive research in various model systems it remains incompletely understood how localized RhoA activation is achieved and regulated in anaphase. Here we delineated the contribution of the central spindle, centralspindlin complex (Mklp1 and mgcRacGAP), Aurora B and Plk1 to cytokinesis initiation in human cells. By disrupting the central spindle via knock-down of the microtubule bundling protein PRC1, we identified two redundant signaling pathways involved in the activation of RhoA and the initiation of cleavage furrow ingression: One dependent on the central spindle and Plk1 as previously described, and a second pathway depending on cortical Aurora B activity and centralspindlin oligomerization, and operating independently of the central spindle and Plk1. We further show that Plk1 inhibition in PRC1-proficient cells sequesters Mklp1 and MgcRacGAP onto the central spindle making centralspindlin unavailable for Aurora B-dependent phosphorylation and oligomerization at the equatorial cortex. We propose that Plk1 activity at the central spindle allows the dynamic exchange of centralspindlin between the central spindle and equatorial cortex allowing centralspindlin to function as a regulator of central spindle formation and activator of RhoA at the equatorial cortex.

M175

Structure and constriction mechanism of the actomyosin ring.

L.T. Nguyen[1,2], G.J. Jensen[1,2];
[1]Biology and Bioengineering, California Institute of Technology, Pasadena, CA, [2]Howard Hughes Medical Institute, Chevy Chase, MD

Cytokinesis is orchestrated by a contractile actomyosin ring, but its structure and mechanism remain elusive. We visualized the 3D structure of the ring in frozen-hydrated dividing yeast cells by electron

cryotomography (ECT). Detailed arrangements of actin filaments within the ring and with respect to the membrane were seen for the first time, providing a crucial spatial constraint for the constriction mechanism of the ring. Using the ECT data and input from the current literature we then explored sixteen mechanistic models by coarse-grained simulations at the 3D molecular details, revealing plausible mechanisms for preventing membrane distortion and protein aggregation. We found that, in the model that best fits experimental data, both bipolar and membrane-attached unipolar myosins exist in the ring, reconciling two different views in the field regarding the myosin configuration. In this model, ring tension is generated primarily by interactions between bipolar myosins and actin, and transmitted to the membrane via unipolar myosins. This model recapitulates a broad distribution of distances from actin filaments to the membrane observed in our tomograms and separation of two different myosin isoforms into the outer and inner subdomains of the ring reported in a previous fluorescence microscopy study. Further, it rationalizes how bundles of actomyosin were able to separate from the membrane in fluorescence microscopy experiments of the same previous study.

M176

Modeling contractile ring dynamics in the Caenorhabditis elegans zygote.

D.B. Cortes[1], S. Ryan[1], F. Nedelec[2], A.S. Maddox[1];
[1]Biology, University of North Carolina Chapel Hill, Chapel Hill, NC, [2]Cell Biology and Biophysics, EMBL Heidelberg, Heidelberg, Germany

Cytokinesis is required for cell proliferation with failures potentially leading to aneuploidy and cancer. The actomyosin contractile ring is a dynamic structure responsible for driving cytokinesis. Proper cytokinesis requires assembly of contractile ring components at the cell equator, in the cortex. Actin, myosin, crosslinkers, and regulators then reorganize as the ring matures from a wide band to a tight cable, becoming contractile. Previous work has provided insight into the changes that occur in ring and cell structure. However, less is known about changes on the mesoscopic and molecular scales due to imaging limitations. Several models have given insight to mechanisms of contractility, including actin treadmilling and minifilament adaptive response to force-load, but the predictive power of these is limited by the simplification of myosin minifilament and actin dynamics. Herein we set out to further bridge the gap between the models of cytokinetic ring components and quantitative cell biology, by first establishing a model of contractility where myosin minifilaments are modeled. We developed this model of cytokinesis using the software Cytosim, which provides unparalleled resolution on a molecular scale. In our model actin treadmilling and shortening, both of which have been implicated in models of contractility, provide for a dynamic actin meshwork. We built upon previous models depicting the catch-slip bond nature of myosin II motors binding to actin filaments and built full minifilaments as multiple motor heads protruding off either end of 300nm rods. To further refine our model we queried the changes in protein concentration of several components of the ring including myosin, actin, anillin, and septin. Previous methods for visualizing cytokinesis result in uneven illumination and detection of structures across the cytokinetic ring, with the illumination plane orthogonal to the contractile ring plane; making quantitative analysis of the ring less precise. To this end we used custom chambers to position *C. elegans* zygotes such that the entire contractile ring forms in the illumination plane of a focused light sheet. This setup allowed us to quantify the contractile ring densities of components over the length of cytokinesis. Using this system, we generate contractile rings, on the scale of *C. elegans* zygote contractile rings, that exhibit protein density dynamics like those measured in our in vivo data and show a model for how these rings may contract. Our initial estimates for all major structural components yield simulated rings that close at biologically-relevant timescales, exert force that constricts a “membrane,” and form mesoscopic contractile foci.

M177

The mechanome of asymmetric cell division.

T.T. Pham[1,2], J. Helenius[3], N. Lee[2], E. Lund[2], D. Mueller[3], C. Cabernard[2];
[1]Biozentrum, University of Basel, Basel , Switzerland, [2]Department of Biology, University of Washington, Seattle, WA, [3]D-BSSE, ETH Zürich, Basel, Switzerland

Asymmetric cell division (ACD) generates cellular diversity and is an important process during development. Stem cells in particular utilize ACD in order to self-renew the stem cell yet generate differentiating siblings. Some stem cells undergo both physical and molecular ACD and it is unknown how biophysical parameters, such as cortical tension, stiffness or osmotic pressure affect the formation of sibling cell size asymmetry. We use Drosophila neural stem cells (neuroblasts) to study the contribution of biophysical parameters on ACD. We are combining fluorescence microscopy with atomic force microscopy (AFM), particle image velocimetry (PIV) and genetically encoded stiffness sensors to measure the dynamics of the actomyosin network and cortical stiffness of cultured neuroblasts. The combination of these measurements allow us to propose a model, explaining how dynamic changes in physical parameters contribute to the establishment of sibling cell size differences during mitosis.

M178

Cell-intrinsic and extrinsic control of cytokinetic diversity in the C. elegans embryo.

T. Davies[1], N. Romano Spica[1], B. Lesea-Pringle[1], J. Dumont[2], M.M. Shirasu-Hiza[3], J.C. Canman[1];
[1]Pathology and Cell Biology, Columbia University Medical Centre, New York, NY, [2]Institut Jacques Monod, Paris, France, [3]Genetics, Columbia University Medical Centre, New York, NY

Accurate cytokinesis, the physical division of one cell into two, is essential for the development of all multicellular organisms. In animal cells, cytokinesis is driven by constriction of an actomyosin contractile ring. It has long been known that animal cells divide using a highly conserved molecular mechanism, but growing evidence suggests that the molecular requirements for, and regulation of, cytokinesis in individual cell types has more diversity than previously realized. Thus, to fully understand cytokinesis, we need to acknowledge this diversity and compare cytokinesis between different cell types. To address this question, we studied division in the 4-to-8 cell *C. elegans* embryo. At this early stage of development, each individual cell already has a distinct cell identity leading to a distinct cell lineage that is controlled by inherited factors, cell-cell contacts, cell polarity, and conserved cell fate-signaling molecules (e.g. Notch, Wnt, Src). Using fast-acting (≤20 sec) temperature-sensitive mutants, we determined the level of activity and time of protein function required for successful cytokinesis in each of the 4 cells. We found reproducible, cell type specific variation in the cytokinetic requirement for the filamentous-actin (f-actin) nucleator formin^{CYK-1}, but not the motor myosin-II^{NMY-2}. Specifically, in two of the four cells (EMS and P2), we found cell division was more robust and able to occur successfully with greatly reduced formin activity and contractile ring f-actin levels. To determine if this cell-type specific cytokinetic robustness was dependent on cell intrinsic or extrinsic regulation, we performed blastomere isolation experiments. We found in one cell (P2), this cytokinetic robustness is dependent on cell intrinsic regulation, whereas in another cell (EMS) this cytokinetic robustness is dependent on cell-fate signaling pathways and direct cell-contact with its neighbor cell, P2. Together, our results demonstrate both cell-intrinsic and cell-extrinsic mechanisms control cytokinetic diversity in individual cell types and can protect against division failure when the contractile ring is weakened.

Minisymposium 18: Molecular Mechanisms of Cell-Cell Signaling

M179

STK25 Activates the LATS Kinases to Inhibit YAP/TAZ.

S. Lim[1], N.J. Ganem[1,2];
[1]Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA, [2]Medicine, Boston University School of Medicine, Boston, MA

LATS kinases restrict the oncoproteins YAP/TAZ in a phosphorylation-dependent fashion in response to numerous extracellular stimuli. However, upstream kinases responsible for LATS activation remain poorly characterized. We performed a focused RNAi-based kinome screen to characterize potential upstream activators of LATS1/2 and identified STK25 as our strongest hit. Knockdown of STK25 expression significantly decreased YAP phosphorylation in response to cytoskeleton-dependent stimulatory cues such as actin depolymerization and contact inhibition and increased levels of active nuclear YAP and expression of YAP-target genes. Genetic deletion of STK25, both in Cre-mediated KO Mouse Embryonic Fibroblasts, as well as in CRISPR/Cas9-mediated KO human cells, recapitulated phenotypes observed in knockdown experiments. By contrast, stable overexpression of wild-type STK25, but not kinase-dead STK25, led to increased YAP phosphorylation, suggesting that the kinase activity of STK25 is critical for exerting its inhibitory effects on YAP. STK25-dependent regulation of YAP was found to be mediated through LATS kinases, as both knockdown and overexpression of STK25 showed no effect when performed in the context of CRISPR-based KO of LATS1/2. Mechanistically, we found that STK25 activates LATS kinases by directly phosphorylating the activation loop at S909/S872. Functionally, we discovered that loss of STK25 confers pro-oncogenic phenotypes to cells, including increased proliferative capacity and resistance to contact-mediated cell cycle arrest. Interestingly, STK25 is significantly focally deleted in human cancers, suggesting that its loss may enable cancer cells to functionally inactivate LATS activity and overcome critical tumor suppressive signaling.

M180

Mechanism of T cell receptor signal transduction by phase transition.

X. Su[1], J.A. Ditlev[2], E. Hui[1], S. Banjade[2], M.K. Rosen[2], R.D. Vale[1];
[1]CMP, UCSF/HHMI, San Francisco, CA, [2]Biophysics, UT Southwestern/HHMI, Dallas, TX

During T cell activation, many signaling molecules coalesce into submicron- or micron-sized clusters on the plasma membrane though the mechanism and function of these microclusters are unclear. Using a biochemical reconstitution system, we demonstrated that multivalent protein-protein interactions drive the formation of T cell microclusters. These microclusters display a liquid-like property and form a separate phase from the environment. The phase separation is triggered by TCR phosphorylation, which is fully reversed by phosphatase treatment. Importantly, microclusters enrich kinases but exclude phosphatases and thus promoting tyrosine phosphorylation. Furthermore, by manipulating microclusters in Jurkat T cells, we demonstrated that microclusters promote ERK activation following TCR triggering. Together, we conclude that T cell microclusters display distinct phase behavior on cell membranes and these microclusters could promote biochemical reactions mediating TCR signaling.

M181

A conserved, transposable ubiquitination signal directs Ras for inhibitory ubiquitination by Rabex-5 and is mutated in tumors.

C. Washington[1,2], Y. Martino-Cortez[1,3], S. Shahar[1], H. Liu[1,4], R. Gokhale[1], C.M. Pfleger[1];
[1]Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, [2]University of Cincinnati College of Medicine, Cincinatti, OH, [3]Tufts University School of Medicine, Boston, MA, [4]Memorial Sloan Kettering Cancer Center, New York, NY

Ras signaling plays an important role in defining cellular outputs of growth, proliferation, survival, and differentiation depending on context making it a crucial signal transduction cascade in normal development and in maintaining health throughout lifespan. Therefore, it is imperative for cells to employ mechanisms to limit the strength and duration of Ras signaling to what is required by the biological context. Negative feedback circuits and antagonistic activities can be engaged to curtail Ras signaling. For example, GAP proteins such as the tumor suppressor NF1 promote GTP hydrolysis to inactivate Ras. The Ras oncogene is frequently amplified and/or mutationally activated in cancer. Common oncogenic mutations in Ras (such as G12V) affect GTP-loading and make these mutant forms insensitive to inactivation by NF1. In contrast, the E3 Rabex-5 (also called RabGEF1) can inhibit both wild-type and oncogenic forms of Ras by promoting Ras mono- and di-ubiquitination. We report here identification of a ubiquitination signal that mediates Rabex-5 ubiquitination of *Drosophila* Ras, *Ras85D*. This signal is transposable and is conserved in mammalian isoforms of Ras. This signal is also found in other *Drosophila* proteins suggesting it represents a general Rabex-5 ubiquitination motif. Mutating this ubiquitination signal to create a Ras protein insensitive to Rabex-5-mediated ubiquitination confers transforming activity *in vivo* in fly epithelial tissues . Although rare, variants in which this signal is mutated have been reported in a handful of gliomas. Taken together, these findings suggest that evading Rabex-5-mediated ubiquitination could be sufficient to promote tumorigenesis. Moreover, because mutation in the Ras ubiquitination signal is rare, promoting Rabex-5 activity could be a therapeutic strategy for down-regulating Ras activity in tumors and developmental syndromes associated with increased Ras signaling.

M182

APC regulates Wnt signaling by inhibiting a constitutive clathrin-mediated activation pathway.

K. Saito-Diaz[1], H. Benchabane[2], A. Tiwari[1], B. Li[3], A. Tian[2], L.M. Sawyer[1], A.S. Hyde[1], A.K. Kenworthy[1], D. Robbins[3], Y. Ahmed[2], E. Lee[1];
[1]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, [2]Department of Molecular and Systems Biology, Dartmouth College, Hanover, NH, [3]Department of Surgery, University of Miami, Miami, FL

The Wnt pathway is a conserved pathway that controls many developmental processes and is mutated in many human diseases (e.g., cancer). In the absence of Wnt ligand, cytoplasmic β-catenin is phosphorylated and targeted for degradation by a complex assembled by the scaffold protein Axin, and the tumor suppressor adenomatous polyposis coli (APC). When a Wnt ligand binds to the co-receptors Frizzled (Fz) and the low-density lipoprotein receptor-related protein 6 (LRP6), a receptor complex is assembled promoting LRP6 phosphorylation. Phosphorylated LRP6 binds to the β-catenin degradation complex in a process mediated by Dishevelled (Dvl), assembling the Wnt signalosome. The signalosome is internalized which blocks β-catenin degradation, leading to the activation of a Wnt-transcription program. Mutations in the APC gene leading to constitutive Wnt pathway activation occur in over 80% of human colorectal cancers (CRC). Our lab developed a monoclonal antibody (mAb7E5) that targets the

co-receptor LRP6 and inhibits Wnt signaling *in vitro* and *in vivo* . Significantly, mAb7E5 inhibits Wnt signaling in APC mutant CRC cell lines. Similarly, LRP6 knockdown inhibits Wnt signaling in APC mutant cells. Consistent with these results, activation of the Wnt pathway by knockdown of APC in human cells can be inhibited by simultaneously knocking down LRP6, or by blocking Fz or Dvl function. These results indicate that loss of APC leads to constitutive ligand-independent Wnt signalosome formation. This mechanism is conserved in *Drosophila* , where intestinal stem cell proliferation by loss of APC is inhibited by depletion of the receptor *Arrow* /LRP6 or Dvl. Using an inducible degradable form of APC, we demonstrate that APC loss is rapidly followed by Wnt receptor activation and increased β-catenin levels. Finally, clathrin inhibition blocks activation of the Wnt pathway in APC mutant cells and in organoids derived from tumors obtained from APC mutant mice. Consistent with a role for clathrin in regulating Wnt signaling upon loss of APC, we show that APC co-immunoprecipitates with clathrin. These results suggest a model in which Wnt receptor activation can occur via a constitutive, ligand independent clathrin-mediated mechanism that is normally inhibited by APC.

M183

Cholesterol-dependent regulation of the oncoprotein Smoothened during Hedgehog signaling.

P. Huang[1], A. Salic[1];
[1]Cell Biology, Harvard Medical School, Boston, MA

The Hedgehog (Hh) pathway controls key events in animal development. Insufficient Hh activity in embryogenesis causes birth defects such as holoprosencephaly and brachydactyly, while hyperactive signaling after birth is implicated in many cancers, including basal cell carcinoma and medulloblastoma. The seven-transmembrane domain oncoprotein Smoothened (Smo) is critical for relaying Hh signals across the plasma membrane. In unstimulated cells, Smo is inhibited by the tumor suppressor membrane protein Patched1 (Ptch1), ensuring that the Hh pathway is repressed. During Hh stimulation, Hh ligand binds and inhibits Ptch1, leading to activation of Smo, which triggers the downstream signal transduction events that ultimately result in activation of target gene transcription. A central question in Hh signaling is how Smo is controlled. It is thought that Smo equilibrates between inactive and active conformations, controlled by an endogenous ligand, which, in turn, is regulated by Ptch1. Sterols have emerged as candidate Smo ligands, as they are required for vertebrate Hh signaling. Furthermore, some oxysterols activate Smo by binding to a site in its extracellular, cysteine-rich domain (CRD). Oxysterols that activate Smo, however, are present in cells at significantly lower levels than the EC50 for Smo activation, and, additionally, they do not synergize with Hh ligand, as expected if they mediated Smo regulation by Ptch1. Thus perhaps another sterol is the endogenous Smo ligand.
We used X-ray crystallography and functional approaches to determine how Smo interacts with sterols, how sterols activate Smo, and which endogenous sterol activates Smo. We first solved the structure of SmoCRD bound to 20(S)-hydroxycholesterol (the most potent activating oxysterol), which we used to precisely define how Smo recognizes sterols. Structure-guided mutagenesis was then used to demonstrate that sterol binding is essential for Hh signaling. We next solved the structure of unliganded SmoCRD, revealing that 20(S)-OHC induces a conformational change of the protein, which we demonstrate is sufficient for Smo activation. Surprisingly, we find that cholesterol binds and activates Smo, and synergizes with Hh ligand. We also find that oxysterol synthesis is not essential for Smo activation. Finally, we use mutagenesis to show that cholesterol, and not 20(S)-hydroxycholesterol, activates Smo in cells. Our results suggest that the endogenous Smo activator is cholesterol itself, providing the first instance in which cholesterol plays a second messenger role in a developmental signaling pathway. We propose that Hh signaling through Ptch1 controls Smo by regulating its interaction with cholesterol.

M184

Defining the structure and stoichiometry of the Wnt-regulatory destruction complex during normal development and the mechanisms by which the complex is regulated by Wnt signaling.

K. Schaefer[1], S. Zhang[2], T.T. Bonello[2], C. Williams[2], D.J. Mckay[1,2], M. Peifer[1,2,3];
[1]Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, [2]Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, [3]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC

Wnt signaling is essential for normal development and tissue homeostasis, while mutations activating Wnt signaling help drive colorectal and other cancers. The key regulated effector of Wnt signaling is the transcriptional coactivator β-catenin (βcat). In the absence of Wnt signaling the destruction complex (DC), comprised of the tumor suppressor APC, the scaffold Axin, and two kinases, phosphorylates βcat, thus targeting it for proteasomal degradation. However, the structure of the DC and the mechanism by which it is regulated by Wnt signaling remain open questions. Most models in the field treat the DC as a four-protein complex, and suggest, based on work in Xenopus egg extract, that Axin's protein levels are >1000-fold lower than any other protein in the complex. In contrast, our data in cultured cells suggest the DC is a large multimeric structure assembled by APC modulation of Axin polymerization, with super-resolution microscopy suggesting relatively similar stoichiometries. We now have taken these studies in vivo into Drosophila embryos. Strikingly, we found that in vivo APC2 and Axin protein levels are within 5-fold of one another, in contrast to the prevailing models in the field. We used these data to systematically vary the levels of APC2 and/or Axin. The sensitivity of Wnt signaling to elevated Axin levels exhibits a tight threshold. A 3-4 fold increase in Axin levels is tolerated, but further Axin over-expression (7-fold), induces embryonic lethality and dramatic changes in cell fate. This occurs because the DC becomes insensitive to inactivation by Wnt signaling. In contrast, over-expressing APC2 has little effect on Drosophila embryonic viability or cell fate. However, APC2 over-expression had a surprising effect on the DC. APC2 over-expression elevated βcat levels in cells that receive Wg signal, suggesting it sensitizes the DC to inactivation by Wnt signaling, and supporting dual negative and positive roles for APC2 in signaling. We also defined how Wnt signaling affects DC assembly and localization. Axin contains a self-polymerization domain, allowing it to self-assemble into larger protein complexes. Using Airyscan microscopy and fluorescence-based molecular counting of GFP tagged Axin puncta, we found that in the absence of Wnt signal, Axin forms large cytoplasmic puncta containing 60-900 Axin molecules, with little remaining cytoplasmic Axin. In contrast, in the presence of Wnt signal, Axin is found in smaller puncta at the plasma membrane, with relatively high levels in the cytoplasm. These data suggest Wnt signaling affects both localization and assembly of the DC. Based on these and other data we propose a model for the assembly and mechanism of action of the Wnt-regulatory DC.

M185

Activating RASopathy mutations lead to both gain and loss of function phenotypes *in vivo*.

G.A. Jindal[1,2,3], Y. Goyal[2,3], V.L. Patterson[1], J.L. Pelliccia[1], E. Yeung[2,3], G.M. Schupbach[1], S.Y. Shvartsman[1,2,3], R.D. Burdine[1];
[1]Molecular Biology, Princeton University, Princeton, NJ, [2]The Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, [3]Chemical and Biological Engineering, Princeton Univeristy, Princeton, NJ

Somatic mutations in the RAS/MAPK pathway are found in almost 1/3 of human cancers. However, germline mutations in RAS/MAPK pathway components cause developmental disorders termed RASopathies, including Noonan and Cardio-Facio-Cutaneous syndromes. These disorders are common,

affecting 1/1000 births, and can include cardiac defects, orbital hypertelorism, neurodevelopmental delays, and increased risk of cancer. While it is generally believed that RASopathies are caused by altered levels of RAS pathway activation, the actual signaling changes, and phenotypic consequences of those alterations, are not well understood.
Here, we utilize zebrafish and *Drosophila* systems to analyze RASopathy mutations during development. We developed a rapid assay in zebrafish to rank human mutations in MEK from RASopathy and cancer patients. We find our zebrafish ranking holds in *Drosophila*, suggesting the rank reflects intrinsic properties of the mutations and not species-specific effects. The assay can be utilized to rapidly assess potential mutations and variants of unknown function in RAS pathway components in the future. Surprisingly, we find that activating variants of MEK, can both increase and decrease signaling levels of the pathway *in vivo* depending on the cellular location. We see this both in zebrafish and in *Drosophila* indicating that RASopathy phenotypes may result from combinations of activated and attenuated signaling during development. Indeed, we show gain-of-function and loss-of-function phenotypes in both systems resulting from the same mutation in MEK. We further show that while these MEK variants are active in *in vitro* assays in the absence of upstream signals, *in vivo* these variants require endogenous signaling for full activation.
Taken together our results have important implications for development of pharmaceutical therapies for these disorders. While MEK inhibitors are rapidly being developed to trial in cancer patients, our work suggests inhibition of MEK may actually worsen some phenotypes in RASopathy patients.

M186

The Semaphorin receptors, Neuropilins and Plexins, promote Hedgehog signaling through distinct cytoplasmic mechanisms.

J.M. Pinskey[1], N.E. Franks[1], R.J. Giger[1], B.L. Allen[1];
[1]Cell and Developmental Biology, University of Michigan, Ann Arbor, MI

Hedgehog (HH) signaling is essential for embryonic and postnatal development, while perturbation of HH pathway function can lead to a variety of developmental diseases, birth defects, and cancers. Neuropilins, which have well-characterized roles in Semaphorin and VEGF signaling, have recently been implicated in the regulation of HH signaling. Neuropilins contain short, catalytically inactive cytoplasmic domains, requiring Plexin co-receptors to regulate small intracellular GTPases during Semaphorin signal transduction. However, the mechanism of Neuropilin function in HH signal transduction remains unclear, and a role for Plexin proteins in HH signaling has not been explored. Here we report that Neuropilins and Plexins both promote HH signaling through distinct cytoplasmic mechanisms. Using HH-dependent luciferase reporter assays in NIH/3T3 cells, we show that the Neuropilin-1 cytoplasmic and transmembrane domains are both necessary and sufficient to regulate HH pathway activity, independently of Plexin and Semaphorin binding. We also show that Neuropilin-1 localizes to the primary cilium, a key platform for HH signal transduction, although this localization does not correlate with HH signaling promotion. Instead, our data suggest that Neuropilin-1 selectively regulates GLI activator function through a novel 12-amino acid cytoplasmic motif. Strikingly, we also find that multiple Plexin family members promote HH signaling, independent of their ability to interact with Neuropilins. Instead, point mutations in the GTPase activating domain of Plexins prevent HH pathway promotion, suggesting that GAP function is required for Plexin-dependent HH regulation. Furthermore, deletion of the autoinhibitory Plexin-A1 extracellular domain significantly increases HH pathway activity, providing additional evidence that Plexin GAP activity regulates HH signaling. Together, our data suggest that Neuropilins and Plexins regulate HH signaling downstream of ligand activation through distinct

cytoplasmic mechanisms. Therapeutic approaches targeting Semaphorin receptors may be useful to regulate overactive HH signaling in cancer and other diseases.

M187

Polymerization of the mitochondrial phosphatase PGAM5 underlies its biological activity.

K. Ruiz[1], T.M. Thaker[1], L. Miller-Vedam[2], C. Agnew[1], A. Frost[2], N. Jura[1,3];
[1]Cardiovascular Research Institute, University of California San Francisco, San Francisco, CA,
[2]Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA,
[3]Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA

Phosphoglycerate mutase family member 5 (PGAM5) is an atypical protein phosphatase localized to the mitochondrial membranes through a transmembrane domain tether. PGAM5 is involved in regulation of mitochondrial fission, and has been shown to promote cell death in response to oxidative stress or mitochondrial damage. The cell death response is believed to be triggered when PGAM5 is cleaved from mitochondrial membranes and relocated to the cytosol. The physiological importance of PGAM5 is underscored by genetic studies in mice, which revealed that PGAM5 deficiency causes a Parkinson's Disease-like disorder. The mechanism for regulation of the PGAM5 phosphatase domain activity has been poorly understood, and as a result the tools for understanding biological functions of PGAM5 or manipulation of its activity in disease do not exist. Recent analysis of crystal structures has identified a dodecameric ring assembly of PGAM5 phosphatase domains in complex with the multimerization domain, which has previously been shown to be necessary for full phosphatase activity. Using electron cryo-microscopy we demonstrate that PGAM5 adopts the dodecameric structure also in solution and we show, for the first time, that this assembly is critical for PGAM5 phosphatase activity and its role in mitochondrial homeostasis. Our new high resolution crystal structure of the PGAM5 dodecamer demonstrates that the multimerization motif serves as an allosteric activation domain. Furthermore, our electron microscopy analysis of the purified PGAM5 reveals that the cleaved form of PGAM5 organizes into long filaments composed of the dodecameric PGAM5 rings. Using super resolution fluorescence microscopy, we demonstrate presence of these filaments in the cytoplasm of intact cells and provide evidence that PGAM5 oligomerization is necessary for function of PGAM5 as activator of cell death. In summary, our work uncovers novel structures of the multimeric PGAM5 assemblies and demonstrates their importance for PGAM5 signaling.

M188

ER translocation of the entire MAPK pathway drives ERK reactivation and autophagy to promote therapy resistance in BRAF mutant cancers.

R. Ojha[1], N.M. Leli[2], A. Onorati[1], I. Verginadis[2], S. Piao[1], F. Tameire[2], V. Rebecca[1], C. Fennelly[1], C.I. Chude[1], C. Koumenis[1], R.K. Amaravadi[1];
[1]Division of Hematology and Oncology, University of Pennsylvania, Philadelphia , PA, [2]Department of Radiation Oncology, Smilow Center for Translational Research, University of Pennsylvania, Philadelphia , PA

BRAF and MEK inhibitors (B+Mi) produce striking response rates in certain BRAF mutant cancers, but heterogeneous resistance mechanisms limit the length and magnitude of response. MAPK reactivation is the most common resistance mechanism identified, but there is a paucity of information regarding how this reactivation occurs. Activation of endoplasmic reticulum (ER) stress-induced autophagy has also been established as a resistance mechanism to these inhibitors, but the mechanism by which B+Mi

engage this stress response is not well characterized. Here we report that B+Mi-induced translocation of the RAS-ERK signaling axis into the ER is necessary for ERK reactivation and resistance. B+Mi promoted binding of cytoplasmic GRP78 to the MAPK scaffold protein, Kinase Suppressor of Ras (KSR). Retrograde early endosomal trafficking shuttled this complex to the SEC61 translocon on the ER. Inducible knockdown of KSR2 or dominant negative RAB5 and CRISPR KO SEC61 all prevented ER translocation of the RAS-ERK pathway. This abrogated ERK reactivation and sensitized BRAF mutant melanoma and colon cancer cells to targeted therapy. Once in the ER, all MPKs were retained, except ERK. Notably, ERK was found to be translocated back to the cytoplasm. The cytoplasmic lipid kinase activity of PERK was found to be required for ERK reactivation in the cytoplasm. Reactivated ERK was found to translocate from the cytoplasm to the nucleus where it phosphorylated and stabilized ATF4. Phosphorylated ATF4 was required to activate ER stress associated autophagy. ER translocation of the MAPK pathway was demonstrated in vivo in patient derived xenograft tumors established from a patient resistant to BRAF+MEKi. Expression of dominant negative ATF4 mutant conferred sensitivity to B+Mi in vivo. This study identifies a new strategy to reverse resistance by targeting ER translocation of the MAPK pathway. Additionally, this demonstrates that one of the key consequences of MAPK reactivation is activation of a non-canonical ER stress response providing a new mechanistic link between two major drug resistance pathways in cancer.

Keywords: RAS, MAPK, ERK reactivation, endosomal trafficking, KSR, GRP78, ATF4, ER stress and autophagy.

Funding: This work was entirely supported by NIH grants R01CA169134; P01CA114046; P30 CA016520; SPORE P50 CA174523; 1R01CA198015.

Minisymposium 19: Organelle Morphogenesis, Targeting, and Distribution

M189

A new pathway for membrane protein insertion at the ER.

A. Guna[1], N. Volkmar[2], J.C. Christianson[2], R.S. Hegde[1];
[1]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom, [2]Ludwig Institute for Cancer Research, University of Oxford, Oxford, United Kingdom

Membrane protein insertion is an essential cellular process. The broad biophysical and topological range of membrane proteins necessitates multiple insertion pathways, which remain incompletely defined. Here, we have discovered a new membrane protein insertion pathway, identified the class of substrates it handles, explained why other known pathways do not work for these substrates and reconstituted the pathway using purified components. We show that tail-anchored proteins with low to moderate transmembrane domain hydrophobicity fail to engage TRC40, the previously known targeting factor for this class of membrane proteins. Instead, these proteins are kept soluble in the cytosol by calmodulin. Dynamic release from calmodulin allowed sampling of the endoplasmic reticulum (ER). At the ER, the conserved ER membrane protein complex (EMC), a broadly conserved ten-subunit complex of unknown function, was shown to be essential for efficient insertion in vitro and in cells. In the absence of an intact EMC, these proteins were shown to mislocalize, aggregate, and get degraded. Purified EMC in synthetic liposomes was sufficient to catalyse insertion of its substrates in a reconstituted system. Therefore, the EMC is a transmembrane domain insertase that is necessary and sufficient for the insertion of roughly half of all tail-anchored proteins that don’t have access to the TRC40-dependent pathway.

M190

Single molecule and ensemble dynamics of the endoplasmic reticulum.

C.J. Obara[1,2], J. Nixon-Abell[1,3], C. Blackstone[1,3], J. Lippincott-Schwartz[1,2];
[1]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA, [2]Cell Biology and Metabolism Program, National Institute of Child Health and Human Development, Bethesda, MD, [3]Cell Biology Section, National Institute of Neurological Disorder and Stroke, Bethesda, MD

The endoplasmic reticulum (ER) is an expansive, membrane-enclosed organelle that plays crucial roles in numerous cellular processes. Besides its clearly defined roles as the major site of cellular translation, the master regulator of calcium homeostasis, and origin of the secretory pathway; it makes contacts with and signals to control the behavior of many other cellular organelles. Despite this diverse set of functions, the spatial location of these processes within the ER network remain poorly characterized due to the highly intricate and dynamic nature of the organelle. Here, we utilize a variety of emerging superresolution imaging technologies to characterize the fine structure and the distribution of ER proteins within the organelle membrane and lumen. We have previously shown that the peripheral ER contains many intricate tubular clusters that cannot be distinguished from continuous sheets by diffraction-limited imaging; we further show that these structures contain diverse biological processes and proteins that are consistent with their complex structure. In fact, the ER is not as homogeneous of a structure as appears with traditional imaging approaches, instead containing a multitude of highly dynamic microdomains throughout the membrane and lumen which carry out individualized biological functions. By performing high speed, simultaneous structured illumination microscopy (SIM) and single particle tracking-photoactivation localization microscopy (sptPALM), we track the behavior of individual proteins within the dynamic membranous network of the ER. We show for the first time a strong effect of organelle structure on the behavior of single ER proteins and protein complexes, suggesting a correlation between the structure of the organelle and the localization of biological functions at the level of individual protein molecules.

M191

Importin α palmitoyolation drives cell surface area to volume dependent scaling during development.

C.W. Brownlee[1], R. Heald[1];
[1]Cellular and Molecular Biology, University of California, Berkeley, Berkeley, CA

Early embryogenesis is accompanied by rapid and reductive cell divisions requiring that subcellular structures adapt to a wide range of cell sizes. The interphase nucleus and mitotic spindle have been shown to scale smaller as cell volume decreases and cytoplasmic composition changes, but factors linking physical and biochemical scaling mechanisms are unknown. We show that the multipurpose nuclear transport receptor importin α is modified by palmitoylation, which mediates association with plasma membrane fractions in Xenopus egg and embryo extracts. Palmitoylation of importin α inhibits its binding to nuclear localization signal-containing proteins that modulate nuclear and spindle size. Reconstitution of importin α targeting to the outer membrane in cell-like compartments recapitulates scaling relationships observed during embryogenesis. These results identify importin α as a cell surface area-to-volume sensor that coordinately scales the size of intracellular structures to cell size.

M192

Spatial organization of ER–PM junctions revealed by super- and high-resolution imaging.

T. Hsieh[1], Y. Chen[1], C. Chang[1], W. Lee[1], J. Liou[1];
[1]Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX

The endoplasmic reticulum (ER) extends widely and forms junctions with virtually all the other membrane-bound organelles in eukaryotic cells. These ER–organelle junctions enable direct interactions and transfer of molecules between the ER and its apposing organelles to fulfill various cellular functions. Particularly, ER–plasma membrane (PM) junctions mediate crucial activities ranging from Ca^{2+} signaling to lipid metabolism. The location and extent of these cellular activities may be modulated by spatial organization of ER–PM junctions in the cell cortex. Nevertheless, the morphology and distribution of ER–PM junctions are not well characterized. Using photoactivated localization microscopy (PALM), we reveal ER–PM junctions are mainly oblong with the dimensions at 100-nm scale. Using total internal reflection fluorescence microscopy (TIRFM) and structure illumination microscopy (SIM), we show that F-actin contributes to spatial distribution and stability of ER–PM junctions. Further functional assays suggest that intact F-actin architecture is required for phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] homeostasis mediated by ER–PM junctions. Taken together, our study provides quantitative information about spatial organization of ER–PM junctions that is in part regulated by F-actin. We envision functions of ER–PM junctions can be differentially regulated through dynamic actin architecture during cellular processes.

M193

ER-PM contacts confine exocytic sites for polarized morphogenesis.

D. Zhang[1], A. Ng[1], A. Ng[1];
[1]Cell Biology, Temasek Life Sciences Laboratory, Singapore, Singapore

Spatial control of exocytosis underlies cell morphogenesis and many other important biological processes. We report a novel role of endoplasmic reticulum (ER)-plasma membrane (PM) contacts in restricting exocytic sites for polarized fission yeast morphogenesis. We show that fission yeast cells lacking both ER-PM contacts and actomyosin-based secretory vesicle transport exhibit globular cell shape due to compromised spatial confinement of exocytosis. By artificially manipulating the strength and extent of ER-PM contacts in wild-type and mutant cells with induced ectopic exocytosis, we demonstrate that exocytosis and ER-PM contact formation are incompatible. Furthermore, extensive ER-PM junctions at the non-growing lateral cell cortex prevent the PM from exocytic vesicle tethering and hence attenuate growth potential at cell sides. We propose that ER-PM contacts function as a new ‘morphogenetic module’ by limiting exocytosis to growing tips in fission yeast. Similar mechanisms could apply to other cell types with prominent ER-PM contacts.

M194

Coupling curved membranes to cytoskeleton: I-BAR proteins and ezrin.

F. Tsai[1,2], C. Prévost[1], C. Stefani[3], E. Lemichez[3], E. Coudrier[2], P. Bassereau[1];
[1]PhysicoChimie Curie, Institut Curie, Paris, France, [2]Subcellular Structure and Cellular Dynamics, Institut Curie, Paris, France, [3]4. Mediterranean Center of Molecular Medecine , University of Côte d’Azur, Nice, France

Cell plasma membranes are highly deformable and form very dynamical thin tubular protrusions on their periphery, called filopodia. Very highly curved membrane structures also form in the endothelial

cells when trans-cellular tunnels called "transendothelial cell macroapertures" (TEM) open upon toxin-induced inhibition of RhoA signaling, or during leukocyte diapedesis. Interestingly, our results show that these apparently very different structures, filopodia and TEM, have common properties: a) filopodia are sustained by actin bundles, while the opening of TEM is limited by an actin cable that builds at its periphery b) both contain I-BAR domains proteins and ezrin connecting plasma membrane to the actin filaments, which are essential for these structures (Stefani et al., 2017). Here, we will address the question of the origin of the localization of these proteins on curved membranes. With in vitro experiments using Giant Unilamellar Vesicles (GUVs), purified proteins and membrane nanotubes of controlled curvature pulled from these GUVs, we have evidenced that the I-BAR domain protein IRSp53 is enriched on negatively curved membrane (Prevost et al., 2015), in agreement with the in vivo observations (Mattila and Lappalainen, 2008), but ezrin is not (Tsai et al., in preparation). However, we show that ezrin can be enriched on negatively curved membrane through its direct interaction with the I-BAR domain (Tsai et al., in preparation). Our work provides a mechanism to target ezrin to specific curved area of the plasma membrane, irrespectively of its large abundance at the plasma membrane. References Mattila, P.K., and Lappalainen, P. (2008). Filopodia: molecular architecture and cellular functions. Nat Rev Mol Cell Biol 9, 446-454. Prevost, C., Zhao, H., Manzi, J., Lemichez, E., Lappalainen, P., Callan-Jones, A., and Bassereau, P. (2015). IRSp53 senses negative membrane curvature and phase separates along membrane tubules. Nat Commun 6, 8529. Stefani, C., Gonzalez-Rodriguez, D., Senju, Y., Doye, A., Efimova, N., Lipuma, J., Hamaoui, D., Maddugoda, M.P., Cochet-Escartin, O., Prévost, C., et al. (2017). Ezrin enhances line tension along transcellular tunnel edges via NMIIa driven actomyosin cable formation Nat Commun 8, 15839. Tsai, F.-C., Bertin, A., Bousquet, H., Manzi, J., Picas, L., Miserey-Lenkei, S., Lemichez, E., Coudrier, E., and Bassereau, P. (in preparation). Ezrin binds to curved membranes via mechanisms involving its phosphorylation and binding to IRSp53.

M195

Super-resolution imaging reveals differential clustering of microtubule motors on vesicle membranes.

G.A. Cordier[1], M. Lakadamyali[1,2], P.A. Gomez-Garcia[1], A. Sandoval[1];
[1]Advanced Fluorescence Imaging Biophysics Group, ICFO, Barcelona, Spain, [2]Physiology, Perelman School of Medicine (UPenn), Philadelphia, PA

Motor proteins play an essential role in cellular organization by carrying organelles along microtubules and delivering them at the right place. Recently, the clustering of dynein motors has emerged as a novel mechanism of regulating retrograde trafficking of large vesicles. We aimed to determine whether similar clustering mechanisms of retrograde and anterograde motor proteins play a role in the trafficking of small vesicles in intact cells. Using multi-color, 3D super-resolution microscopy and quantitative analysis, we revealed the organization of dynein, Kif5 and Kif3 on the membrane of lysosomes at the nanoscale level. Our results show that all motor proteins are organized in small teams composed of between 1-4 motor proteins on the lysosome membrane. However, the total number of motor teams on the membrane is differentially regulated for the three motors. In addition, this number scales differentially with the size of the lysosome among the different motors. Overall, our results reveal for the first time how various motor proteins are organized on the membrane of small vesicles in intact cells, giving new insights into mechanisms of transport regulation by motor protein clustering.

M196

Diffusion as a ruler: Modeling kinesin diffusion as a length sensor for intraflagellar transport.

N.L. Hendel[1], M. Thomson[2], W.F. Marshall[1];
[1]Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA, [2]Biology and Biological Engineering, California Institute of Technology, Pasadena, CA

An important question in cell biology is whether cells are able to measure size, either whole cell size or organelle size. Perhaps cells have an internal chemical representation of size that can be used to precisely regulate growth, or perhaps size is just an accident that emerges due to constraint of nutrients. The eukaryotic flagellum is an ideal model for studying size sensing and control because its linear geometry makes it essentially one-dimensional, greatly simplifying mathematical modeling. The assembly of flagella is regulated by intraflagellar transport (IFT), in which kinesin motors carry cargo adaptors for flagellar proteins along the flagellum and then deposit them at the tip, lengthening the flagellum. The rate at which IFT motors are recruited to begin transport into the flagellum is anticorrelated with the flagellar length, implying some kind of communication between the base and the tip and possibly indicating that cells contain some mechanism for measuring flagellar length. Although it is possible to imagine many complex scenarios in which additional signaling molecules sense length and carry feedback signals to the cell body to control IFT, might the already-known components of the IFT system be sufficient to allow length dependence of IFT? Here, we investigate a model in which the anterograde kinesin motors unbind after cargo delivery, diffuse back to the base, and are subsequently reused to power entry of new IFT trains into the flagellum. By modeling such a system at three different levels of abstraction we are able to show that the diffusion time of the motors can in principle be sufficient to serve as a proxy for length measurement. In all three implementations, we found that the diffusion model can not only achieve a stable steady-state length without the addition of any other signaling molecules or pathways, but also is able to produce the anticorrelation between length and IFT recruitment rate that has been observed in quantitative imaging studies.

M197

Mechanical force induces mitochondrial fission via the canonical fission machinery.

Q. Feng[1], S.C. Helle[1], B. Kornmann[1];
[1]Institute of Biochemistry, ETH Zurich, Zurich, Switzerland

Eukaryotic cells are densely packed with macromolecular complexes and intertwining membranous organelles that continually change shape and engage in active trafficking. It is intriguing that organelles avoid clashing and entangling with each other during such dynamic movements in such limited space. Here we describe a mechanism that explains how mitochondria orderly cohabit with other organelles in the crowded space of the cytoplasm. Mitochondria form extensive networks that are constantly remodeled by fission and fusion events. While the molecular machineries that execute mitochondrial fission and fusion processes are relatively well documented, little is known about what triggers these events and determines the fusion and fission sites. We show here that mechanical stimulation of mitochondria – via the encounter with motile intracellular pathogens, via external pressure applied by an atomic force microscope, or via cell migration across uneven microsurfaces – resulted in the recruitment of the canonical mitochondrial fission machinery and subsequent fission. The mitochondrial fission factor (MFF) acts as a membrane-bound force sensor preferentially accumulating at mitochondria of reduced diameter, then recruiting the fission machinery to sites of mechanical strain. Thus, mitochondria may avoid entanglement with itself and other cellular structures by responding to biomechanical cues. These results shed new light on mitochondrial dynamics, an important process that

has been shown to influence cell migration, cardiovascular functions, neuronal plasticity, aging and more. That mechanical triggers can be coupled to biochemical responses in membrane dynamics also provides a new perspective in studying organelle communication in general.

M198

DYRK3 kinase regulates dissolution and condensation of membrane-less organelles during mitosis.

A.K. Rai[1], J. Chen[2], M. Selbach[2], L. Pelkmans[1];
[1]IMLS, University of Zurich, Zurich, Switzerland, [2]MDC, Max Delbruck Center, Berlin, Germany

Cell undergoes many biochemical reactions in compartments that are not surrounded by any lipid membranes called membrane-less organelles. Recently, it has been proposed that the mechanism of condensation of such compartments relies on a physical principle called phase separation, which is driven by weak molecular interactions between low-complexity domains (LCDs) within proteins and RNA. However, the cellular mechanisms regulating the dynamic condensation and dissolution of these compartments remains poorly understood. Interestingly during early mitosis , concomitant with nuclear envelope breakdown, multiple membrane-less organelles undergo dissolution, and then re-condense towards the end of mitosis. We show that the dual-specificity kinase DYRK3 interacts with, and localizes to multiple membrane-less organelles like stress granules, splicing speckles and pericentriolar satellites. Inhibition of DYRK3 kinase activity during mitosis results in co-condensation of multiple proteins from these compartments, and poly-adenylated RNA into aberrant hybrid structures. The formation of such aberrant structures in mitotic cells results in mitotic delay because of extended metaphase. We have been able to demonstrate that DYRK3 acts as a dissolvase during mitosis and increase in DYRK3 kinase to its substrate concentration ratio as a consequence of nucleo-cytoplasmic dilution post nuclear envelope breakdown allows cells to undergo rapid dissolution of organelles during early mitosis. Further, the re-condensation of organelles during late mitosis can be explained by our observation that the APC/C-CDH1 driven ubiquitination degrades DYRK3 during late mitosis, decreasing DYRK3 to substrate ratio. Thus, changes in relative concentration of the dissolvase (DYRK3) to the phase separating proteins (DYRK3 substrate) can explain the phenomenon of complete dissolution of multiple organelles during early mitosis and their re-condensation later in mitosis.

Minisymposium 20: RNA Biology

M199

A new class of repeat-enriched non-coding RNAs regulating activity of RNA-binding proteins.

K. Yap[1], E.V. Makeyev[1];
[1]Centre for Developmental Neurobiology, King's College London, London, United Kingdom

RNA-binding proteins (RBPs) have a wide range of cellular functions that must be tightly regulated. Some RBPs are known to accumulate in non-membrane-bound compartments but the mechanisms underlying this process are only beginning to be elucidated. Here we developed a customized bioinformatics workflow to systematically identify mammalian RNAs containing repeated RBP interaction motifs. Most of the transcripts discovered by this approach were enriched in so-called short tandem repeats that consist of 2-12 nt-long sequence units concatenated in a head-to-tail manner. A top-scoring example of this category was a long noncoding RNA predicted to bind multiple copies of polypyrimidine tract-binding protein (PTBP1), an important regulator of pre-mRNA splicing in the nucleus and pro-apoptotic mRNA translation in the cytoplasm. The new transcript, which we named

PNCTR, is expressed at elevated levels in various types of cancer cells where it localizes to the perinucleolar cap (PNC), a nuclear body previously shown to sequester PTBP1 by an unknown mechanism. Loss of PNCTR abrogates PTBP1 localization to the PNC thus suggesting that this RNA plays a critical role in the PNC assembly. Importantly, PNCTR knockdown reduces cancer cell proliferation at least in part by altering PTBP1 activity and promoting programmed cell death. Overall, our work uncovers a new class of short tandem repeat-enriched RNAs that may play a critical role in cancer progression by recruiting their RBP partners to specific subcellular compartments.

M200

RNA structure drives stress granule assembly in yeast extracts.

K. Begovich[1], J.E. Wilhelm[1];
[1]Biological Sciences, University of California, San Diego, La Jolla, CA

Stress granules are membrane-less organelles that are comprised of proteins involved in RNA metabolism and their mRNAs targets. Formation of stress granules is associated with down-regulation of global translation and act as a storage center for mRNAs stalled in translation initiation resulting in major changes in cell physiology. Additionally, alterations in the phase separation behavior of many stress granule proteins contribute to several neurodegenerative diseases. Thus understanding stress granule formation and function is likely to provide insights into this class of diseases.

While most biochemical studies have focused on how individual proteins in stress granules can phase-separate, there is little understanding of how multiple proteins and transcripts come together in a stress granule. In order to understand the how stress granules assemble, we have developed a yeast extract system that allows stress granules to be assembled in vitro when RNA is added to the extract. We validated our in vitro assembled stress granules in two ways. First, in vitro assembled stress granules contain specific stress granule markers while excluding proteins that are specific to other RNA granules arguing that they are not non-specific RNA aggregates. Furthermore, extracts made from yeast with mutations that disrupt stress granule assembly fail to form stress granules in vitro. This argues that the in vitro stress granules assemble via the same pathways as the in vivo stress granules.

Access to this extract system has allowed us address the role of RNA in stress granule formation. We have found that not all RNAs are capable of triggering stress granule assembly. The ability to assemble a stress granule is not tied to specific sequence motifs, translatability, or the propensity of a message to phase separate. Instead, the presence of secondary structure appears to be a key feature in triggering stress granule assembly. This suggests a simple model for stress granule assembly where energy depletion decreases the activity of factors that unwind RNA. This leads to an increase in RNA secondary structure triggering stress granule formation. We are currently using our system to further test this model as well as identify proteins and metabolites that regulate of stress granule assembly

M201

RNA sequence determines specificity and identity of polyQ-driven phase separations.

E.M. Langdon[1], A.S. Gladfelter[1];
[1]Biology, University of North Carolina, Chapel Hill, NC

Cells rely upon compartmentalization for the spatial and temporal control of their biochemistry. A newly appreciated mechanism for this organization is the formation of non-membrane bound organelles through the condensation of macromolecules. In the multinucleate fungus, *Ashbya gossypii*, nuclear

division occurs asynchronously, and multiple sites of polarized growth coexist in a continuous cytoplasm. These phenomena are reliant upon the RNA-binding protein, Whi3, undergoing liquid de-mixing with its target mRNAs to form phase-separated droplets, positioning cyclin (*CLN3*) transcripts near nuclei and formin (*BNI1*) transcripts at sites of polarized growth. We are interested in how Whi3 functions in two separate cellular processes and how co-existing Whi3 droplets remain physically distinct. We have shown in vitro that *CLN3* and *BNI1* mRNAs drive Whi3 to phase separate into droplets with different biophysical properties, suggesting different mRNAs promote physically and functionally distinct droplets. We have additionally observed, using single molecule mRNA FISH, that *CLN3* and *BNI1* transcripts do not co-localize in the cell. Consistent with this, we find that pre-formed droplets containing Whi3 with *BNI1* mRNA are immiscible with Whi3 droplets containing *CLN3* mRNA in vitro. Remarkably, another Whi3 target mRNA involved in cell polarity, *SPA2*, readily mixes into *BNI1* droplets, indicating functionally related mRNAs share features that enable them to co-condense. This provides evidence that the nucleotide sequence or other features of RNAs may be encoding Whi3-droplet identity and spatial segregation within the cell. Recently, others have shown that mRNAs can self-assemble in pathological contexts to form liquid droplets. We find that *CLN3*, *BNI1*, and *SPA2* can all phase separate in a Whi3-free context. Notably, when *CLN3* is mixed with *BNI1*, the two mRNAs form distinct structures and do not co-localize, whereas *BNI1* and *SPA2* readily mix. This supports the idea that this behavior is intrinsic to the mRNAs themselves. We further speculated that Whi3 is binding to specific secondary structures of the mRNAs, forming distinct complexes depending on the bound mRNAs. Supporting this, we find that *CLN3* with disrupted secondary structure is completely miscible with BNI1 droplets, suggesting that indeed, secondary structure is critical for the separation of distinct Whi3-mRNA complexes. These data are the first example of how specificity can be achieved in intracellular condensates and shows that mRNAs can encode this specificity based on their secondary structure.

M202

Probing the search dynamics of RNA polymerase in live E. coli cells.

K.E. Bettridge[1], C.H. Bohrer[1], J. Xiao[1];
[1]Biophysics and Biophysical Chemistry, Johns Hopkins School of Medicine, Baltimore, MD

Gene expression and gene regulation of E. coli in response to environmental stimuli relies on the ability of RNA polymerase (RNAP) to navigate the crowded milieu of the cytoplasm to find its target in a responsive, efficient manner. While the kinetics of transcription and promoter search have been extensively studied in vitro, the details of the search mechanism in living cells is not well understood. In E. coli, proteins make up approximately 50% of the total volume, which leads to noticeable effects such as anomalous diffusion. To probe the search mechanism of RNAP in the context of a crowded environment, we use high-resolution single molecule tracking in combination with statistical modeling to provide a detailed dynamic model of RNAP movement in live cells. We found that RNAP exists in three distinct diffusive modes, each with their own diffusion coefficient, that corresponded to particular search modes of RNAP- DNA-bound RNAP, RNAP that is undergoing rapid association and dissociation from the nucleoid, and freely diffusing RNAP. Furthermore, RNAP can undergo diffusive mode state-switching with defined kinetic rates, enabling us to parse out a clear kinetic model of RNAP movement. We utilize genetic manipulations and drug treatments to perturb the transcriptional state of the cell in predictable ways to verify these diffusive modes as well as the kinetics of state-switching. Noticeably, we find that RNAP state-switching is likely not in steady state, with implications that non-specifically bound RNAP is the rate-limiting step in promoter search.

M203

Ire1 RNase specificity separates transcriptional and post-transcriptional regulation of ER protein homeostasis.

W. Li[1], V. Okreglak[1], J. Peschek[1], P. Kimmig[1], P. Walter[1,2];
[1]Department of Biochemistry and Biophysics, University of California San Francisco , San Francisco, CA, [2]Howard Hughes Medical Institute, University of California San Francisco , San Francisco, CA

The endoplasmic reticulum (ER) is the major folding compartment for most secretory and plasma membrane proteins in the cell. A conserved signaling pathway, the unfolded protein response (UPR), senses and modulates the folding capacity of the ER. To maintain ER protein homeostasis under ER stress conditions, the ER membrane embedded sensor, Ire1, binds unfolded proteins through its ER-lumenal domain and initiates two distinct mRNA processing programs through its cytoplasmic kinase/RNase domains. First, in both metazoans and *S. cerevisiae*, Ire1 catalyzes the unconventional cytoplasmic mRNA splicing of *XBP1* (metazoans) or *HAC1* (*S. cerevisiae*)—thereby initiating a transcriptional response that increases the ER folding capacity. Second, in metazoans and *S. pombe*, Ire1 selectively degrades ER-localized mRNAs—thereby post-transcriptionally reducing the ER's protein folding burden through regulated Ire1-dependent mRNA decay. Thus, Ire1 homologs in *S. cerevisiae* and *S. pombe* are specialized to only one of the two functional outputs, while Ire1 in metazoans can perform both. To understand how Ire1 can regulate protein homeostasis through distinct RNA processing programs mechanistically, we characterized Ire1 from *S. cerevisiae* and *S. pombe* with *in vivo* and *in vitro* experiments. Surprisingly, despite relatively low sequence conservation in the lumenal domains, these domains share conserved ER-stress sensing mechanism. Conversely, despite high sequence conservation, Ire1 cytoplasmic domains recognize distinct RNA sequence and structural features, which leads to functional divergence in RNA processing. Finally, by applying our new findings, we successfully reconstituted unconventional mRNA splicing in *S. pombe* cells. Therefore, we engineered *S. pombe* into a metazoan-like Ire1 system, where unconventional mRNA splicing and selective mRNA decay co-exist. Our results provide new insights into a mechanistic understanding of Ire1 function and its interplay with RNA substrates.

M204

THE RNA EXOSOME REGULATES DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS.

C. Belair[1,2], K. Kim[3], Y. Tanaka[3], I. Park[3], S.L. Wolin[1,2];
[1]RNA Biology Laboratory, National Cancer Institute, Frederick, MD, [2]Cell Biology, Yale School of Medicine, New Haven, CT, [3]Yale Stem Cell Center, Yale School of Medicine, New Haven, CT

The unique abilities of human embryonic stem cells (hESCs) to self-renew and to differentiate into all three germ layers (endoderm, mesoderm, ectoderm) are linked to their "open chromatin", which allows high levels of transcription and contributes to pluripotency by keeping many tissue-specific genes in permissive transcriptional states. As a consequence, hESCs express many RNAs at higher levels than differentiated cells, including potentially harmful RNAs such as endogenous retrotransposons and mRNAs that encode proteins promoting differentiation. Although both the proteasome and RNA interference pathway contribute to controlling this pervasive transcription, the role of RNA surveillance pathways in degrading potentially deleterious RNAs is largely unknown. Here we report that the RNA exosome, a major ribonuclease complex, restrains hESCs from differentiating into endoderm, mesoderm and ectoderm. We generated clonal hESC lines expressing doxycycline-inducible shRNAs directed against the core exosome subunit EXOSC3/hRRP40. Although EXOSC3 depletion does not affect the proliferation of hESCs or the expression of pluripotency markers, we uncovered a role for the exosome in preventing

differentiation. In these experiments, we allowed hESCs to differentiate into embryoid bodies and assayed for the expression of markers corresponding to each of the three germ layers. Interestingly, embryoid bodies derived from EXOSC3-depleted hESCs exhibited up to 10-fold level increases in mRNAs encoding ectoderm, endoderm and mesoderm markers, compared to control cells. Consistent with the idea that the exosome is critical for preventing hESC differentiation, we found that downregulation of exosome subunits is an early event in the differentiation of wild-type hESCs. To determine the set of RNAs targeted by the exosome, we combined whole transcriptome analysis (RNA-Seq) with high throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP). We found that the exosome reduces the levels of specific miRNAs, long noncoding RNAs and mRNAs as well as potentially active retrotransposon transcripts. Importantly, we showed that the exosome restrains differentiation in part by degrading pre-mRNA and mRNA encoding a transcription factor crucial for mesendoderm formation. Together, our data establish the RNA exosome as a new regulator of hESC differentiation and reveal the importance of RNA decay pathways in maintaining ESC pluripotency.

M205

Asymmetric Distribution of Hexose Transporter mRNA Provides a Growth Advantage.

T. Stahl[1], S. Hümmer[1], A. Spang[1];
[1]Biozentrum, University of Basel , Basel, Switzerland

Asymmetric localization of mRNA is important for cell fate decisions in eukaryotes and provides the means of localized protein synthesis in a variety of cell types. Here we show that hexose transporter mRNAs are retained in the mother cell until metaphase-anaphase transition (MAT) and then released in the bud in *S. cerevisiae*. The retained mRNA was translationally inactive but was already ribosome-bound before MAT. Treatment with a translation initiation inhibitor caused degradation of HXT mRNA, while blocking elongation, prevented release of the mRNA into the bud, indicating translational control of mRNA localization. Consistently, unlike in large added cell, in small and medium budded cells, HXT proteins were not found at the plasma membrane in the bud. Importantly, when cells were released from starvation into rich, glucose containing medium, HXT2 mRNA, but none of the other HXT mRNAs tested, was enriched in the bud after MAT. This enrichment was dependent on the cAMP/Ras2/PKA pathway, microtubules and correct nuclear segregation into the bud. Using strains that only expressed one hexose transporter allowed us to demonstrate that Hxt2 only strains grow faster than their counterparts. Therefore, asymmetric distribution of HXT2 mRNA provides a growth advantage for young daughters who are better prepared for nutritional changes in the environment. Our data provides strong evidence that asymmetric mRNA localization is an important factor in determining cellular fitness and may influence lifespan as well as aging.

M206

Ribosome Heterogeneity in Translating the Genetic Code.

M. Barna[1];
[1]Developmental Biology and Genetics, Stanford University School of Medicine, Stanford, CA

The central dogma of molecular biology has for decades served as an explanation for the flow of genetic information within a biological system. In so far as the transmission of biological information, the ribosome has been perceived to decode the genome with machine-like precision; serving as an integral but largely passive participant in the synthesis of all proteins across all kingdoms of life. Our research has fundamentally changed this view, by demonstrating that not all of the millions of ribosomes within each cell are the same and that ribosome heterogeneity provides a novel means for diversity of the

proteins that can be produced in specific cells, tissues, and organisms. I will present our work centered on providing a roadmap for the characterization of ribosome composition at a single cell level and during cellular differentiation. We employed a highly quantitative mass spectrometry-based approach to precisely quantify the abundance of each ribosomal protein (RP) as well as a large cohort of ~ 400 ribosome associating proteins (RAPs) belonging to actively translating ribosomes within stem cells. This led to the identification of subsets of ribosomes that are heterogeneous for RP composition. To further address the functional role of ribosome heterogeneity in translational control of the mammalian genome, we employed CRISPR/Cas9 to endogenously tag and purify heterogeneous ribosome populations. We then developed an adapted ribosome profiling method to precisely quantify and characterize the nature of mRNAs translated by distinct heterogenous ribosomes genome-wide. This led to the identification of subpools of transcripts, critical for key cellular processes including cell signaling, metabolism, growth, proliferation and survival, which are selectively translated by specific types of ribosomes. Most remarkably, there are specific signaling pathways where almost every single component is selectively translated by specialized ribosomes demarcated by a single RP. I will further present recent findings on the mechanisms by which ribosome-mediated control of gene expression is encoded by structured RNA regulons within 5'UTRs. Our findings suggest that 3D shapes present in the mRNA template provide a rich language that is read by different classes of ribosomes. Together, these studies reveal a critical link between ribosome heterogeneity and specialized translational control of the mammalian genome, which adds an important layer of control to the post-transcriptional circuitry of gene regulation.

M207

mRNAs that transfer between mammalian cells are translated in acceptor cells.

G. Haimovich[1], S. Dasgupta[1], J.E. Gerst[1], R.H. Singer[2,3];
[1]Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel, [2]Anatomy Structural Biology, Albert Einstein College of Medicine, Bronx, NY, [3]Janelia Research Campus of the Howard Hughes Medical Institute, Ashburn, VA

We recently demonstrated that full-length mRNAs undergo transfer between mammalian cells through a mechanism that involves cytoplasmic extensions, called membrane nanotubes that connect donor and acceptor cells. However, the biological significance of intercellular transfer hinges on whether these mRNAs undergo translation and affect acceptor cell physiology. By using a reporter mRNA that allows for the detection of translating single mRNA molecules, we show that a transferred mRNA is translated in acceptor cells. Also, using a complementation assay to test the effect of mRNA transfer on acceptor cell physiology, we co-cultured cells expressing Heat-Shock Factor-1 (HSF1) with $HSF1^{-/-}$ knockout (KO) cells. HSF1 is a transcription factor that induces the transcription of several genes, including HSP70, upon heat-shock. Our preliminary results show that in co-culture, HSP70 transcription in $HSF1^{-/-}$ cells is increased when compared to KO cells cultured alone, indicating that HSF1 mRNA was translated after transfer. Other complementation assays are currently being tested. In parallel, a whole-proteome approach is being developed to assess the extent of translating transferred mRNAs and the results will be presented. This phenomenon may prove important for the proper development and functioning of tissues, regulation of the tumor microenvironment, as well as for host-parasite or symbiotic interactions. The work which shows that mRNAs transfer through membrane nanotubes was posted on bioRxiv (#137836) and is currently under revision in PNAS. This data, on nanotube-mediated transfer, will be presented in the ASCB-EMBO special interest subgroup symposium "Tunneling nanotubes: intercellular highways, new frontiers for deciphering intercellular communication in disease."

M208

Long-range function of secreted small nucleolar RNAs that direct 2'-O-methylation.

J.M. Rimer[1], J. Lee[1], C.L. Holley[1], R.J. Crowder[1], D.L. Chen[2], P.I. Hanson[3], D.S. Ory[1], J.E. Schaffer[1];
[1]Medicine, Washington University, St Louis, MO, [2]Mallinckrodt Institute of Radiology, Washington University, St Louis, MO, [3]Cell Biology Physiology, Washington University, St Louis, MO

Small nucleolar RNAs (snoRNAs) are non-coding RNAs that guide chemical modifications of structural RNAs in cells. While snoRNAs are primarily localized in the nucleolus, where their canonical function is to target nascent ribosomal RNAs for 2'-O-methylation, recent studies provide evidence that snoRNAs traffic out of the nucleus. Furthermore, high-throughput sequencing data indicate that extracellular vesicles released from cells contain snoRNAs. However, it is not known whether snoRNA secretion is regulated or whether secreted snoRNAs are functional. We found that box C/D snoRNAs from the Rpl13a locus are secreted in response to treatment with lipopolysaccharide, an inflammatory stimulus, in cultured macrophages, in mice, and in human subjects. Secreted Rpl13a snoRNAs co-fractionate with extracellular vesicles, float in a continuous sucrose gradient with extracellular vesicle membrane protein markers at a density of 1.17 g/cm2 , and are protected from RNAse treatment. In a murine parabiosis model in which shared circulation was established between Rpl13a-snoRNA knockout and wild type mice, we demonstrate that snoRNAs travel through the circulation to function in distant tissues. These findings support a previously unappreciated link between inflammation and snoRNA secretion in mice and humans and uncover a potential role for secreted snoRNAs in cell-cell communication.

Microsymp 13: Tissue Structure and Cell-Cell Interactions

E85

Characterization of the mechanically-induced shape change of erythrocytes into polyhedrocytes.

V. Tutwiler[1], A.R. Mukhitov[2], A.D. Peshkova[2], G. Le Minh [2], J. Vicksman[1], C. Nagaswami[1], R.I. Litvinov[1], J.W. Weisel[1];
[1]University of Pennsylvania , Philadelphia, PA, [2]Kazan Federal Research University , Kazan, Russia

Polyhedral structures have been observed in nature since the time of Aristotle; however, most research has focused on Platonic and Archimedean solids rather than irregular polyhedra. Moreover, these tightly packed cells were recently observed in contracted blood clots, where the highly deformable erythrocytes were compressed into the core of the clot and formed a tessellated network. This resulted in a shape change from the normal biconcave cell to a polyhedral shape; consequentially the terminology polyhedrocytes was coined. Since hemostatic and thrombotic disorders are leading causes of death and disability worldwide, exploring the nature of the shape change from biconcave erythrocytes to polyhedrocytes has the potential to inform therapeutic targets. Here, we use histology, transmission electron microscopy, scanning electron microscopy and confocal microscopy to visualize and quantify the mechanical deformation of erythrocytes. We determined that this shape change is linked to the presence of contractile forces that are generated by the platelets and highly influenced by the composition of the clot. The presence of more platelets or more platelet activity, as induced by a higher thrombin concentration, resulted in a direct correlation with the percentage of polyhedrocytes present. Likewise, the inhibition of contractile proteins or the ability of platelets to propagate contractile force resulted in softer clots with fewer polyhedrocytes present on the inside of the clot. Through the

use of confocal microscopy, we were able to reconstruct the three-dimensional structure of erythrocytes from contracted blood clots and compare bioconcave cells, polyhedrocytes and intermediate forms. Interestingly, as the erythrocytes undergo deformation, there was no change in the surface area, volume, or sphericity of the cells. The deformed erythrocytes and polyhedrocytes became less oblate and more prolate than the biconcave cells. Collectively, these studies reveal that polyhedrocytes are convex, irregular polyhedra. The polyhedrocytes had a total number of faces that ranged from 10 to 16 with a median of 13 faces. The faces were made up of polygons with 3 to 6 sides, with the majority of the faces being quadrilaterals. Taken together, these results point to the importance of studying the deformation of erythrocytes as a result of clot contraction into a tightly packed network, since these structures could provide a key function of replicating the barrier function of the endothelial layer post injury and/or influence the outcome of thrombotic conditions.

E86

A role for desmosomal cadherins in creating complex tissues.

J.A. Broussard[1], O. Nekrasova[1], J.L. Koetsier[2], K.J. Green[1];
[1]Pathology and Dermatology, Northwestern University, Chicago, IL, [2]Pathology, Northwestern University, Chicago, IL

The evolution of multicellularity was facilitated by the emergence of cell-cell adhesion molecules in the cadherin family, which couple cell-cell adhesion sites to the actin cytoskeleton to regulate tissue integrity, polarity, and morphogenesis. As metazoans evolved, tissue morphologies with increasingly specialized functions appeared in concert with desmosomal (DSM) cadherins. One of the best examples is the epidermis, in which multiple DSM cadherins give rise to specific types of intercellular connections and cytoskeletal attachments throughout the cell layers. We hypothesized that the functional overlay of these patterned DSM cadherins onto classic cadherins facilitated new mechanisms to increase tissue structural and functional complexity during evolution.
We addressed this question for the DSM cadherin desmoglein 1 (Dsg1), which is first expressed as keratinocytes initiate a program of epidermal differentiation and exit the basal layer, and is later concentrated in the superficial layers. Using a genetically manipulable epidermal reconstitution model, we show that Dsg1 promotes delamination of basal cells, inducing stratification. We identified two Dsg1-associated modules required for Dsg1 function: a dynein motor complex and an Arp2/3/cortactin actin remodeling complex. Using genetic, pharmacologic, FRET, and laser ablation approaches, we show that dynein-dependent positioning of Dsg1/cortactin/Arp2/3 drives actin remodeling to reduce cortical tension and promote delamination of basal cells. Importantly, ectopic expression of Dsg1, normally absent in simple epithelia, is sufficient to induce stratification and formation of a second adherent cell layer in simple epithelial MDCK cells. Based on its role in regulating cortical tension during stratification, we tested whether Dsg1 coupling to the actomyosin system also contributes to suprabasal functions. Molecular indicators of tension and microablation experiments revealed the existence of a mechanical gradient in stratified control epidermis, with highest tension in the superficial layers. Loss of Dsg1 shifts the mechanical gradient towards the basal layers of the epidermis. Dsg1 silencing altered indicators of the force-sensitive adherens junction component vinculin and tight junction (TJ) component ZO1, the latter which contributes to formation of the superficially restricted TJ barrier. Moreover, our data suggest that Dsg1 regulates TJs through the ErbB family member ErbB2, which in human skin is localized to cell-cell contacts in the TJ-forming layer. Together, our data suggest a model in which Dsg1 regulates the formation and function of the epidermis by controlling the distribution of mechanical inputs to drive morphogenesis and the development of the life-essential epidermal barrier.

E87

Flares of active Rho and F-actin locally reinforce the tight junction barrier in response to mechanical stress.

R.E. Stephenson[1], T. Higashi[1,2], I. Erofeev[3], B. Coy[1], T.R. Arnold[1], A. Goryachev[3], A.L. Miller[1];
[1]Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, [2]Fukushima Medical University, Fukushima, Japan, [3]Centre for Systems Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom

The epithelial barrier is important for generating specialized compartments in multicellular organisms. The barrier property of vertebrate epithelia is dependent on tight junctions, which restrict the flow of ions, water, and small molecules between epithelial cells. Epithelia are subject to a number of cell- and tissue-scale forces, such as cell divisions, extrusions, wound healing, and morphogenetic events. During these events, cell-cell junctions must remodel to accommodate changes in cell shape and mechanical force. However, very little is known about how tight junctions are able to maintain barrier function during these events. In order to examine this question, we developed a highly sensitive barrier assay compatible with live imaging. When we applied this barrier assay to gastrula-stage *Xenopus laevis* embryos, we found that the epithelial barrier is not uniform across space and time. Instead, small barrier breaches occur – often around dividing cells – and persist for minutes before barrier function is restored.
We have previously reported that "flares" of active Rho accumulate at cell-cell junctions, particularly in situations where the cell-cell junctions are compromised (1, 2). Because Rho flares occur on similar time scales and at similar locations as barrier breaches, we investigated whether the two may be correlated in space and time. Indeed, we found that local barrier breaches are followed by local increases in Rho activity. As Rho returns to baseline levels, the barrier function is restored. Thus, we hypothesized that Rho may be involved in restoring barrier function on subcellular scales. To further investigate this possibility, we examined fluorescently-tagged tight junction proteins ZO-1 and Occludin with respect to Rho. Intriguingly, we observed local discontinuities in ZO-1 and Occludin prior to the flare, and both proteins remained locally increased over baseline, or reinforced, following the flare. In order to investigate how active Rho contributes to reinstatement of the barrier and reinforcement of ZO-1, we perturbed actin polymerization and junction contraction using pharmacological tools. Both junction contraction and actin polymerization appear to contribute to ZO-1 reinforcement, as disruption of either results in only partial ZO-1 reinforcement. When both junction contraction and actin polymerization are lost, ZO-1 fails to be reinforced, and the barrier function is not reinstated. Taken together, these data indicate that transient breaches of the tight junction barrier arise in response to mechanical force, and Rho flares serve to rapidly repair these breaches, preserving the overall barrier function of the epithelium.

E88

A non-canonical Notch signaling complex regulates adherens junctions and endothelial barrier function.

M.L. Kutys[1,2], W.J. Polacheck[1,2], J. Yang[1], J. Eyckmans[1,2], Y. Wu[3], K.K. Hirschi[3], C.S. Chen[1,2];
[1]Biomedical Engineering, Boston University, Boston, MA, [2]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, [3]Cardiovascular Research Center, Yale University, New Haven, CT

The vascular barrier that separates blood from tissues is actively regulated by the endothelium and is essential for transport, inflammation, and hemostasis. Hemodynamic shear stress plays a critical role in

maintaining endothelial barrier function, but how this occurs remains unknown. Here, using an engineered organotypic model of perfused microvessels and confirming in mouse models, we identify that activation of the Notch1 transmembrane receptor directly regulates vascular barrier function through a non-canonical, transcription independent signaling mechanism that drives adherens junction assembly. Shear stress triggers proteolytic activation of Notch1 and the release of the Notch1 transmembrane domain, which is the key domain that mediates barrier establishment. Expression of the Notch1 transmembrane domain is sufficient to rescue Notch1 knockout-induced defects in barrier function, and does so by catalyzing the formation of a novel receptor complex in the plasma membrane consisting of VE-cadherin, the transmembrane protein tyrosine phosphatase LAR, and the Rac1 GEF Trio. This complex locally activates Rac1 at cell-cell contacts to drive the formation of cortical actin fibers, strengthen adherens junctions, and establish barrier function. Canonical Notch transcriptional signaling is highly conserved throughout metazoans and is required for many processes in vascular development, including arterial-venous differentiation, angiogenesis, and remodeling; here, we establish the existence of a previously unappreciated non-canonical cortical signaling pathway for Notch1 that regulates vascular barrier function, and thus provide a mechanism by which a single receptor might link transcriptional programs with adhesive and cytoskeletal remodeling.

E89

Cell-cell adhesion and myosin activity controls the curvature-dependent cortical actin assembly in mammary gland epithelium.

W. Jung[1], K. Elawad[1], S.H. Kang[1], Y. Chen[1];
[1]Mechanical Engineering, Johns Hopkins University, Baltimore, MD

It has been demonstrated in recent studies that geometry might play an important role in cell behaviors, ranging from mucus production, cell migration and the origination and prorogation of cancer stem cells. Despite the studies on potentially curvature-sensitive proteins at the nanoscale, it is not clear how cells sense curvature and other geometric parameters at cellular and multi-cellular-level. To systematically study whether and how curvature determines cell behaviors, we developed a technique combining 3D printing and soft lithography to fabricate open channels with radii ranging from 40µm to 90µm. We examined various protein expression levels of mouse mammary epithelial cells EPH4-EV cells at different curvatures. EPH4-EV cells are used to simulate the formation of mammary gland epithelium, which has been observed previously to exhibit differential phenotypes depending on the local geometry of the mammary ducts. We found that cortical actin is 1.3-fold more enriched in cells grown on curvature of 1/60µm and 1.5 fold more enriched in 1/40µm when compared to curvature of 1/90µm. Inhibiting myosin phosphorylation via blebbstatin restored the cortical actin formation in cells cultured in channels of high curvatures, implying myosin is involved in curvature-dependent cortical actin assembly. Furthermore, it was also observed that cell-cell contact is required in the curvature-dependent cortical actin assembly. The prominent cortical actin assembly usually seen at 1/40µm and 1/60µm was not detected when the cell seeding density resulting in sparse cell-cell contact. Our finding suggests myosin-mediated cortical actin assembly might be a length scale-appropriate machinery for curvature sensing at the multi-cellular level.

E90

Desmosomal Regulation of Gap Junctions via Ras: Implications for Cardiocutaneous Disease.

C.Y. Kam[1], A.D. Dubash[2], F. Sheikh[3], P.D. Lampe[4], S. Polo[5], K.J. Green[1,6];
[1]Department of Pathology, Northwestern University, Chicago, IL, [2]Department of Biology, Furman University, Greenville, SC, [3]Department of Medicine, University of California-San Diego, La Jolla, CA, [4]

Translational Research Program, Fred Hutchinson Cancer Research Center, Seattle, WA, [5]FIRC Institute of Molecular Oncology, Milan, Italy, [6]Department of Dermatology, Northwestern University, Chicago, IL

The mammalian heart is a vital organ comprising specialized muscle cells, or cardiomyocytes (CMs) that adhere to each other via intercalated discs, specialized junctions made up of elements from desmosomes, adherens junctions, and gap junctions. These junctions are crucial for integrating mechanical, chemical, and electrical signals to coordinate cardiac rhythmic contraction. Desmoplakin (DP) is an essential component of desmosomes, that tethers intermediate filaments to sites of cell adhesion. DP is a frequent target for mutation in cardiocutaneous disorders. In particular, DP mutations cause arrhythmogenic cardiomyopathy (AC), an inherited disorder characterized by replacement of healthy myocardium by fibro-fatty deposits that presents with or without cutaneous defects. During AC pathogenesis, a loss of electrical conduction between CMs can occur prior to fibro-fatty infiltration, an event that has been dubbed the “concealed phase”. Providing a possible explanation for the conduction defect, DP loss has been linked to decreased levels of the gap junction protein Connexin-43 (Cx43). However, the mechanism by which loss of DP decreases Cx43 expression is unknown.
We utilized *in vitro* and *in vivo* models of DP deficiency in heart and skin to elucidate the mechanism by which DP regulates Cx43 protein levels. Depletion of DP in rat CMs led to lowered Cx43 protein levels, consistent with prior *in vivo* studies. Gap junction dynamics are regulated by a number of phosphorylation sites on the Cx43 C-terminus. Systematic analysis of known Cx43 phospho-sites in DP-deficient CMs revealed a specific increase in the MAPK sites S279/282, previously shown to signal internalization and degradation of Cx43. Elevated phosphorylation was also conserved in cardiac and epidermal tissues from mice with conditional ablation of DP. Furthermore, elevated p-Cx43 to total Cx43 signal ratio is observed in a cardiac patient sample harboring a pathogenic AC mutation. DP depletion in CMs accelerated loss of Cx43, which was prevented by inhibitors of lysosomal, but not proteosomal, degradation.
RNA-sequencing analysis revealed Ras-GTPases as candidates for DP-dependent activation of the ERK1/2 MAPK pathway. DP loss led to elevation of K-ras transcript, protein, and activity. We utilized a novel reagent derived from the bacterium Vibrio vulnificus termed RRSP that is able to cleave Ras isoforms to test if dampening of Ras activity could ameliorate DP-dependent Cx defects. Cx43 levels were restored in CMs treated with RRSP, supporting a mechanistic connection between ERK activation and Cx43 expression. Collectively, our results reveal a novel mechanism for the regulation of Cx43 levels in cardiac disease caused by loss of DP and highlight a potential novel therapeutic for AC patients.

E91

A contractile hoop stress aids in balancing sudden hydrostatic pressure perturbation in a tubular epithelium.

D. Maity[1], S.X. Sun[1,2], Y. Chen[2];
[1]Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, [2]Mechanical Engineering, Johns Hopkins University, Baltimore, MD

Epithelium lines major organs where functional tubular structures consist of polarized and differentiated epithelial cells, such as endothelium lining the arterial walls and kidney proximal tubule epithelium. These epithelia are constantly subjected to hydrostatic pressure stresses which can result in ruptures. As seen in aging and inflammation, epithelium rupture imposes detrimental effects. Therefore it is important to understand how epithelium maintain homeostasis upon rapid changes in hydrostatic pressure that might result in ruptures.

We have developed a microfluidic device to investigate the response of epithelium to hydrostatic pressure changes. A mono-layered MDCKII epithelium lines a tubular channel in collagen (7mg/ml rat-tail Type-I) of diameters ranging from 75um to 150um. Via time-lapse imaging, the dynamics of the collagen channel with and without an epithelium were measured in response to varied hydrostatic pressure perturbations (~1kPa). A rapid increase in channel diameter was first detected because of the elasticity of the tissue, followed by the drop from the maximum to a constant value for a long period, dictated both by elasticity and permeability of the material. The dynamics can be modeled in terms of poro-elastic responses. Our hypothesis was tested in collagen gels formed at different gelation temperatures and cross-linking conditions. While the system without cells deformed by ~5%, the epithelium deformed by ~2% upon sudden increase in hydrostatic pressure. It was found that epithelium balances the effect of sudden perturbation by a contractile hoop stress. Theoretical modelling revealed that this constant hoop stress acts in a way that the outcome is an increment in the effective stiffness of the epithelial layer (previously reported to be ~10-20kPa) and that it exists in a pre-stressed condition. This suggests that the epithelium behaves in a non-linear regime even at low levels of perturbation as opposed to previous findings.
Quite intriguingly, upon perturbation by higher hydrostatic pressures(~2-5kPa), the tubular epithelium manifested identical responses as the collagen, suggesting the contractile hoop stress by epithelium is disrupted under such condition. In summary, we have devised a platform to simulate the tissue responses to hydrostatic perturbations and characterized the differential dynamic behaviors upon variable parameters.

Microsymp 14: Cell Metabolism

E92

The tumor suppressor Lkb1 controls cell fate through pyruvate-alanine transamination.

A.G. RADU[1,2], S. Torch[1,2], F. Fauvelle[3], P. Hainaut[1], L. Larue[4], C. Thibert[1,5], M. Billaud[5,6];
[1]Tumor molecular pathology and biomarkers, Institute for Advanced Biosciences, INSERM/UJF U1209 , Grenoble, France, [2]cofirst authors , Grenoble, France, [3]MRI Facility IRMage, Grenoble Neurosciences Institut , INSERM/CEA U817, Grenoble, France, [4]Normal and Pathological Development of Melanocytes, Institut Curie, CNRS UMR3347; INSERM U1021, Orsay, France, [5]co-senior and co-corresponding authors, Grenoble, Lyon, France, [6]Centre de Recherche en Cancerologie, Université Claude Bernard Lyon I, INSERM 1052, CNRS 5286, Lyon, France

The tumor suppressor *LKB1* (also named *STK11*) codes for a serine/threonine kinase. Germline mutations of *LKB1* are responsible for the Peutz-Jeghers syndrome, a dominantly inherited cancer disorder. Somatic mutations of this gene have been associated with various cancers including lung and cervical tumors. Lkb1 acts as a key regulator of energy metabolism through the activation of the AMP-activated protein kinase (AMPK), a sensor that adapts energy supply to the nutrient demands of cells facing situations of metabolic stress. To achieve metabolic adaptations, AMPK phosphorylates numerous substrates which inhibit anabolic processes while activating catabolic reactions. In particular, AMPK inhibits the nutrient-sensor kinase mTOR. In addition to AMPK, Lkb1 also phosphorylates 12 AMPK-related kinases that regulate cell polarization, axon branching of cortical neurons and hepatic neoglucogenesis.
To determine how Lkb1 coordinately regulates cell polarity and energy metabolism during cell fate decision, we spatio-temporally deleted the *Lkb1* gene in embryonic multipotent neural crest cells (NCC). These cells originate from the neural tube and give rise to various tissues including the peripheral nerves and the enteric nervous system (ENS).We showed that Lkb1 governs several aspects of cephalic NCC

development that are crucial during vertebrate head formation (Creuset et al., 2016). We also reported that mutant mice exhibited hypopigmentation, hindlimb paralysis and intestinal pseudo-obstruction. We described that Lkb1 is crucial for the differentiation and maintenance of two NCC-derivatives, Schwann cells and the ENS. Using a model of neural crest stem cell line, we demonstrated that Lkb1 is key for glial differentiation.Mechanistically, *Lkb1* loss led to increased alanine levels as also observed *in vivo* . Interestingly, inhibition of pyruvate-alanine transamination rescued glial differentiation of *Lkb1* -null NCC, in a dependent manner of mTOR. Furthermore, AICAR rescued glial differentiation of *Lkb1* -deficient NCC and treatment of *Lkb1* -deficient mice with AICAR corrected the Schwann cells and ENS phenotypes (Radu et al., submitted).
Altogether, these findings highlighted the novel and crucial role of Lkb1 during neural crest cell fate and uncovered a link between Lkb1-mediated pyruvate-alanine cycling and glial differentiation. These results provide new insights to understand the metabolic regulations exerted by Lkb1 during development and tumorigenesis.

E93

Mechanical modulation of glycolysis through phosphofructokinase and its activators in a KRAS-dependent manner.

J. Park[1], T. Isogai[1], C.J. Burckhardt[1], B. Gao[1], R. Bachoo[1], G. Danuser[1];
[1]The Lyda Hill Department of Bioinformatics, University of Texas Southwestern Medical Center, Dallas, TX

Cells can actively sense the microenvironment by cytoskeletal remodeling and by actomyosin contractility. Both processes consume a significant amount of energy, which is supported by the cell's metabolic activity. However, it is unclear if cells adjust their metabolism directly to the mechanical conditions of the environment. To begin to test such a link this study investigates metabolic changes as a function of extracellular matrix stiffness using untransformed human bronchial epithelial cells (HBECs) and non-small cell lung cancer cells (NSCLCs). We show that HBECs have more glucose metabolites and exhibit higher rates of glycolysis when placed on stiff substrate in comparison to soft substrate. Pharmacological inhibition of focal adhesion kinase and actomyosin contractility both disrupt glycolysis. Moreover, we show that expression of phosphofructokinase platelet (PFKP), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase3 (PFKFB3), and AMP-activated protein kinase (AMPK) are down-regulated upon decreased focal adhesion or intracellular tension, which could be partially rescued by proteasome inhibition. However, both mutant Kras-transformed HBECs and NSCLCs with the KRAS mutations assume consistent rates of glycolysis independent of cell substrate stiffness. Interestingly, the expression of PFKP is maintained by the mechanical desensitization driven by the Kras mutations. Knockdown of PFKP in Kras-mutant NSCLCs hindered tumor growth in mice as expected. Thus, while untransformed lung epithelial cells appear to adjust their metabolic activity in response to the mechanical properties of the microenvironment, Kras-mutated, transformed lung cancer cells loose this responsiveness, which may support the conditions of tumorgenesis and metastasis.

E94

Cell signaling involved in acute glycolytic response to immune cell activation.

Y. Kam[1], P.M. Swain[1], B.P. Dranka[1];
[1]Cell Analysis, Agilent Technologies , Lexington, MA

Immune cell activation is precisely regulated to respond toward a specific stimulus only and turn on a series of cellular processes required. The cellular metabolism is one of the emerging factors required for

the proper immune cell activation and differentiation. Several recent reports suggested that there is an immediate glycolytic response upon activation of T cells or macrophages not only as an indicative event but also as an essential component of activation. In the real-time monitoring of glycolysis and OXPHOS using a Seahorse XF analyzer, both CD4+ and CD8+ T cells as well as JurKat immortalized T cell line showed a rapid increase in the basal glycolytic activity within 6 min by in-situ administration of anti-CD4+ and CD8+ antibody-conjugated beads. Similarly, macrophages derived from human peripheral blood monocyte as well as RAW264.7 immortalized murine macrophage cell line showed a significant escalation in glycolytic activity within an hour by lipopolysaccharide with or without interferon γ. The increase in glycolysis was well correspond to the key cytokine production in both cell types. The further real-time cell metabolism analysis using various inhibitors for cell signaling showed that Akt activation is an essential factor for both T cell and macrophage activation. Interestingly, T cell activation also requires actin cytoskeletal polymerization which is recently suggested to support PI3K-mediated glycolysis increase through aldolase. In contrast, macrophage activation-related glycolytic response was not affected by actin polymerization inhibitors. These results suggest that Akt activation can be a core player mediating the early glycolytic response. They also imply the glycolytic response upon immune cell activation is mediated by diverse signaling pathways depending on cell types and the real-time analysis of cellular metabolism can provide useful kinetic information to understand the cell signaling of immune cell metabolism.

E95

Posttranslational Arginylation Enzyme Ate1 Controls Mitochondrial Functions and Cellular Warburg Effects.

C. Jiang[1], D.M. Patel[1], A. Kumar[1], B.T. Moorthy[1], M. Birnbaum[1], B. Alfonso[2], J. Huang[2], A. Barrientos[3,4], T. Lampidis[5,6], F. Fontanesi[4], F. Zhang[1,6];
[1]Molecular Cellular Pharmacology, University of Miami, Miami, FL, [2]Human Genetics, University of Miami, Miami, FL, [3]Department of Neurology, University of Miami, Miami, FL, [4]Biochemistry Molecular Biology, University of Miami, Miami, FL, [5]Cell Biology, University of Miami, Miami, FL, [6]Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL

The Warburg effect is the preference of glycolysis over mitochondrial respiration in the presence of oxygen. This phenotype is commonly seen in cancer cells but its cause still remains less clear. Here we report that the down-regulation of an evolutionarily conserved enzyme, arginyltransferase 1 (Ate1), is sufficient to cause the Warburg effect at the cellular level.
As a central component of the N-end rule, Ate1 is the sole enzyme in mammalian cells mediating posttranslational addition of an extra arginine in a process called arginylation. In this study, by measuring the rates of glucose intake and lactate production, and by measuring glycolytic profiles with the Seahorse metabolic analyzer, we found that a knockout (KO) or a down-regulation of Ate1 is sufficient to up-regulate glycolysis. Consistently, by using glycolysis inhibitors 2-DG and 2-FDG, we found that the portion of glycolysis derived ATP was increased by the KO or down-regulation of Ate1, and such a phenotype can be rescued when recombinant Ate1 is expressed in the Ate1-KO cells. Therefore our data indicates that a down-regulation of Ate1 increases glycolysis. In contrast, the KO of Ate1 appears to impair the function and morphology of mitochondria. By using Western blot on whole cells and isolated mitochondria separated on denaturing or native PAGE, we found that the Ate1-KO, while not affecting the quantity of mitochondria in the cell, changed its quality with defects in the formation of respiration chain complexes and super complexes. Particularly, Ate1-KO appears to compromise the formation and the function of succinate dehydrogense (complex II) by impairing the import and modification of several subunits. Importantly, we found that the level of hypoxia-inducible factor 1a (HIF1a) is increased in

Ate1-KO cells and the knockdown of HIF1a was able to suppress the glycolytic phenotype in these cells. We also found that the increase of HIF1a in Ate1-KO cells are mediated by two pathways, the arginylation-dependent degradation of this protein by the N-end rule, and the increase of succinate level as expected by the compromise of succinate dehydrogense. Finally, by confocal microscopy and by biochemical assays, we found that a portion of Ate1 is located inside mitochondria, and this may be explained by the evolutionary origin of Ate1 as a gene transferred from the mitochondrial ancestors. Our study shows for the first time that Ate1 is tightly connected to mitochondrial function, established HIF1a as a previously unknown substrate of the N-end rule, and discovered a novel pathway in mediating the cancer Warburg effect by an evolutionarily posttranslational modification enzyme.

E96

Environmental availability of cystine drives usage of glutamine as a TCA cycle substrate and causes glutamine addiction.

A. Muir[1], L.V. Danai[1], D.Y. Gui[1], C.Y. Waingarten[1], C.A. Lewis[2], M.G. Vander Heiden[1,3];
[1]Koch Institute for Integrative Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, [2]Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, [3]Dana-Farber Cancer Institute, Boston, MA

The tri-carboxylic acid (TCA) cycle is primarily known for its role in generating NADH from the oxidation of pyruvate, which is used to power ATP production by oxidative phosphorylation. Beyond ATP production, the TCA cycle also supplies biomass precursors needed for the growth and proliferation of cells. Thus, supplying the TCA cycle with carbon is an essential metabolic feature of proliferating cells. Many mammalian cancer cell lines depend on extracellular glutamine as a major TCA cycle substrate to support their proliferation in vitro. However, recent studies have suggested that some cells that depend on glutamine to fill the TCA cycle and proliferate in culture rely much less on glutamine catabolism for these functions when growing as tumors in vivo. This has led to the conclusion that environmental differences between tumors and cell culture influence the extent of glutamine catabolism. We sought to identify such environmental differences that cause differential dependence on glutamine for TCA cycle maintenance. We hypothesized that differences in available nutrients between standard culture conditions and tumors could account for differences in glutamine utilization. Therefore, we cultured cancer cell lines in 100% adult bovine serum, a condition that more closely reflects the nutrients available to cells in vivo. Cells cultured in adult bovine serum have decreased glutamine catabolism and rely less on glutamine metabolism for proliferation compared to growth under standard tissue culture conditions. The small molecule nutrient component of adult bovine serum was responsible for the differential utilization of glutamine. By analyzing the nutrient differences between bovine serum and media, we find that levels of a single nutrient, cystine, can account for the differential dependence on glutamine in these different environmental contexts. We show that increasing cystine availability to cancer cells growing as tumors in vivo increases glutamine catabolism in these tumors. Lastly, we find that cystine levels dictate glutamine dependence via the cystine/glutamate antiporter xCT/SLC7A11, and that environmental cystine levels in conjunction with xCT/SLC7A11 expression are necessary and sufficient to drive increased glutamine anaplerosis. Collectively, these results define important determinants of glutamine metabolism and glutamine dependence in cancer cells, and highlight that cellular metabolism is determined not only by cellular genetics, but by the interplay of the cell with the nutrient environment.

E97

A cleavage product of Polycystin-1 is a mitochondrial matrix protein that regulates mitochondria morphology and function.

C. Lin[1], M. Kurashige[1], Y. Liu[2], T. Wang[1], V. Choudhary[1], R. Hobbs[1], L. Liu[1], P. Lee[3], P. Outeda[4], F. Zhou[1], N.P. Restifo[3], T.J. Watnick[4], W. Prinz[1], X. Hong[2], L.F. Menezes[1], G.G. Germino[1];
[1]Kidney Disease Branch; National Institute of Diabetes and Digestive and Kidney Disease, National Institutes of Health (NIH), Bethesda, MD, [2]Laboratory of Molecular Genetics; National Heart, Lung and Blood Institute, National Institutes of Health (NIH), Bethesda, MD, [3]Center for Cell-Based Therapy; National Cancer Institute, National Institutes of Health (NIH), Bethesda, MD, [4]Department of Medicine; Division of Nephrology, University of Maryland School of Medicine, Baltimore, MD

Background: Kidney function is dependent on the proper structure of its tubule system. Among the genetic diseases that disrupt nephron architecture, Autosomal Dominant Polycystic Kidney Disease (ADPKD; MIM ID's 173900, 601313, 613095) is the most common. Caused by mutations in either *PKD1* or *PKD2*, ADPKD is a life-threatening condition estimated to affect almost 1/1000 that often results in renal failure by the sixth decade. *PKD1* encodes polycystin-1 (PC1), a large transmembrane protein that is autocatalytically cleaved into 3,048-aa N-terminal (NTF; ~ 325 kDa) and 1,254-aa C-terminal fragments (CTF; ~150 kDa) that remain non-covalently associated. Additional CTF cleavage products containing the cytoplasmic tail (CTT) have also been described, including a ~28 to ~34 kDa fragment reportedly triggered by mechanical stimuli and localized to the nucleus; and a ~100 kDa ER product (P100) likely including the final 6 transmembrane (TMs) domains. Recent studies have reported intrinsic metabolic reprogramming in *Pkd1* knock-out cells, implicating dysregulated cellular metabolism in the pathogenesis of polycystic kidney disease. However, the exact nature of the metabolic changes and their underlying cause remains controversial.**Results**: Pairs of proximal or distal tubule epithelial kidney cell lines in which the *Pkd1* knockout was derived from its control counterpart were used for *in vitro* studies. We show herein that *Pkd1*$^{ko/ko}$ renal epithelial cells have distinct metabolic flux, impaired fatty acid utilization, increased fragmentation of the mitochondrial network and elevated mitochondrial membrane potential. Consistent with the *in vitro* results, transmission electron microscopy (TEM) images of kidneys from fifth-generation C57/BL6 *Pkd1*^{tm2Ggg} (stock 010671; The Jackson Laboratory); ER-cre (stock 004682; The Jackson Laboratory) mouse also showed abnormal mitochondrial morphology. Using live cell imaging, immunofluorescence, and biochemical methods, we further show that a C-terminal cleavage product of polycystin-1 (CTT) translocates to the mitochondria matrix, not the nucleus as had been previously reported. Overexpression of CTT rescues the fragmented mitochondria phenotype in *Pkd1*$^{ko/ko}$ renal epithelial cells. Transgenic expression of mouse CTT in Drosophila results in decreased viability and exercise endurance but increased CO2 production.**Conclusion**: Our results suggest a direct role for PC1 in regulating mitochondrial function and cellular metabolism and provide a framework to understand how impaired mitochondrial function can be linked to the regulation of tubular diameter in both physiological and pathological conditions.

E98

AMPK Regulates Peroxisomal Cargo Proteins Import via PEX5 Phosphorylation.

J. Jing[1], D. Tripathi[1], R. Dere[1], C. Walker[1,2];
[1]CPEH, Baylor college of Medicine, Houston, TX, [2]CTCR, Texas AM University , houston, TX

Peroxisomes are highly metabolic, autonomously replicating organelles. While their function as metabolic organelles is well known, their place in the world of cell signaling is only just beginning to emerge. AMP-activated protein kinase (AMPK) is a major regulator of cellular energy homeostasis, but

has yet to be linked to peroxisome biology. In exploring targets for AMPK phosphorylation, we identified the peroxisome import receptor PEX5, which delivers peroxisome proteins to this organelle to regulate peroxisome function and homeostasis, as a target for this kinase. An optimal AMPK substrate motif used to analyze all PEX proteins in Peroxisome DB 2.0 identified a potential AMPK phosphorylation motif in PEX5 at serine 279 (S279). Using an AMPK-substrate-specific antibody, in vitro kinase assays, and a phospho-specific antibody generated by our group (anti-S279 on PEX5), we identified S279 as a bona fide site for PEX5 phosphorylation by AMPK. Furthermore, we found that AMPK phosphorylation of PEX5 regulated translocation of cargo proteins with a peroxisome targeting sequence (PTS1) recognized by PEX5 to the peroxisome. These data provide a model where under conditions of nutrient/energy stress, activation of AMPK increases the import of proteins essential for peroxisome functions such as beta-oxidation. Thus, we have uncovered a previously unappreciated linkage between the AMPK signaling pathway and the peroxisome, which opens new horizons for understanding peroxisomal homeostasis and its role in cellular metabolism.

Microsymp 15: Chromosome Structure, Centromeres and Kinetochores

E99

Channel Nucleoporins recruit the Polo-like kinase PLK-1 to the Nuclear Pore Complexes in prophase to direct Nuclear Envelope Breakdown in *C. elegans* embryos.

L. Martino[1], S. Morchoisne-Bolhy[2], D.K. Cheerambathur[3], L. Van Hove[1], J. Dumont[4], N. Joly[1], A.B. Desai[3], V. Doye[2], L. Pintard[1];
[1]UMR7592 CNRS - Université Paris Diderot, Sorbonne Paris Cité, Team Cell Cycle and Development, Institut Jacques Monod, Paris, France, [2]UMR7592 CNRS - Université Paris Diderot, Sorbonne Paris Cité, Team Non-conventional Functions of Nuclear Pore, Institut Jacques Monod, Paris, France, [3]Department of Cellular and Molecular Medicine, University of California, Ludwig Institute for Cancer Research, San Diego, La Jolla, CA, [4]UMR7592 CNRS - Université Paris Diderot, Sorbonne Paris Cité, Team Cell Division and Reproduction, Institut Jacques Monod, Paris, France

In animal cells, nuclear envelope breakdown (NEBD) is required for the assembly of the mitotic spindle and for proper chromosomes segregation. Whereas mitotic kinases have been implicated in NEBD, how they coordinate their activity in space and time to trigger NEBD is still unclear. Here we show that both in human cells and *C. elegans* embryos, the mitotic Polo-Like Kinase 1 (PLK-1) is recruited to the nuclear pore complexes in prophase, just prior to NEBD, through its Polo-Binding Domain (PBD). We identified the *C. elegans* nucleoporins NPP-1/Nup58, NPP-4/Nup54 and NPP-11/Nup62, which form a trimeric complex localized at the central channel of the nuclear pore, as the critical factors anchoring PLK-1 to the Nuclear Envelope (NE). In particular, NPP-1 NPP-4 and NPP-11 primed at multiple polo-docking sites by Cdk1 and PLK-1 itself, physically interact with the PLK-1 PBD. Finally, we provide evidences that PLK-1 localization to the NE is required for efficient NEBD. We conclude that nucleoporins play an unanticipated regulatory role in NEBD, by recruiting PLK-1 to the Nuclear Envelope in prophase, thereby facilitating phosphorylation of critical downstream targets.

E100

Chromosome dynamics simulations reveal the role of condensin and cohesin in building the bottle-brush chromosome architecture.

J.G. Lawrimore[1,2], A.B. Doshi[2], B.S. Friedman[2], A. Fulp[2], E.Y. Yeh[2], K.S. Bloom[2];
[1]Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, [2]Biology Department, University of North Carolina at Chapel Hill, Chapel Hill, NC

Despite their similar molecular structure, cohesin and condensin occupy geometrically distinct regions in mitotic chromosomes across eukaryotes. In budding yeast, this separation is apparent at the pericentric region, the cohesin and condensin-rich region surrounding the centromere. Mitotic chromosomes of higher eukaryotes, and the pericentric region of budding yeast, contain a high density of radial chromatin loops extending from an axial core region, causing the chromosome to resemble a bottle-brush. We constructed novel chromatin dynamics simulations modeling cohesin as a slip ring and condensin as either a static or dynamic loop extruder on a bottle-brush model of the pericentric region. Centromere simulations in which condensin persistently binds to chromatin while cohesin passively slides along chromatin recapitulate the geometric separation observed in live cells. Critically, the physical segregation of condensin and cohesin relies on an extensional force on the chromatin. In the pericentric region of budding yeast, the main extensional force is provided by the mitotic spindle. In the highly condensed chromosomes of higher eukaryotes, and to a lesser extent in the pericentric region of budding yeast, this extensional force is provided the repulsion of the high number or densely packed radial loops extending from a central axis.
The physical segregation of cohesin and condensin within the pericentric region predicts the motion of chromatin within the pericentric region would be deferentially regulated by cohesin and condensin based on the chromatin's position. We found the dynamics of a 10 kb lacO/LacI-GFP array placed 1.8 kb from CEN15, which co-localized with pericentric cohesin, is primarily confined by cohesin. In contrast, we found the dynamics of a 5.5 kb tetO/TetR-GFP array within a conditionally dicentric plasmid, which co-localizes with condensin, is primarily confined by condensin. We constructed chromatin dynamics simulations of the dicentric plasmid to explore how several molecules of cohesin and condensin could directly alter the plasmid's dynamics. Simulations containing condensin resembled wild-type experimental dynamics, whereas simulations lacking condensin resembled the plasmid dynamics of cells containing the temperature sensitive condensin mutation, brn1-9. These studies support a model where condensin persistently binds to chromatin while extruding loops, while cohesin slides along chromatin. Extensional force on the chromatin centers condensin within the chromosome's axis, while cohesin can slip along the chromatin to diffuse to the less crowded radial edges of chromatin loops.

E101

The molecular requirements for epigenetic establishment of centromeres depend on the type of underlying DNA.

G.A. Logsdon[1], C.W. Gambogi[1], E.J. Barrey[2], P. Heun[2], B.E. Black[1];
[1]Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, [2]Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, United Kingdom

Human artificial chromosomes (HACs) are exciting tools for current and planned synthetic biology efforts and could have important future clinical applications. A key element in a HAC is the centromere because it directs the faithful segregation of the HAC to daughter cells in mitosis. Functional centromeres are defined by the presence of nucleosomes containing the histone variant, CENP-A. The acquisition of CENP-A nucleosomes on naked DNA HAC templates is the critical step for HAC formation

because it initiates the self-propagating epigenetic pathway for centromere inheritance. We sought to artificially promote centromere formation by locally seeding CENP-A nucleosome assembly via its specific histone chaperone, HJURP. We reasoned that this would allow us to more efficiently form HACs to test whether or not it would relax the DNA sequence requirements reported for previous versions (i.e. a reported strict requirement for highly repetitive centromeric α-satellite DNA). Our epigenetic seeding approach successfully stimulated HAC formation on α-satellite DNA, providing an example in a mammalian system for epigenetic spreading of CENP-A-containing chromatin. Earlier studies had reported a strict requirement in HAC formation for the DNA binding protein, CENP-B, and the 17 bp element within α-satellite monomers to which it binds, the CENP-B box (Ohzeki et al., 2002. *J. Cell Biol.* 159, 765-775; and Okada et al., 2007. *Cell* 131, 1287-1300). We found that artificial epigenetic centromere seeding completely bypasses the requirement for CENP-B. We also found that HACs can form on complex DNA that lacks any detectable α-satellite DNA or CENP-B protein, both findings in stark contrast to the longstanding models of HAC formation. Surprisingly, targeting local HJURP recruitment did not stimulate HAC formation on complex DNA. Moreover, unlike their earlier α-satellite counterparts, complex DNA HACs allowed us to define the sequence composition, copy number, and precise location of CENP-A nucleosome assembly, providing important insight into HAC formation and stability. Complex DNA HACs also circumvent the cloning difficulties caused by highly repetitive α-satellite DNA that has limited the broader utility of the earlier generation of HACs. Together, our study innovates the HAC "toolbox" and reveals molecular requirements for centromere formation that unexpectedly vary depending on DNA sequence.

E102

Assess the mechanisms that lead to de novo deposition of centromere identity in real time.

D. Fachinetti[1];
[1]Department of Cell Biology, Institut Curie, Paris, France

The preservation of centromere function is a crucial challenge for cells in order to maintain a correct chromosome karyotype. Indeed, defects in centromere formation or function lead to numerical and structural chromosome alterations, common features of cancer cells and developmental disease. In most species, centromeres are established on a series of repetitive DNA sequences and on a specialized centromeric chromatin. This chromatin is enriched with the histone H3 variant CENP-A, which was demonstrated to be the epigenetic mark that maintains centromere identity and function via a two-step mechanism1. However, it still remains unclear if CENP-A is the sole mark for centromere identity. Indeed, the presence in the majority of eukaryotes of specific centromeric DNA sequences suggests that they play a fundamental role in determining centromere position. While the pathways required for centromere maintenance are very well characterized, nothing is known about how a centromere is formed de novo in human cells. Using genome editing and inducible protein degradation2 to achieve rapid, complete3 and reversible CENP-A depletion, we have generated models of centromere inheritance and neocentromere establishment in human cells. First, we have dissected the pathways that control de novo deposition of the centromeric epigenetic mark in oder to uncover the role of DNA sequences. Second, we showed that temporary loss and re-activation of CENP-A promotes neocentromere formation in certain genetic backgrounds. Using these unique models, we are now studying centromere (and neocentromere) composition, genomic localization, and epigenetic landscape in human cells.

References

1. Fachinetti, D. et al. A two-step mechanism for epigenetic specification of centromere identity and function. Nat Cell Biol 15, 1056–1066 (2013).

2. Holland, A. J., Fachinetti, D., Han, J. S. & Cleveland, D. W. Inducible, reversible system for the rapid and complete degradation of proteins in mammalian cells. Proc. Natl. Acad. Sci. U.S.A. 109(49):E3350–7 (2012).
3. Hoffmann, S. et al. CENP-A Is Dispensable for Mitotic Centromere Function after Initial Centromere/Kinetochore Assembly. Cell Rep 17, 2394–2404 (2016).

E103

Dynamically switching protein interaction networks during M-phase progression in vertebrate kinetochores.

M. Hara[1], T. Hori[1], T. Fukagawa[1];
[1]Frontier Biosciences, Osaka University, Osaka, Japan

Chromosomes duplicated in S-phase have to be faithfully divided into daughter cells to transmit genomic information to the next generation through cell divisions. The accurate chromosome segregation requires kinetochore, a large protein complex, which is formed at centromere region on each chromosome to attach to spindle microtubules. Among kinetochore proteins, Constitutive Centromere Associated Network (CCAN) proteins, including 16 subunits and KMN (Knl1, Mis12 and Ndc80 complexes) network proteins form a core architecture in vertebrate kinetochore. The CCAN proteins constitutively localize to centromere throughout the cell cycle and the KMN network proteins are recruited to the CCAN during M-phase to establish a functional kinetochore that binds to microtubules. We previously showed that artificial tethering of CCAN components CENP-C or -T onto a non-centromeric region established a functional artificial kinetochore without other CCAN subunits, respectively. Given CENP-C and -T directly bind to the KMN network, we have proposed a two-pathway model (CENP-C- and -T-dependent pathways) for recruitment of the KMN network to establish functional kinetochores in M-phase. However, it remains unknown how the two pathways coordinatelywork in the native kinetochores in which both two pathways exist together.
To address this question, we expressed CENP-C or -T mutants, which lacked in their KMN-binding domains, into CENP-C- or -T- deficient chicken DT40 cells, respectively. Surprisingly, the CENP-C-dependent KMN network recruitment was dispensable for cell viability and mitotic progression, whereas the CENP-T-dependent KMN network recruitment was essential, suggesting that the KMN-binding of CENP-T, but not of CENP-C, is necessary for kinetochore assembly and chromosome segregation during M-phase in DT40 cells. Furthermore, we found that localization profile for Mis12 and Ndc80 was dynamic and their localization dependency to the CCAN proteins was changed during mitotic progression. At the onset of anaphase most of the KMN network appeared to bind to CENP-T, not to CENP-C, which is consistent with our data that chromosome segregation largely relies on the CENP-T-dependent pathway in DT40 cells. These results led us to a model for kinetochore assembly in which the kinetochore protein interaction networks dynamically switch during M-phase progression.

E104

Ndc80 complex auto-inhibition is opposed by MIND to increase microtubule binding.

E.A. Scarborough[1], C.L. Asbury[2], T.N. Davis[1];
[1]Biochemistry , University of Washington, Seattle , WA, [2]Physiology and Biophysics , University of Washington , Seattle , WA

Kinetochores are comprised of an intricate system of proteins that form into multiple sub-complexes. They serve to form strong, load-bearing attachments between DNA and dynamic microtubules in order to faithfully segregate chromosomes during mitosis. As such, kinetochore complexes are highly

regulated in order to avoid aneuploidy. Previous literature has focused on the outer kinetochore complex, Ndc80, as a target for this regulation—its role in microtubule binding, in maintaining tension across the kinetochore-microtubule interface, and in correction of erroneous microtubule attachments. Prior studies have also indicated that this complex can adopt multiple conformations due to a flexible internal loop, and *in vivo* evidence suggests that these structural changes are physiologically relevant. However, the biological consequences of these structural rearrangements of the Ndc80 complex, and how they help to regulate mitotic fidelity, are still unclear. The Ndc80 complex is an elongated rod-shaped heterotetramer comprised of two dimers: Ndc80/Nuf2 and Spc24/Spc25. We show in two ways that the Spc24/25 dimer inhibits microtubule binding of the Ndc80/Nuf2 dimer. First, a dimer of Ndc80/Nuf2 (lacking Spc24/25) has an increased residence time on microtubules. Second, adding excess Spc24/25 dimer decreases the residence time of the Ndc80 complex on microtubules. This inhibition was further explored with bulk and single molecule FRET assays. A version of the Ndc80 complex was labeled with FRET probes on opposite ends of the complex as a proxy for folding. When measured in a bulk or single molecule FRET assay, the labeled Ndc80 complex exhibits energy transfer. These results suggest that auto-inhibition of microtubule binding by the complex occurs by bending at the loop. We have previously shown that the central kinetochore component MIND is able to increase the microtubule-binding affinity of a single Ndc80 complex by 4-fold. Here we show that MIND likely acts by opposing the auto-inhibition of the Ndc80/Nuf2 microtubule binding. Under single molecule FRET conditions, the addition of MIND to the Ndc80 complex decreases the interaction between the two ends of the Ndc80 complex. Also, the addition of excess Spc24/Spc25 dimer to the Ndc80-MIND co-complex reduces the residence time to that of the Ndc80 complex alone, again implying that the addition of MIND is opposed to a folded, inhibited state of the Ndc80 complex. The first analysis of the kinetics of the conformational changes of the Ndc80 complex is underway using our single molecule FRET assay. We propose that MIND regulation of the Ndc80 complex is important during loading of the Ndc80 complex at the kinetochore in order to ensure proper temporal and spatial microtubule binding of the complex.

E105

Force-dependent changes in the 3D architecture of a kinetochore.

E. Roscioli[1], C. Smith[1], N.J. Burroughs[1,2], A. McAinsh[1];
[1]Centre for Mechanochemical Cell Biology, Division of Biomedical Sciences, Warwick Medical School,, University of Warwick, Coventry, United Kingdom, [2]Mathematics Institute, University of Warwick, Coventry, United Kingdom

The accurate segregation of sister chromatids into daughter cells is ensured by proper kinetochore-microtubule interactions. The growth and shrinkage of attached microtubules and the stepping of molecular motors imposes pushing and pulling forces on the kinetochore structure. Recent studies have shown that bi-orientated (amphitelic) kinetochores are non-compliant structure in that the distance between inner and outer markers is invariant to changes in inter-kinetochore distance. However, we found that the kinetochore will undergo conformational changes (in the Ndc80 complex) when microtubule binding is eliminated. Moreover, we revealed how the outer kinetochore can undergo rotation relative to the sister-sister axis (we termed this Swivel). Here we have expanded this work into diploid human RPE1 cells and investigated the position and swivel of multiple kinetochore components, including the KMN network, RZZ complex and components of the spindle assembly checkpoint. We will discuss our efforts to construct a 3D architectural map of a bi-orientated kinetochore, explore how the structure responds to different mechanical inputs and relate this to the activation/silencing of the spindle assembly checkpoint.

Microsymp 16: Novel Approaches in Studying the Cytoskeleton

E106

Developing Tunable Bioink for Versatile 3D Bioprinting.

K. Elawad[1], W. Jung[2], S.H. Kang[2], Y. Chen[2];
[1]Materials Science and Engineering, Johns Hopkins University, Baltimore, MD, [2]Mechanical Engineering, Johns Hopkins University, Baltimore, MD

With the advances in instrumentation, 3D bioprinting technology has matured over the years for potential applications that require high precision in cell placement and geometry. For instance, in order to study cell migration, tissue repair, and tumor progression at physiological conditions, it is desirable to print organoids in mimicry of the in vivo tissue conditions. However, currently available bioinks lack the tenability in adjusting mechanical properties such as stiffness and porosity to represent in vivo conditions. We have developed biocompatible and tunable alginate-based bioink for in vitro tissue printing. By adjusting the concentration, gelling, and crosslinking conditions of the alginate/gelatin composite bioink, it can be used for direct ink writing to produce printed tissues at a 500 micron scale. The bioink is extruded from the printing head to form 3D geometries as supportive scaffolds for 3D cell culture. Gelatin is used as a rheological modifier to enhance the printability of the alginate ink. Complex, tissue-like 3D architectures can be achieved by embedding multiple cell types in our bioink. We also verified that fibroblasts were able to stay viable and replicate during a 7-day incubation period. In summary, our bioink is biocompatible, mechanically tunable and can form stable structures for 3D bioprinting. It is suitable to be used in organoid fabrication where physiological biophysical microenvironments can be recreated faithfully.

E107

A new method for large-volume high-resolution intravital imaging using multiphoton microscopy identifies microenvironment-driven tumor cell phenotypes leading to metastasis.

D. Entenberg[1,2,3], Y. Wang[1,2,3], J. Pastoriza[4], M.H. Oktay[1,2,3,4], J.S. Condeelis[1,2,3];
[1]Department of Anatomy and Structural Biology, Einstein College of Medicine/ Montefiore Medical Center, Bronx, NY, [2]Gruss-Lipper Biophotonics Center, Einstein College of Medicine/ Montefiore Medical Center, Bronx, NY, [3]Integrated Imaging Program, Einstein College of Medicine/ Montefiore Medical Center, Bronx, NY, [4]Department of Surgery, Einstein College of Medicine/ Montefiore Medical Center, Bronx, NY

We report, for the first time, a new approach to intravital imaging that successfully stabilizes tumors in living animals and isolates them from imaging artifacts associated with living tissue. This allows the capture, over days, of many high-magnification, sub-cellular resolution images in a mosaic pattern that maintains their spatial relationships. These images are then stitched together to produce a low-magnification, but high resolution, large volume image over time: a process we call Large-Volume, High-Resolution Intravital Imaging (LVHR-IVI). LVHR-IVI does not suffer from the magnification and spatial resolution artifacts inherent with mesoscopic and light sheet intravital imaging. LVHR-IVI is applicable to many different tissues, which includes soft tissues such as the mammary fat-pad, lymph nodes, and salivary gland which are particularly challenging as they are extremely compliant and easily transmit motion and vibrations from the animal's involuntary movements. Furthermore, using permanent imaging windows in mice in combination with LVHR-IVI extends imaging sessions from hours to multiple days over several weeks.

We have used LVHR-IVI to identify and quantitate modes of tumor cell motility, invasion and systemic dissemination in transgenic breast tumors which maintain the clinically observed histology presented by patients. High-resolution time-lapse imaging of these tumors identify the tumor microenvironment of metastasis (TMEM) - mediated transient vascular permeability events as the mechanism of tumor cell intravasation and hematogenous dissemination
Furthermore, we used LVHR-IVI to evaluate the percent contribution of each of the proposed origins of circulating tumor cells and tumor cell clusters in vivo. Potential mechanisms of migration toward blood vessels include: 1) single cell migration; 2) streaming migration; 3) collective migration. Potential mechanisms of intravasation of blood vessels include: 1) Cell crowding of individually arriving tumor cells at sites of intravasation; 2) Collective vascular invasion by collectively migrating tumor cells; 3) Single cell intravasation followed by cluster formation in the blood.
The results indicate streaming migration resulting in cell crowding at TMEM intravasation sites are the major mechanisms associated with intravasation. Furthermore, the arrival of single tumor cells at distant sites in close temporal proximity can result in cluster formation within blood vessels there. Finally, the survival of single cells during metastatic seeding is 10-100 fold greater than previously believed. These new insights narrow the targets associated with metastatic progression and allow for better prognostic markers, treatment strategies and associated companion diagnostics.

E108

Examining mechanisms regulating microtubule organization in dividing cells using lattice light sheet microscopy.

M.C. Pamula[1], S. Forth[2], S. Suresh[1], W.R. Legant[3], E. Betzig[3], T.M. Kapoor[1];
[1]Laboratory of Chemistry and Cell Biology, The Rockefeller University, New York , NY, [2]Department of Biological Sciences, Rensselaer Polytechnic Institute, Troy, NY, [3]Howard Hughes Medical Institute, Janelia Research Campus, Ashburn, VA

Accurate chromosome segregation must be maintained over multiple cell division cycles to stably propagate genetic information. At anaphase onset, microtubules with opposite polarity interdigitate to form the spindle midzone, a specialized array that functions to keep chromosomes apart and position the cell division plane. The proper assembly of this structure depends on PRC1 (protein regulator of cytokinesis 1), a nonmotor microtubule-associated protein that selectively crosslinks antiparallel microtubules, and Kinesin-4, a plus-end directed motor protein. Prior studies have shown that these proteins form a stable complex in vitro and are thought to “measure” features of microtubule arrays. For example, PRC1/Kinesin-4 complex in vitro can both autonomously regulate the length of antiparallel microtubule overlap in dynamic filament networks and also can form micron-scale “end tags” of different lengths on stable microtubules depending on the size of the filament. However, we do not know how the length-dependent regulation of individual filaments or overlap regions between filaments contributes to spindle midzone assembly in human cells. Here, we use lattice light sheet microscopy to image whole cell volumes of dividing cells at rates varying from 1.8 to 4.4 seconds per volume. We quantitatively investigate the association of fluorescently-labeled PRC1 on microtubule bundles during the metaphase to anaphase transition in hTERT-RPE1 cells. In metaphase, approximately 40 PRC1 bundles are established that span the cell equator and measure 4.6 +/- 1.2 microns in length. Immediately after anaphase onset, PRC1 bundles shrink five-fold to 0.9 +/- 0.3 microns in between the separating chromosomes. We are designing PRC1 mutant constructs that have reduced binding to microtubules which we hypothesize may alter the number of bundles established in metaphase or the length of PRC1 bundles in metaphase and anaphase.

E109

Stereotyped morphological structure detection from high-resolution, live-cell, 3D images.

M.K. Driscoll[1,2], E.S. Welf[1,2], K.M. Dean[1,2], R. Fiolka[2], G. Danuser[1,2];
[1]Bioinformatics, UT Southwestern Medical Center, Dallas, TX, [2]Cell Biology, UT Southwestern Medical Center, Dallas, TX

3D cell migration is principally governed by environmental cues via subcellular mechanisms such as intracellular signaling and cytoskeletal dynamics. Directly observing the regulation of 3D cell migration therefore requires simultaneously imaging across multiple spatial and temporal scales, ranging from the small (<1 micrometers) and quick (<1 seconds) scales regulating cytoskeletal turnover to the large (>10 micrometers) and slow scales (>10 minutes) of the cell interacting with its environment. Recent advances in high-resolution light-sheet microscopy have begun to allow us to image cells in 3D over these scales with single molecule sensitivity. However, the large size and the difficulty of visualizing 3D objects with complex geometries on 2D computer screens prohibit interpretation of this microscopy data by eye. To facilitate automated analysis of these images, we developed a 3D morphological structure detector to locate and characterize protrusions. This detector combines techniques from computer graphics, machine learning, and computer vision to identify stereotyped protrusions, such as filopodia, blebs, and lamellipodia. The detector decomposes the cell surface into approximately convex patches, merges those patches via machine learning, and then, again via machine learning, classifies the merged patches by protrusion type. A surface patch is convex if any two points on the patch can be connected by a line that does not exit the cell. Since it relies on machine learning, to detect new protrusion types researchers do not need to mathematically describe protrusions, but rather click on examples of these structures in their own data. A support vector machine generalizes these examples to a model that can be used to detect this protrusion type in new data sets. Using this detector, we analyzed the membrane distribution of PIP2 and KRAS in blebbing melanoma cells embedded in 3D collagen. We found that PIP2 associates with individual blebs, whereas KRAS localizes to regions of high bleb surface density, but does not associate with individual blebs. This detector will facilitate the study of the subcellular regulation of 3D cell migration.

E110

Nanofiber Curvature Enables Quantitating Single Protrusions.

A. Mukherjee[1], B. Koons[1], P. Sharma[2], Z. Ye[1], B. Behkam[1], A.S. Nain[1];
[1]Mechanical Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA, [2]Department of Biomedical Engineering and Mechanics, Virginia Polytechnic Institute and State University, Blacksburg, VA

Our native extracellular matrix (ECM) is primarily comprised of fibrous proteins; the size and organization of which is now widely appreciated to be key in cellular biophysical sensing and motility. Despite decades of in vitro investigation using 2 and 3D substrates, a comprehensive description of initial protrusive events in metastatic invasion using nanofibers mimicking ECM dimensions is still critically missing. This is due to the inability of current approaches to (i) isolate single protrusions and (ii) provide controlled curvatures to quantitate protrusion sensitivity to geometry. Using contrasting diameter fibers deposited orthogonally on top of each other, we quantitate single protrusion dynamics at high spatiotemporal resolution in both breast adenocarcinoma MDA-MB-231 and brain glioblastoma DBTRG-05MG. Specifically, we constrain the cell body to the larger diameter base fiber (~2 μm), while allowing individual protrusions to form on protrusive high curvature small diameter fibers (~100, 200 and 600 nm) and low curvature flat ribbons of corresponding widths. Quantitating at ~minute temporal

scales, our strategy allows us to quantitatively distinguish ('protrutype') between the protrusive dynamics of two cancer cell lines and report that the MDA-MB-231 is more sensitive to the fiber curvature compared to DBTRG-05MG. We find that in all cell types, protrusions are rich in actin and tubulin, but intermediate filament vimentin localizes only in mature protrusions. Furthermore, we show that vimentin depleted cells can still form long length protrusions, thus negating the currently understood role of vimentin in protrusion dynamics. Quantitating at ~second temporal scales, we report that protrusion initiation and maturation occurs by wrapping-around the nanofibers in a helical manner with the width and rotational speed of each turn regulated by curvature. Specifically, high curvature fibers (~100-300 nm) result in narrower widths (2.1 ± 0.1 µm), whereas low curvature fibers (~600-1000 nm) result in wider widths (4.4 ± 0.2 µm). Interestingly, we find that intracellular transport coincides with protrusion maturation and the kinetics of vesicle cytoplasmic trafficking occur inside protrusions at speeds of 0.7 ± 0.1, 1.0 ± 0.1, and 1.7 ± 0.1 µm/s on fiber diameters of 100, 600 and 1000 nm respectively. Mean square displacement measurements reveal a rapid shift from a sub-diffusion regime (exponents <1) for vesicles outside the protrusions to a super-diffusive regime (exponents >1) as vesicles travel within the protrusion at high persistence. In summary, we report a multiscale and integrative platform which allows the quantification of protrusion morphodynamics decoupled from cell migration and enables identifying the utility of sensory protrusions.

E111

Development and implementation of a Förster Resonance Energy Transfer based biosensor for measuring intracellular tension and force.

R.G. Hart[1], D. Kota[2], L. Brunmaier[2], J. Liu[2], I. Chandrasekar[1];
[1]Sanford Childrens Health Research Center, Sanford Research, Sioux Falls, SD, [2]Department of Nanoscience and Nanoengineering, South Dakota School of Mines and Technology, Rapid City, SD

During cellular processes such as cell adhesion, migration and cell division, the actin cytoskeleton exhibits varying changes in tension to generate mechanical force. Nonmuscle myosin 2 (NM2) is an actin binding motor protein that generates the contractile force to facilitate these cellular processes. We have developed a Forster Resonance Energy Transfer (FRET) based biosensor probe of Nonmuscle Myosin 2 (NM2); this probe enabled us to measure the dynamic changes in intracellular tension during various cellular processes. We utilized the recently published tension sensor module (TSMod) in which a 40 amino acid long elastic domain from a spider silk protein was inserted between fluorophores mTFP1 (Donor probe) and venusA206K (acceptor probe) that undergoes low or high FRET depending on the state of the protein (tensed or relaxed) attached to it. We strategically designed and cloned the tension sensor module into the coiled-coil domain of NM2A and NM2B cDNA. The biosensor was then introduced into osteosarcoma (U2OS) and kidney epithelial cell lines (HEK293 and MDCK) by stable and transient transfections. Live cell fluorescence lifetime imaging microscopy (FLIM) was used to quantify the molecular forces by measuring the FRET efficiency, and cells expressing NM2A-TSMod or NM2B-TSMod tension sensors indicated higher and varying FRET efficiency (11.3±4.5%) compared to the donor only control probe (mTFP1-NM2A or mTFP1-NM2B) (2.79±1.4%). Our NM2 biosensor has identified dynamic changes in tension and force within the cell during various activities. First, molecular forces in the range of 3-8 pN were identified in different regions of the cell, as well as along the individual actin filaments in both adherent and migrating cells. The mechanotransduction on a single actin filament was characterized by the fluorescence correlation spectroscopy (FCS), and indicates a fast force transmission (~500 ms) on a single actin filament in U2OS cells. In addition, we measured the mechanical force map of the MDCK cells during cellular migration, molecular forces of actin on the focal adhesion sites exhibit an alternative pattern varying from 2.5 pN to 7.2 pN within 7.2±3 mins. Our NM2 TSMod biosensors can

be applied to visualize and measure dynamic tension changes in cells during various physiological processes.

E112

Improved and tunable molecular tension sensors reveal extension-based control of vinculin loading.

A.S. LaCroix[1], A.D. Lynch[1], M.E. Berginski[1], B.D. Hoffman[1];
[1]Biomedical Engineering, Duke University, Durham, NC

Despite the importance of applied forces and stiffness of the cellular microenvironment in many developmental, physiological, and pathophysiological processes, the molecular mechanisms enabling cells to sense and respond to mechanical stimuli are poorly understood. Forster resonance energy transfer (FRET)-based molecular tension sensors, which enable the visualization of the forces experienced by specific proteins in living cells, are uniquely suited to study these mechanisms. However, the limited dynamic range in existing sensors has hindered their widespread use. The most common design of these sensors involves two fluorescent proteins (FPs) linked by an unstructured, extensible domain. In response to applied load, the domain extends, reducing FRET, and producing a readily detectable optical signal. To enable the rational design of novel tension sensors with improved force sensitivities, we developed a first principles model based on standard descriptions of polymer mechanics to describe the behavior of these sensors under load. Guided by this model, we altered aspects of the tension sensors, such as the optical properties of the FPs as well as the length and composition of the extensible domain, to improve sensor sensitivity and create a collection of sensors for use in living cells. By utilizing a sensor optimized for studying vinculin mechanobiology, we readily observe that loads supported by vinculin are not uniformly distributed within individual adhesion structures. Also, comparison of multiple sensors with distinct mechanical properties reveals the existence of an extension-based, instead of the traditionally assumed force-based, mechanism regulating vinculin loading. Simple simulations of the mechanical characteristics of FAs suggest that the extension-based control is most likely due to extension-controlled mechanical inputs, such as the step size of a molecular motor or the discrete increases in length associated with a growing actin filament, regulating the loading of vinculin. The model also can predict the behavior of readily achievable FRET-based tension sensor designs. Based on the optical properties of commonly used FPs, the length of springs typically used in FRET-based tension sensors, and estimates of the persistence length of unstructured polypeptides, we also calculate the mechanical behavior of an additional 1020 possible designs. The various sensors are predicted to have sensitivities covering the range of 1-25 pN (1-14 nm) with different sensors exhibiting wide varieties of characteristics, including extremely sharp transitions or broad responsiveness. These rationally-designed sensors should uniquely enable a wide variety of novel studies and be very useful in elucidating key mechanisms of mechanotransduction

Microsymp 17: Membrane Dynamics and Trafficking

E113

Compartmentalization of Plasma Membrane by a Self-Similar Cortical Actin Fractal.

S. Sadegh[1,2], J.L. Higgins[3], P.C. Mannion[3], M.M. Tamkun[4,5], D. Krapf[2,3];
[1]Department of Neurosciences, University of California, San Diego, San Diego, CA, [2]Electrical and Computer Engineering, Colorado State University, Fort Collis, CO, [3]School of Biomedical Engineering, Colorado State University, Fort Collins, CO, [4]Department of Biomedical Sciences, Colorado State University, Fort Collins, CO, [5]Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO

A broad range of membrane proteins display anomalous diffusion on the cell surface. Different methods provide evidence for obstructed subdiffusion and diffusion on a fractal space, but the underlying structure inducing anomalous diffusion has never been visualized because of experimental challenges. We addressed this problem by imaging the cortical actin at high resolution while simultaneously tracking individual membrane proteins in live mammalian cells. Our data confirm that actin introduces barriers leading to compartmentalization of the plasma membrane and that membrane proteins are transiently confined within actin fences. Furthermore, superresolution imaging shows that the cortical actin is organized into a selfsimilar meshwork. These results present a hierarchical nanoscale picture of the plasma membrane.

E114

Signalling via membrane receptors generate functional nanodomains at the plasma membrane of living cells.

J. Kalappurakkal[1], A.A. Anilkumar[1,2], T.S. van Zanten[1], M.P. Sheetz[3], S. Mayor[1,4];
[1]National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, India, [2]St. Johns Research Institute, Bangalore, India, [3]Mechanobiology Institute, National University of Singapore, Singapore, Singapore, [4]Institute for Stem Cell Biology and Regenerative Medicine, Bangalore, India

The plasma membrane (PM) of the cell is a dynamic structure that exhibits lateral heterogeneities at various length and time scales [1]. The Outer-leaflet tethered GPI-anchored proteins (GPI-APs) are one of several such components that exhibit a non-random distribution on the PM [2,3,4]. In a resting cell membrane, about ~40% of GPI-APs form transient (<1 sec) nanoclusters driven by the dynamics of the contractile actin machinery operating close to the inner leaflet of the plasma membrane via a trans bilayer acyl-chain-mediated linkage mechanism [3,5,6,7]. While an understanding of the physical principles behind the active mechanics of actin filaments and myosin that drives this behavior is emerging [8], the molecular machinery behind the generation of this dynamic pool of actin and its mode of coupling to inner-leaflet lipids is lacking. Here we show that localized activation of the β1-integrin receptor generates localized nanoclusters of GPI-APs. Surface-tethered and soluble RGD-containing ligands engage the integrin receptor and activate FAK-src kinases and the RhoGTPase, RhoA. RhoA in-turn triggers actin-nucleation via specific formins and activates myosin-II to generate contractile actin filaments. In parallel, integrin signaling results in the talin-mediated opening of vinculin, which potentiates the coupling of actin to the negatively charged lipids at the inner leaflet. This provides a regulated machinery required to create as well as couple dynamic actin activity at the inner leaflet to the organization of outer leaflet GPI-APs. Upon integrin activation, ~80% of GPI-APs from nanoclusters in its vicinity [5]. Consistent with a functional relevance of this machinery, mutant cells that are unable to

generate GPI-AP nanodomains at the cell surface, despite their normal integrin activation status, exhibit severe defects in integrin-mediated cell spreading behavior.
References: [1] Rao, M., and Mayor, S. (2014). Curr. Opin. Cell Biol. 29, 126–132. [2] Varma, R., and Mayor, S. (1998). Nature 394, 798–801. [3] Sharma, P., Varma, R., Sarasij, R.C., Ira, Gousset, K., Krishnamoorthy, G., Rao, M., and Mayor, S. (2004). Cell 116, 577–589. [4] van Zanten, T.S., Cambi, A., Koopman, M., Joosten, B., Figdor, C.G., and Garcia-Parajo, M.F. (2009). Proc. Natl. Acad. Sci. U. S. A. 106, 18557–18562. [5] Goswami, D., Gowrishankar, K., Bilgrami, S., Ghosh, S., Raghupathy, R., Chadda, R., Vishwakarma, R., Rao, M., and Mayor, S. (2008). Cell 135, 1085–1097. [6] Gowrishankar, K., Ghosh, S., Saha, S., C, R., Mayor, S., and Rao, M. (2012). Cell 149, 1353–1367. [7] Raghupathy, R., Anilkumar, A.A., Polley, A., Singh, P.P., Yadav, M., Johnson, C., Suryawanshi, S., Saikam, V., Sawant, S.D., Panda, A., et al. (2015). Cell 161, 581–594. [8] Köster, D. V., and Mayor, S. (2016). Curr.

E115

The Structural Basis of an ESCRT-III Membrane Assembly.

H.C. Nguyen[1], N. Talledge[1,2], J. McCullough[2], D.M. Wenzel[2], J.J. Skalicky[2], W.I. Sundquist[2], A. Frost[1,2,3];
[1]Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA, [2]Department of Biochemistry, University of Utah, Salt Lake City, UT, [3]Chan Zuckerberg Biohub, San Francisco, CA

The Endosomal Sorting Complexes Required for Transport (ESCRT) mediate critical membrane remodeling events throughout the mammalian cell cycle, including HIV budding, cytokinetic abscission, and sealing of the nuclear envelope, among other roles. ESCRT-III proteins assemble into membrane-binding filaments to catalyze these reactions, but the structures and functions of these assemblies remain poorly understood. Our collaborative team recently determined the first atomic-resolution structure of an ESCRT-III filament – a hetero-polymer consisting of CHMP1B and IST1/CHMP8. Our previous structure demonstrated how one of these ESCRT-III subunits, CHMP1B, transitions from a “closed” to an “open” state to form an interlocked and domain-swapped filament. Moreover, we and others have shown that the CHMP1B-CHMP8 copolymer participates in non-canonical, positive-curvature membrane fission pathways. Very recent work on other ESCRT-III proteins indicated that the mechanisms of opening and assembly we reported are conserved, but also raised questions regarding membrane binding and remodeling activities, as well as and the generality of hetero-polymerization. To address these gaps in our understanding, we have determined the high-resolution structure of a membrane-bound CHMP1B-CHMP8 assembly by cryo electron microscopy (cryoEM). We find that CHMP1B induces a high degree of curvature alone, and that deposition of the CHMP8 strand further constricts the membrane tubule by more than 2-fold – almost to the fission point. Notably, the distance between outer leaflet lipid headgroups is ~10 nm and the distance between inner leaflet lipid headgroups is reduced to only ~4 nm. Conserved residues along helix α1 of CHMP1B serve as the major membrane binding surface and exploit both electrostatic as well as hydrophobic interactions with the convex leaflet of the membrane tubule. Our atomic-resolution cryoEM study reveals the structural mechanisms governing ESCRT-III assembly, membrane-binding, and positive-curvature membrane deforming activities. By analogy with the structural features of other ESCRT-III proteins, we propose general rules for membrane-shaping by the ESCRT-III family.

E116

Architecture of the PI4KIIIα lipid kinase complex.

J.A. Lees[1], Y. Zhang[2], M. Oh[1,3,4], C.M. Schauder*[1], X. Yu[5], J. Baskin[1,3,4], K. Dobbs[6], L.D. Notarangelo[6], P. De Camilli[1,3,4,7,8], T. Walz[2], K.M. Reinisch[1];
[1]Department of Cell Biology, Yale University School of Medicine, New Haven, CT, [2]Laboratory of Molecular Electron Microscopy, The Rockefeller University, New York, NY, [3]Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT, [4]Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, CT, [5]Department of Cell Biology, Harvard Medical School, Boston, MA, [6]Immune Deficiency Genetics Section, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, [7]Department of Neuroscience, Yale University School of Medicine, New Haven, CT, [8]Kavli Institute for Neuroscience, Yale University School of Medicine, New Haven, CT

Plasma membrane (PM) phosphoinositides, including phosphatidylinositol-4-phosphate (PI4P) and its metabolites, phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) and phosphatidylinositol-3,4,5-trisphosphate ($PI(3,4,5)P_3$), play important roles in cell physiology. PI4P and $PI(4,5)P_2$ act as key determinants of PM identity, recruiting effector proteins to the PM to control membrane and cytoskeletal dynamics, while $PI(4,5)P_2$ and $PI(3,4,5)P_3$ play crucial roles in cellular signaling pathways. The PI4KIIIalpha lipid kinase complex catalyzes the first step in the synthesis of PM phosphoinositides, converting phosphatidylinositol (PI) to PI4P. Conserved broadly from yeast to humans, the mammalian kinase (Stt4p in yeast) forms a complex with the regulatory subunits TTC7 and FAM126. These three subunits are recruited to the PM by a fourth protein called EFR3. To gain insights into its function, we have determined the structure of the human PI4KIIIalpha complex at a resolution of 3.6 Å using cryo-electron microscopy. We find that the lipid kinase homodimerizes, bringing together two PI4KIIIalpha/TTC7/FAM126 heterotrimers to form a ~700-kDa complex. Large conserved interaction surfaces between each of the components suggest that the complex remains stably associated in the cell. The conformation of the kinase is constrained by its interactions with the TTC7/FAM126 complex, which are required for its stability *in vivo*. The complex forms a flat surface suited for binding to the membrane, which orients the kinase catalytic domains ideally for reaction with PM PI. Our results offer insights relevant to human disease, including combined immunodeficiency with multiple intestinal atresias (CID-MIA) and Hepatitis C Virus (which requires PI4KIIIa for its replication), while providing a framework for understanding a key regulator of PM phosphoinositide homeostasis.

E117

A kinesin-3 motor transports newly synthesized basement membrane proteins specifically to a basal subregion of the lateral plasma membrane in epithelial cells.

A.L. Zajac[1], A.J. Isabella[1,2], K.E. Sy[1], S. Horne-Badovinac[1];
[1]Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, [2]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA

Basement membranes (BMs) are sheet-like extracellular matrices (ECMs) that play many important roles in tissue organization. In addition to providing attachment sites to cells, these structures act as a reservoir for growth factors, provide polarity information to cells, and provide instructive cues for tissue morphogenesis. Polarized secretion of BM proteins ensures that the BM matrix assembles exclusively along the basal surface of epithelial cells. However, BM proteins rely on a poorly understood polarized secretion pathway that is distinct from that used by general basolateral cargo. The follicular epithelium of the Drosophila egg chamber secretes all major BM proteins and assembles a BM along its basal

surface on the outside of the tissue. This geometry places the trafficking events involved in BM secretion near the tissue's exterior and allows high-resolution imaging of the secretion process in a living, intact organ. We previously identified the GTPase Rab10 as a central regulator of BM polarized secretion. Here we show that a surprisingly small region of the basolateral membrane is targeted for BM secretion and that a kinesin-3 family motor is required for transport to this location. To identify the site of BM protein secretion, we blocked the final tethering stage of exocytosis using mutations in the exocyst complex, a known Rab10 interaction partner. In exocyst mutant cells, BM proteins accumulate just inside the basal-most ~0.5 um of the lateral plasma membrane (PM). To understand how this small region is targeted for secretion, we investigated the dynamics of Rab10+ vesicles leaving the Golgi. Rab10+ vesicles move rapidly and processively on microtubules (MTs) and accumulate near the basal-most region of the lateral PM. Since MT plus-ends are enriched basally, we performed an RNAi screen against plus-end-directed kinesin motors and identified the kinesin-3 family member Khc-73 as a novel regulator of BM trafficking. Khc-73 colocalizes with Rab10, and loss of Khc-73 reduces the basal accumulation of Rab10. We therefore propose that Khc-73 transports Rab10+ vesicles from the Golgi to the basal-most region of the lateral PM for secretion. To test the importance of this targeted secretion, we examined the structure of the BM after loss of Khc-73. In this mutant condition, BM proteins accumulate in between cells along the entire lateral domain and form an intercellular network of BM proteins that disrupts tissue organization. Altogether, this work highlights the importance of precisely targeting BM protein secretion, as this precise targeting ensures that the BM is assembled in the correct location to properly organize a developing tissue.

E118

Dynamics of Exocyst Subunit Assembly and Vesicle Fusion, using CRISPR-edited GFP Tagging of Endogenous Loci.

H. Nishida-Fukuda[1], I.G. Macara[1], S.M. Ahmed[1];
[1]Cell and Developmental Biology, Vanderbilt University, Nashville, TN

The exocyst is an essential complex required for delivery of proteins to the plasma membrane. There is a continuing controversy about whether the exocyst is a stable octamer that binds to the membrane and tethers exocytic vesicles, or is a dynamic complex in which subcomplexes are assembled at the fusion site. To explore the dynamics of exocyst function we created sfGFP-tagged alleles of 5 exocyst subunits (Sec3, Sec5, Sec6, Sec8 and Exo70) in a mouse mammary epithelial cell, NMuMG, using the CRISPR/Cas9 system, and tracked the arrival of these subunits and of vesicles at the plasma membrane by TIRF microscopy. We transfected VAMP-pHluorin and TfR-pHuji to visualize vesicle fusion and mApple-Rab11 to visualize vesicles. We were unable to recover viable cells with fusions of 3 vesicle-associated exocyst subunits (Sec10, Sec15 and Exo84), suggesting that these constructs are non-functional. We discovered that vesicle fusion occurs about 15 seconds after arrival at the membrane. Exo70 and Sec6 arrive at a similar time and disappear immediately after fusion. Interestingly, Sec5 arrives earlier - about 19 seconds prior to fusion - and Sec3 disappears about 4 seconds before fusion occurs. These data suggest that the exocyst is not pre-assembled but instead that subunits arrive and disappear at different times. Using double-tagged cells we are now cross-correlating these events, and can begin to catalog in unprecedented detail the itinerary for vesicle delivery to the plasma membrane, and the mechanisms by which tethering and fusion are regulated.

E119

The role of membrane curvature in topography-induced cellular signaling.

B. Cui[1], W. Zhao[1], H. Lou[1], F. Santoro[1];
[1]Department of Chemistry, Stanford University, Stanford, CA

Many biomedical applications require direct contact between the cells and non-biological materials. For example, medical implants inserted into the patient's body have intimate contact with adjacent cells and tissues. Synthetic materials are recognized by cells through their chemical compositions as well as their physical properties. The importance of physical properties in modulating cellular behavior, such as surface topography and material rigidity are increasingly recognized. In particular, studies show that surface topography in the scale of tens of nanometers to a few micrometers significantly affect cell adhesion and tissue integration. As topographic features are stable over long-term and easier to control, they offer unique advantages for modulating cell responses for tissue engineering. Despite a large body of observations, little is known about the origin or underlying mechanisms of the effect of topographical cues on cellular behavior. We explore the underlying mechanisms by employing advanced nanotechnology that generates precisely controlled nanotopography. Our results shows that local membrane curvature induced by nanoscale topographical features significantly affect the distribution of curvature-sensitive proteins and stimulate several cellular processes in live cells including clathrin-mediated endocytosis and actin dynamics. We propose that local membrane curvature is the critical player in topography-induced intracellular signaling. Furthermore, our studies show a strong interplay between biological cells and nano-featured surfaces, which is an essential consideration for future development of interfacing devices.

Microsymp 18: Bioengineering and Signaling

E120

Mechanisms connecting the conserved protein kinases Kin1, Pom1, and Ssp1 in fission yeast cell polarity and division.

M. Lee*[1], S.F. Rusin[1], N. Jenkins[1,2], A.N. Kettenbach[1,2], J.B. Moseley[1];
[1]Department of Biochemistry and Cell Biology, The Geisel School of Medicine at Dartmouth, Hanover, NH, [2]Norris Cotton Cancer Center, The Geisel School of Medicine at Dartmouth, Lebanon, NH

Connections between the protein kinases that function within complex cell polarity and division networks are poorly understood. Rod-shaped fission yeast cells grow in a highly polarized manner, and genetic screens have identified multiple protein kinases required for polarized growth and cell shape in this organism. Kin1, the sole MARK/PAR-1 family kinase in fission yeast, regulates cell polarity and cytokinesis through unknown mechanisms. Here, we show that the CaMKK-like Ssp1 acts as an upstream activator of Kin1 by directly phosphorylating the Kin1 activation loop to promote cell polarity. To define the downstream targets of Ssp1-Kin1 signaling, we performed large-scale phosphoproteomics screens that identified Kin1 substrates in cells. We found that Kin1 phosphorylates itself and the endocytic adaptor protein Pal1 to promote growth at cell tips, and these proteins were interdependent for localization to growing cell tips. Additional Kin1 substrates for cell polarity and cytokinesis (Tea4, Mod5, Cdc15 and Cyk3) were also directly phosphorylated by a second cell polarity kinase, the DYRK-family member Pom1. Interestingly, Kin1 and Pom1 were enriched at opposite ends of growing cells, and they phosphorylated largely non-overlapping sites on shared substrates. Combined inhibition of both Pom1 and Kin1 led to synthetic defects in Cdc15 and Cyk3, confirming a non-redundant functional connection through shared substrates. Thus, connections between protein kinases can be direct (Kin1-Ssp1) or

indirect (Kin1-Pom1), and contribute to dynamic control of robust cellular processes such as polarized growth and division. These findings identify connections between Kin1 and other conserved protein kinases in the polarity and division network, providing insights into similar mechanisms in other higher organisms.

E121

Phosphorylation of the HPV E6 oncoprotein by DNA damage response kinases links the E6 interaction with 14-3-3 proteins and p53.

J.V. THATTE[1], P. Massimi[1], L. Banks[1];
[1]Tumour Virology , International Centre for Genetic Engineering and Biotechnology (ICGEB), TRIESTE, Italy

Human Papillomavirus (HPV) is a major cause of human cancers, with cervical cancer being the most prevalent. The virus encodes two oncoproteins, E6 and E7, together which are responsible for the initiation and maintenance of malignancy. The cancer-causing E6 oncoproteins are characterized by the presence of a PDZ binding motif (PBM) on the C-terminus. This motif confers interaction with a variety of cellular proteins involved in the regulation of cell polarity, and phosphorylation by PKA switches E6 substrate specificity to members of the 14-3-3 protein family. In this study we have been interested in analyzing the conditions under which E6 is subject to phosphorylation within the PBM. Using a variety of approaches, we demonstrate a striking increase in the levels of E6 phosphorylation by a number of DNA damage response kinases, including those that are induced following treatment with chemotherapeutic agents. In order to understand the biological relevance of these phospho-modifications of E6, we have analysed their effects upon the ability of E6 to inhibit p53 transcriptional activity. We show that phospho-E6 has increased interaction with 14-3-3 proteins, a direct consequence of which is an inhibition of p53 transcriptional activity. These studies demonstrate that, under conditions that induce the DNA damage response, E6 has acquired an alternative means of disrupting p53 activity, through its PBM phosphorylation and subsequent sequestration of 14-3-3 from p53. This has important implications for how E6 contributes towards the development of malignancy: it provides an alternative means of inhibiting p53 activity, explains why the PBM is specifically conserved in cancer causing E6 oncoproteins, and explains why the residual levels of p53 in cervical cancer are nonetheless subject to inactivation by E6.

E122

Mad1 promotes tumor progression through destabilization of p53.

J. Wan[1], B.A. Weaver[1];
[1]Cell and Regenerative Biology , University of Wisconsin-Madison, MADISON, WI

Mitotic arrest deficient 1 (Mad1) plays a well-characterized role in the mitotic checkpoint. However, interphase roles of Mad1 that do not impact mitotic checkpoint function are still largely uncharacterized. We have found that overexpression of Mad1, which is common in human breast cancer, results in decreased protein stability of the tumor suppressor p53 in multiple cell types. To gain mechanistic insight into this process, we first determined whether increasing expression of Mad1 alters its localization. Upregulated Mad1 localizes to puncta within interphase nuclei in both breast cancer tissue and cultured cells. We found that upregulated Mad1 localizes to ProMyelocytic Leukemia Nuclear Bodies (PML NBs), which have been implicated in the stabilization of p53. Immunoprecipitation results indicate that Mad1 and PML interact and that the C-terminal domain (CTD) of Mad1 and the N-terminal domain of PML are required for this interaction. Within the CTD of Mad1, the SUMO-Interacting Motif

(SIM) is critical for the localization of Mad1 to PML NBs. MDM2 is an E3 ubiquitin ligase that targets p53 for degradation. In response to DNA damage, PML sequesters Mdm2 to the nucleolus, which stabilizes p53. In cells with elevated levels of Mad1, Mad1 interrupts the interaction between PML and MDM2. Mad1, rather than MDM2, is sequestered to the nucleolus after DNA damage, suggesting that Mad1 displaces MDM2 from PML. The displaced MDM2 is no longer sequestered in the nucleolus, freeing it to ubiquitinate p53, resulting in p53 destabilization. Upregulation of Mad1 accelerates growth of orthotopic mammary tumors, which show decreased levels of p53 and its downstream effector p21. In addition to causing a low rate of chromosome missegregation, our results show an unexpected interphase role for Mad1 in promoting tumor formation and progression by destabilizing p53.

E123

A novel window for high resolution imaging of the lung reveals mechanisms of metastatic breast cancer progression.

L. Borriello[1,2], S. Voiculescu[3], Y. Wang[1,2,4], M.H. Oktay[1,4,5], J.S. Condeelis[1,2,4], D. Entenberg[1,2,4];
[1]Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY, [2]Gruss-Lipper Biophotonics Center, Albert Einstein College of Medicine, Bronx, NY, [3]Department of Surgery, Albert Einstein College of Medicine, Bronx, NY, [4]Integrated Imaging Program, Albert Einstein College of Medicine, Bronx, NY, [5]Department of Pathology, Albert Einstein College of Medicine, Bronx, NY

It has become clear that the tumor microenvironment dominates the metastatic phenotype of tumor cells. In addition, the cell biology mechanism behind tumor cell dissemination from the primary tumor is becoming well understood thanks to high resolution multiphoton intravital imaging. Imaging shows that therapeutic agents, including cytotoxic chemotherapy and receptor tyrosine kinase inhibitors, have the ability to alter this microenvironment to either promote or reduce metastatic dissemination from the primary tumor. Unknown however, is if the mechanism of tumor cell dissemination from the primary tumor is conserved in metastatic tumors and if the above treatments impact the metastatic site differently from the primary tumor, essential information for the design of long term treatment strategies. Each of the major metastatic sites (bone, lung, and liver) are inaccessible internal organs limiting analysis to standard assays (FACS, histopathology, etc.) which destroy the tissue and thus only give single time point, snap-shot analyses without vital information about the tumor microenvironment. Only intravital imaging through implantable imaging windows gives the ability to return to the tissue multiple times over course of disease progression and treatment. The lung, the central metastatic site in breast cancer patients, is the most difficult organ to study using intravital microscopy due to its perpetual motion. Recent advances in intravital microscopy have enabled visualization of the live intact lung, but are limited in time over which they can be utilized (hours) and require major, invasive surgeries. Both of these limitations reduce their usefulness to the study of single cell events in the lung and are susceptible to introducing artifacts. We have addressed these limitations by developing an implantable, permanent, lung imaging window which allows high-resolution multiphoton imaging of the intact, breathing (without ventilation), murine lung over days to weeks of repeated imaging. This window does not use vacuum to immobilize the lung tissue, thereby avoiding artifacts associated with vacuum lung windows. Using our new window, we have documented over weeks, and for the first time, tumor cell arrival, the cell motility steps used during extravasation, tumor cell survival, dormancy, and progression to micro-metastases, all at subcellular resolution. Further, we have observed, for the first time, the presence and intravasation activity of the Tumor MicroEnvironment of Metastasis (TMEM) in metastatic lung lesions directly demonstrating use of the same mechanism of tumor cell dissemination from metastatic lung tumors as observed in the primary tumor opening new strategies for the treatment of metastatic tumors to prevent metastatic progression and death.

E124

Eyes in the cell: Visualizing active kinases using genetically encodable fluorescent biosensors.

A. Mukherjee[1,2], R. Singh[1], S. DilipKumar[1], P. Pothula[1], S. Udayan[1], R. Das[3], B. Rao[4], A. Gulyani[1];
[1]technology for the advancement of science, institute for stem cell biology and regenerative medicine, bangalore, India, [2]school of chemical and biotechnology, SASTRA University, thanjavur, India, [3]National Centre for Biological Sciences, bangalore, India, [4]Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC

Cell fate and physiology are regulated by cell signaling wherein signaling proteins play a central role. On receiving cues (internal and/or external) proteins are known to form complex interactomes thereby regulating many processes with immense specificity. Very often the same protein/family of proteins gives rise to very different cellular outcomes and responses downstream of these cues. Therefore, to understand a given cellular pathway, understanding the stringent spatio-temporal regulation of these signaling proteins becomes essential. One such approach is to selectively detect and report the "active" state of the protein. Using this approach, our lab has generated a toolbox of fluorescent biosensors for the Src family of kinases. Src kinases are a family of non-receptor tyrosine kinases and signals emanating from these kinases are known to feed into multiple pathways. Fyn kinase (an SFK member) is ubiquitously expressed in all cell types wherein it controls a myriad of functions ranging from control of myelination of neurons by oligodendrocytes to regulation of adhesion structures in keratinocytes making it an interesting candidate protein. However, a better understanding of its role in this process has been lacking due to the paucity of specific tools available owing to the high degree of homology amongst the Src kinases. By adapting a stable scaffold based high through-put screening regime we have been able to generate a very specific FRET based "biosensor" for Fyn kinase. Using this sensor we find that cells plated on fibronectin (an integrin ligand) are highly polarized with respect to Fyn activity and that on addition of Platelet-derived growth factor (PDGF), an amplification in this polarized signal is achieved. This is the first conclusive evidence showing involvement of Fyn in mediating cross-talk between different types of receptors and such an approach could help uncover a general mechanism for Fyn's biological action.

E125

Investigating the Conformational Landscape in a Protein-Signaling Complex with Optical Tweezers.

J.P. England[1], Y. Hao[1], S.S. Taylor[2], R.A. Maillard[1];
[1]Chemistry, Georgetown University, Washington, DC, [2]Chemistry, University of California San Diego, San Diego, CA

Protein Kinase A (PKA) is a critical molecular switch that regulates cell-signaling pathways (e.g. Hedgehog Signaling). PKA is composed of catalytic and regulatory subunit that combine to form an inactive complex. Allosteric binding of two molecules of cAMP to domain A and B in the regulatory subunit triggers a conformational change and facilitates the release of the active catalytic subunit. Mutations that affect the interaction between the regulatory and catalytic subunit result in the misregulation of PKA and consequently in tumors that are associated with Cushing's syndrome and Fibrolamellar Hepatocellular Carcinoma. Despite its importance in disease, the details of the cooperativity involved in the allosteric mechanism of PKA activation still remains unclear.
In order to study the cooperativity between domains, single molecule optical tweezers were applied to selectively manipulate domains A and B in the absence and presence of catalytic subunit. Our results show that in the absence of catalytic subunit (apo), the regulatory subunit unfolds in two sequential, but

independent unfolding transitions in which domain B unfolds first with a transition state energy barrier ($\Delta G^{\ddagger}$) of 18.5 k_BT, followed by domain A at 33 k_BT. Interestingly, in the presence of catalytic subunit (bound), the regulatory subunit unfolds cooperatively, where domain B unfolds first at 30 k_BT, followed by the immediate unfolding of domain A at 17.5 k_BT. Additional single molecule studies that unfold the bound regulatory subunit with a mutation that decreases the affinity of domain B for cAMP results in a decoupling of the unfolding energies, where $\Delta G^{\ddagger}$ for domain B and A is 23 k_BT and 20 k_BT.
Our study provides the first observation of a thermodynamic switching mechanism for a protein-signaling complex. This study shows that cooperative interactions between domain A and B are mediated by the presence of the catalytic subunit and is necessary for PKA activation. This cooperativity between domains was quantified thermodynamically by a switch in the energetics of the unfolding energy landscape between the apo and bound conformation. The switch towards a higher $\Delta G^{\ddagger}$ for domain B in the bound state provides insight into the thermodynamic origin of the gatekeeper function of domain B in the PKA complex. Bulk and computational studies of the PKA complex show that binding of cAMP must first occur at domain B before the second cAMP molecule can bind to domain A. Additionally, the decoupling of domain A and B in the mutant protein construct suggest that domain B is propagating an effect to domain A. Future studies include investigating the conformational changes in the PKA Holoenzyme as a function of cAMP concentration.

E126

Three-dimensional modeling of metastatic breast cancer dormancy using tunable PEG-based hydrogels.

S. Pradhan[1], J.H. Slater[1];
[1]Department of Biomedical Engineering, University of Delaware, Newark, DE

Metastasis is the leading cause of breast cancer-related mortality, with only a few palliative treatment options available for patients. One of the major challenges of targeting metastatic disease is the presence of disseminated tumor cells (DTCs), that lie dormant in secondary organotropic niches within the body for extended periods of time. Specifically targeting these DTCs as an approach towards reducing metastatic outbreaks is difficult owing to their quiescent behavior, poorly understood cellular characteristics, and limits of clinical detection. Tumor dormancy is manifested in one of two ways: cellular dormancy (G0-G1 arrest) or dormant micrometastasis (angiogenic dormancy). Targeting the dominant mechanism determining metastatic breast tumor dormancy is of prime interest toward suppressing metastatic outbreaks and improving patient survival rates.
To establish an in vitro system for improved understanding, detection, and treatment of dormant breast cancer, we have developed a three-dimensional (3D) hydrogel culture platform for the recapitulation of key microenvironmental features of the dormant niche. In this study, we encapsulated MDA-MB-231 metastatic breast cancer cells within cell-responsive peptide-conjugated poly(ethylene glycol diacrylate) hydrogels and maintained them in 3D culture for 15 days. The hydrogel stiffness and cell-adhesiveness were modulated through the introduction of additional crosslinking polymer networks and cell-adhesive ligands to cover a wide range of extra-cellular matrix (ECM) characteristics. MDA-MB-231s encapsulated within these hydrogel matrices were investigated for viability (via Live/Dead staining), early-stage apoptosis (via Annexin V staining), and proliferation (via EdU staining) every 5 days post-encapsulation for a total of 15 days.
Our results indicate that cancer cell dormancy is dually dependent on cell-adhesiveness and matrix stiffness. Increasing stiffness leads to reduced proliferation but also increased apoptosis while increasing matrix adhesiveness leads to higher proliferation. The cell proliferation and viability were quantified to vary between 5-10% and 80-90% respectively under the tested conditions, thereby demonstrating that

an optimum matrix formulation has been devised that restricts proliferation of cancer cells through cellular dormancy while still maintaining high viability in 3D culture. This platform is being further implemented to provide insight into the cellular and molecular characteristics of dormant breast cancer cells and to facilitate investigation of organotropic dormancy behavior that could aid in future cancer drug discovery initiatives.

Wednesday, December 6

Symp 6: Quality Control

S14

Orchestration of mitosis by Anaphase-Promoting Complex, a fascinating molecular machine.

B.A. Schulman[1];
[1]Molecular Machines Signaling, Max Planck Institute of Biochemistry, Munich, Germany

The massive, multiprotein E3 ligase Anaphase-Promoting Complex/Cyclosome (APC/C) is a dazzling molecular machine that regulates mitosis, meiosis, and numerous facets of neurobiology by targeting key regulatory proteins for ubiquitin-mediated degradation. APC/C initiates chromosome segregation by promoting ubiquitin-mediated proteolysis of anaphase inhibitors such as Securin, and of B-type cyclins to terminate CDK1 activity. The timing of their degradation is crucial for faithful cell division, which is ensured by extensive regulation of APC/C E3 ligase activity. This involves appropriately timed APC/C phosphorylation, inhibition, activation, and unprecedented mechanisms of ubiquitylation. I will present our current understanding of the structural and molecular mechanisms explaining how this fascinating molecular machine elegantly orchestrates accurate stepwise progression through the cell cycle.

S15

Mechanism of ER-associated protein degradation (ERAD).

T. Rapoport[1,2];
[1]Cell Biology, Harvard Medical School, Boston, MA, [2]Harvard Medical School, Howard Hughes Medical Institute, Boston, MA

Misfolded proteins of the endoplasmic reticulum (ER) are retro-translocated into the cytosol, poly-ubiquitinated, and degraded by the proteasome, a process called ER-associated protein degradation (ERAD). We have used purified components from Saccharomyces cerevisiae to analyze the mechanism of retro-translocation of luminal and membrane substrates (ERAD-L and -M). Our results show that the ubiquitin ligase Hrd1p forms a ubiquitin-gated protein-conducting channel that allows a substrate segment to move into the cytosol. An electron cryo-microscopy structure indicates that the Hrd1 channel has a deep cytosolic cavity and a lateral gate. Once the substrate is poly-ubiquitinated, the Cdc48p ATPase complex is recruited and extracts the substrate from the membrane. The hexameric Cdc48 ATPase moves the substrate through its central pore, exerting a pulling force and resulting in substrate unfolding. Finally, substrate is released from Cdc48 by a deubiquitinase, which trims poly-ubiquitin to an oligo-ubiquitin chain that is then also translocated through the pore. Taken together, these results clarify important aspects of ERAD.

Minisymposium 21: Border Dynamics: Nuclear Envelope Organization and Remodeling

M209

In situ structural analysis of the nuclear pore complex.

M. Beck[1];
[1]Structural and Computational Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany

Nuclear pore complexes (NPCs) are fundamental components of all eukaryotic cells. They mediate nucleocytoplasmic exchange, regulate gene expression and are of high human health relevance. Elucidating their 110 MDa structure imposes a formidable challenge and requires in situ structural biology approaches. Fifteen out of about thirty nucleoporins (Nups) are structured and form the Y- and inner ring complexes. These two major scaffolding modules assemble in multiple copies into an eight-fold symmetric structure that fuses the inner and outer nuclear membranes to form a central channel of ~60 nm in diameter. The scaffold is decorated with transport channel Nups that often contain phenylalanine (FG)-repeat sequences and mediate the interaction with cargo complexes. We combined cryo-electron tomography with mass spectrometry, biochemical analysis, perturbation experiments and structural modeling to investigate nuclear pore architecture in situ. We obtained the most comprehensive architectural model of the NPC to date. We conclude that, similarly to coated vesicles, multiple copies of the same structural building block - although compositionally identical - engage in different local sets of interactions and conformations.

M210

DNA-origami based platforms reveal mobility of FG-nups within nuclear pore complex-like architectures.

Q. Shen[1], P.E. Fisher[1], B. Akpinar[2], T.J. Melia[1], B. Hoogenboom[2], C. Lin[1], P. Lusk[1];
[1]Cell Biology, Yale School of Medicine, New Haven, CT, [2]London Centre for Nanotechnology, London, United Kingdom

Intrinsically disordered “FG-nup” proteins fill the central transport channel of the nuclear pore complex (NPC). These proteins are rich in repetitive peptide motifs of Phe-Gly (FG) amino acid residues, and collectively establish a size-selective diffusion barrier while simultaneously allowing the rapid transport of cargo-carrying nuclear transport receptors (a.k.a. karyopherins/importins/exportins). The molecular mechanisms that underlie the permeability properties of the NPC remain to be fully determined. For example, the form or “phase” of the FG-nup collective remains largely defined by common physical descriptors, such as “polymer brushes” and “hydrogels”, that are predominantly derived from in vitro experimental data where FG-nups are grafted on 2D surfaces or phase-separate out of solution. But, it remains unknown how the individual properties (ex. cohesiveness) of different “flavors” of FG-nups (ex. GLFG and FxFG) combine within the confinement of a ~40 nm-diameter pore architecture as, thus far, there has been no experimental platform capable of mimicking NPC-like geometries in which the stoichiometry and anchor points of the FG-nups can be accurately controlled and defined. Here, we take advantage of DNA origami, a programmable self-assembly technique that folds DNA strands into designer 2D and 3D shapes, to build “nuclear pores on DNA” or “NuPODs.” Our first generation NuPODs are capable of housing up to 48 copies of individual, and combinations of up to two FG-nups, at precise positions that mimic the 8-fold symmetry and grafting densities of FGs within a native NPC. By transmission electron and super-resolution microscopy, we observe unique conformations of FxFG and

GLFG nups with the latter capable of sampling a greater volume and the former filling the central pore of the NuPODs. Most strikingly, high speed atomic force microscopy shows that FG-nups exhibit remarkable mobility within the DNA-origami ring forming "clumps" that can reversibly occlude the "channel"; we are currently testing how nuclear transport receptors influence this mobility. These data suggest a rationale for how the FG-nups can simultaneously act as a diffusion barrier to inert macromolecules while allowing the passage of large cargos. We have thus developed a platform for investigating the sieving properties of complex networks of FG-nups within a well-controlled 3D architecture – an important first step to developing a fully engineered NPC mimic.

M211

Transcription factor-mediated targeting of genes to the nuclear pore complex is the major pathway controlling peripheral localization of genes in budding yeast.

D.G. Brickner[1], M. LeBrun[1], C.R. Hinchliff[1], J.H. Brickner[1];
[1]Molecular Biosciences, Northwestern University, Evanston, IL

Hundreds of genes physically interact with the nuclear pore complex (NPC) and many inducible genes reposition to the nuclear periphery when they are activated. In budding yeast, interaction with the NPC is controlled by *cis*-acting targeting elements (or "DNA zip codes") and requires both NPC proteins and transcription factors (TFs). Here, we describe the mechanistic dissection of the function of TFs and transcriptional regulators/mRNA export factors in mediating targeting to the nuclear periphery. Further, we have performed a global screen of all ~200 yeast transcription factors to test the generality of this phenomenon. Each TF was tagged with the DNA binding domain from LexA and crossed against a strain having the LexA binding site integrated at a locus that normally localizes to the nucleoplasm. The position of this locus with respect to the nuclear envelope was scored using confocal microscopy. Approximately 50% of the tested transcription factors were sufficient to cause localization to the nuclear periphery. The major pathway by which this targeting was mediated required the NPC; CRISPR-mediated mutations in the NPC protein Nup2 blocked peripheral targeting by >95% of these TFs. This suggests that controlling gene positioning within the nucleus is an important and unappreciated function of TFs and that interaction with the NPC is the major pathway by which genes localize at the nuclear periphery. The TFs identified in the screen regulate over 1000 genes, including housekeeping genes as well environmentally or developmentally regulated genes. The TFs are found in all transcription factor families in budding yeast and a large subset (50%) of the transcription factors identified have human homologs. A human TF was able to mediate targeting to the nuclear periphery in yeast, suggesting that the molecular mechanism of gene recruitment to the NPC has been deeply conserved and likely plays a fundamental role in genome organization.

M212

Lamin mutations linked to muscular disease result in mechanically-induced, progressive nuclear envelope rupture and DNA damage in muscle fibers.

A. Earle[1], T.J. Kirby[2], G.R. Fedorchak[1], P. Isermann[2], J. Lammerding[1,2];
[1]Biomedical Engineering, Cornell University, Ithaca, NY, [2]Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY

Mutations in the nuclear envelope proteins lamin A/C cause a broad spectrum of human diseases that include various muscular dystrophies and dilated cardiomyopathy. Despite the near ubiquitous expression of lamin A/C, the majority of mutations lead to skeletal and cardiac muscle phenotypes. The molecular mechanisms underlying these muscle specific defects remain unclear. One potential explanation, the 'mechanical hypothesis', states that lamin mutations result in structurally impaired nuclei that become damaged in mechanically active tissue such as cardiac and skeletal muscle. Patient biopsies have revealed anecdotal evidence of damaged and 'ruptured' nuclei in laminopathies, but this phenomenon has never been systematically investigated. Here, we show that loss of lamins and mutations linked to muscular dystrophy reduced nuclear stability in primary mouse myoblasts, and resulted in severe nuclear damage in muscle fibers in vitro and in vivo. The nuclear defects included severe loss of nuclear envelope integrity and chromatin protrusion across the nuclear lamina that span up to tens of micrometers into the cytoplasm. Combining live-cell imaging with a novel in vitro muscle fiber differentiation assay that allows for longer term differentiation, we found that both frequency and extent of nuclear damage progressively increased with muscle fiber differentiation and contraction in lamin mutant cells, indicating that the observed defects are linked to the increased mechanical stress in contracting muscle fibers. Further supporting this hypothesis, mechanical overload of hindlimb muscle in lamin mutant mice accelerated nuclear rupture and damage. To investigate whether chromatin protrusions were associated with a loss of nuclear compartmentalization, we performed time-lapse imaging of cells expressing NLS-GFP or mCherry-cGAS nuclear rupture reporters. In lamin mutant myoblasts, the incidence of nuclear envelope rupture progressively increased during differentiation, while nuclear rupture was absent in wild-type cells. Staining for HSP90, an endogenous cytoplasmic protein that was found within the nuclei of lamin-mutant myofibers but not wild-type controls, confirmed nuclear envelope rupture in vitro and in vivo. Concurrently, we found that nuclear defects were associated with increased DNA damage in vitro and in vivo, measured by γH2AX and 53BP1 accumulation, resembling our previous findings in cells that experienced nuclear envelope rupture during confined migration. Taken together, our findings suggest that lamin mutations associated with muscle disease structurally weaken the nucleus, and result in severe and progressive nuclear damage in muscle fibers that could contribute to the muscle-specific phenotypes seen in many laminopathies.

M213

Mechano-protection by lamin-A against DNA damage as the developing heart stiffens and strengthens.

S. Cho[1], S. Majkut[2], A. Abbas[1], K. Vogel[1], M. Vashisth[1], J. Irianto[1], M. Tewari[1], B.L. Prosser[3], D.E. Discher[1];
[1]Molecular Cell Biophysics Lab, University of Pennsylvania, Philadelphia, PA, [2]Department of Physics Astronomy, University of Pennsylvania, Philadelphia, PA, [3]Department of Physiology, Pennsylvania Muscle Institute, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Across all terminally differentiated adult tissues, the lamins are the main structural proteins of the nucleus that increase in A- to B-type stoichiometry ('lamin-A:B') when plotted versus collagen-I and tissue stiffness [Swift *et al*, 2013]. To understand mechanisms and functional contributions, beating

hearts from chick embryos at different stages were characterized with various perturbations, revealing that lamin-A:B again scales with the progressive increase in collagen-I, and that lamin-A levels change within ~1 hr when tissue is softened or stiffened by perturbing collagen or actomyosin contractility. Upon tissue softening, phosphorylation and solubilization of lamin-A increase to facilitate its degradation by constitutive matrix metalloproteinase 2 (MMP-2) within the nucleus. DNA damage decreases upon inhibition of actomyosin contractility and moreso if lamin-A is kept high by inhibiting its solubilization. Transcriptional control of lamin-A using retinoids likewise influences DNA damage levels. Isolated cardiomyocytes on collagen-coated soft or stiff gels show that lamin-A again increases monotonically versus matrix stiffness with regulation by phosphorylation and by MMP-2, while decoupling from rhythmic beating. Knockdown of lamin-A in iPS-derived cardiomyocytes on stiff matrices increases frequency of nuclear rupture, loss of DNA repair factors from the nucleus, and DNA damage, with rescue by actomyosin inhibition and by soft matrix. Lamin-A thus couples to actomyosin tension and matrix stiffness in order to minimize DNA damage.

M214

Evolution of mitotic nuclear envelope dynamics through Lem2-ESCRT-III/Vps4 interaction.

G. Pieper[1,2], S. Sprenger[3], D. Teis[3], S. Oliferenko[1,2];
[1]Francis Crick Institute, London, United Kingdom, [2]Randall Division of Cell and Molecular Biophysics, King's College London, London, United Kingdom, [3]Division of Cell Biology, Medical University of Innsbruck, Innsbruck, Austria

Chromatin tethering LEM-domain proteins are conserved molecular components of the inner nuclear membrane that play an important role in nuclear envelope (NE) function throughout eukaryotes. We use *Schizosaccharomyces pombe* and *Schizosaccharomyces japonicus*, two closely related fission yeast species with different mitotic strategies, to investigate how LEM-domain proteins have evolved to sustain distinct modes of mitosis. *S. pombe* undergoes ‘closed’ mitosis, whereas *S. japonicus* ruptures the NE during anaphase.
Here we show that the LEM domain protein Lem2 together with ESCRT-III and the AAA-ATPase Vps4 mediate post-mitotic NE resealing in *S. japonicus*. We demonstrate that ESCRT-III and Vps4 localize at sites of NE resealing and spindle pole body extrusion from the NE at the end of mitosis. Furthermore, ESCRT-III/Vps4 are frequently recruited to the heterochromatin-rich NE domains during interphase and appear to function in restructuring of chromatin-NE attachments during mitosis. Curiously, the *S. japonicus* but not the *S. pombe* Lem2 contains MIM1, a short motif found on ESCRT-III subunits and recognised by Vps4. In *S. japonicus* Lem2 is required for all instances of ESCRT-III recruitment to the NE. In turn, Lem2 localization and function in this organism are disrupted in ESCRT-III mutants or when MIM1 is mutated and Vps4 recruitment is abolished.
Our data suggest a mechanism in which *S. japonicus* Lem2 directs NE dynamics by multimodal recruitment of ESCRT-III and Vps4 to sustain a ‘semi-open’ mitosis in this fission yeast species. I will also present our recent results on the surprising evolutionary fluidity of this interaction.

M215

Dual spindle formation around zygotic pro-nuclei explains parental genome separation.

J. Reichmann[1], B. Nijmeijer[1], J.M. Hossain[1], M. Eguren[1], I. Schneider[1], A.Z. Politi[1], L. Hufnagel[1], T. Hiiragi[2], J. Ellenberg[1];
[1]Cell Biology and Biophysics, The European Molecular Biology Laboratory (EMBL), Heidelberg, Germany, [2]Developmental Biology, The European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

Intriguingly, in the early mammalian embryo, chromosomes are compartmentalised in a parent-of-origin specific manner. In contrast to many other animal species, karyogamy in mammals does not occur through fusion of the pronuclei after fertilisation but instead parental chromosomes are brought together after replication on the metaphase plate of the first mitosis. Surprisingly, maternal and paternal chromatin occupies distinct hemispheres in the nuclei of the two-cell embryo, and this separation only gradually decreases during subsequent stages of development. Both the mechanism underlying parental genome separation and its functional importance for embryonic development are currently unclear. Utilising the high spatio-temporal resolution of our recently developed inverted light-sheet microscope, we reveal that the formation of two separate bipolar spindles around each parental pronucleus keeps maternal and paternal genomes apart during the first cleavage of the zygote. Based on this mechanistic understanding we were able to experimentally mix parental chromosomes during the first embryonic division in order to understand if separate nuclear compartments are required for normal development.

M216

Mixing of parental genomes after fertilization in *C. elegans* involves fusion and fenestration of pronuclear membranes.

M.M. Rahman[1], I. Chang[2], A.S. Harned[2], K. Narayan[2], O. Cohen-Fix[1];
[1]Laboratory of Cell Molecular Biology, National Institute of Diabetes Digestive Kidney Diseases, Bethesda, MD, [2]Center for Molecular Microscopy, Frederick National Laboratories for Cancer Research, Frederick, MD

In a fertilized embryo, the nuclear envelopes (NEs) of the maternal and paternal pronuclei must disassemble to allow mixing of two haploid genomes, leading to the formation of a single diploid nucleus in the zygote. The regulation and mechanics of this process are poorly understood but they are clearly important as premature or delayed disassembly could result in failure to incorporate chromosomes in a zygotic nucleus. It is assumed that NEBD occurs once the two pronuclei are in close apposition, but the precise order of events, and in particular the architecture of the pronuclear membranes, is unclear. To address this, we used the *C. elegans* embryo and followed NE dynamics and architecture using two orthogonal techniques, live cell fluorescence microscopy and 3D electron microscopy, specifically Focused Ion Beam - Scanning Electron Microscopy (FIB-SEM). After fertilization, the maternal and paternal pronuclei migrate towards each other and form a stable interface where NEs are closely juxtaposed. The NE between maternal and paternal pronuclei is breached via a membrane fenestration in the vicinity of the chromosomes only after the maternal and paternal chromosomes have aligned on their respective metaphase plates. This membrane gap then expanded as chromosomes moved in opposite directions in anaphase. Using FIB-SEM we visualized the 3D architecture of the NE fenestration through the four juxtaposed pronuclear membranes. We found that breaching the nuclear membranes between the two pronuclei involves the formation of membrane gaps that span all four membranes, the most prominent of which is in the vicinity of the aligned chromosomes. Moreover, we detected multiple sites where the membranes of the two pronuclei physically interact, forming membrane junctions. Our

preliminary data suggest that NE fenestration involves membrane fusion between the two pronuclei at multiple sites, leading to the formation of gaps across all four membranes. We further speculate that the presence of the chromosomes, and perhaps signals emanating therefrom, contribute to the expansion of NE gaps in the vicinity of the chromosomes.
We previously reported that inactivation of the conserved Polo-like kinase 1 (PLK1) results in a failure in pronuclear membrane fenestration and inhibition of parental chromosome mixing in the *C. elegans* zygote (Rahman et al., MBoC 2015). Recently we identified five additional genes that upon down-regulation by RNAi fail to fenestrate the pronuclear membranes. We are currently investigating how these genes contribute to the timely NEBD after fertilization in the *C. elegans* embryo.

M217

The coordination of chromosome segregation and nuclear envelope assembly: implications for nuclear envelope integrity and genome stability.

S. Liu[1,2], M. Kwon[1,2], N. Yang[3], A. Khodjakov[3,4], D. Pellman[1,2,5];
[1]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA, [2]Department of Cell Biology, Harvard Medical School, Boston, MA, [3]Wadsworth Center, New York State Department of Health, Albany, NY, [4]Rensselaer Polytechnic Institute, Troy, NY, [5]Howard Hughes Medical Institute, Boston, MA

Coordination of nuclear envelope (NE) assembly with chromosome segregation is critical for the normal architecture of eucaryotic nuclei. Defects in this coordination generate “nuclear atypia”, a major feature of human cancer used to establish tumor grade and prognosis. An extreme example of nuclear atypia is micronucleation, where one or a few chromosomes become physically isolated from the rest of the genome because of mitotic errors or chromosome breakage. The NE of micronuclei are fragile, prone to spontaneous disruption, leading to DNA damage and, ultimately, to an extensive form of chromosome rearrangement known as chromothripsis.
Here we report that the NE assembly defects of micronuclei are a consequence of the normal patterned subdomain organization of NE assembly during telophase. A “non-core” set of proteins, which include nuclear pore complexes (NPCs), initially assemble at the periphery of the chromosomes, away from the spindle. Another “core” group of NE proteins assemble where chromosomes are in contact with dense bundles of spindle microtubules. During interphase, remnants of the core domain persist as “pore-free islands”, which slowly acquire NPCs because of import from adjacent NPC-containing NE regions. We find that lagging chromosomes avidly recruit core NE proteins but are markedly deficient in recruiting non-core proteins. Thus, the NE of many micronuclei essentially become isolated core domains, with limited ability to acquire non-core NE components. Consequently, micronuclei exhibit nuclear transport defects and defects in nuclear protein accumulation, including important DNA repair/replication factors. We demonstrate that microtubules are responsible for inhibiting non-core protein recruitment to lagging chromosomes. This microtubule inhibitory effect is independent of chromosome position and the Aurora B phosphorylation gradient. Thus, rather than a “chromosome separation checkpoint” that dynamically monitors the position of lagging chromosomes, we propose that chromosome segregation and NE assembly are coordinated by a simple dependency relationship, with complete NE assembly requiring the disassembly of spindle microtubules.

M218

Taking Apart the Nuclear Envelope during Open Mitosis.

U. Kutay[1];
[1]Institute of Biochemsitry, ETH Zurich, Zurich, Switzerland

NE breakdown (NEBD) is a major event during the rapid intracellular reorganization in preparation of cells for open mitosis. Disassembly of the nucleus directly exploits the activity of protein kinases involved in mitotic entry and is supported by microtubule-dependent restructuring of the NE. To study the disintegration of nuclear pore complexes (NPCs) during NEBD, we had previously established a visual in vitro assay relying on semi-permeabilized cells. Exploiting this system, we have now reconstituted the initial steps of mitotic NPC disassembly using purified soluble factors. Further, we have assessed the importance of membrane dissociation from chromatin for chromosome segregation and cell division.

Minisymposium 22: Cellular Metabolism

M219

Geranylgeranyl-Regulated, ER-to-Golgi Transport of UBIAD1: Implications for Cholesterol Homeostasis and Schnyder Corneal Dystrophy.

R.A. DeBose-Boyd[1];
[1]Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX

Multiple feedback mechanisms converge on the ER (endoplasmic reticulum)-localized enzyme HMG CoA reductase, which catalyzes a key step in synthesis of cholesterol and essential nonsterol isoprenoids including Fpp (farnesyl pyrophosphate) and GGpp (geranylgeranyl pyrophosphate). These two isoprenoids can become attached to many proteins and are utilized in synthesis of dolichol, ubiquinone, heme, and vitamin K2. One mechanism for feedback control of reductase involves sterol-induced ubiquitination, which marks the enzyme for ERAD (ER-associated degradation) that is augmented by GGpp, but not by Fpp. Here, we show that sterols trigger binding of a subset of reductase molecules to UBIAD1 (UbiA prenyltransferase domain-containing protein-1), a prenyltransferase that utilizes GGpp to synthesize the vitamin K2 menaquinone-4. Sterol-induced binding to UBIAD1 protects reductase from accelerated degradation, permitting continued synthesis of nonsterol isoprenoids in sterol-replete cells. GGpp triggers release of UBIAD1 from reductase, allowing for maximal ERAD of reductase.
Mutations in UBIAD1 cause SCD (Schnyder corneal dystrophy), an autosomal dominant eye disease in humans characterized by corneal accumulation of cholesterol. SCD-associated mutants of UBIAD1 resist GGpp-induced displacement from reductase and thereby inhibit its sterol-accelerated ERAD. In the course of our studies, we discovered that GGpp also stimulates translocation of UBIAD1 from the ER to Golgi. SCD-associated mutants of UBIAD1 are refractory to GGpp-induced translocation to the Golgi and remain sequestered in ER membranes where they inhibit reductase ERAD in a dominant-negative fashion. These results support a model that posits upon sensing GGpp depletion in membranes of the ER, UBIAD1 becomes trapped in the organelle and inhibits reductase ERAD so as to stimulate mevalonate synthesis for replenishment of GGpp. This novel sensing mechanism directly controls ERAD of reductase and becomes disrupted in SCD, which likely contributes to the accumulation of cholesterol that characterizes the eye disease.

M220

Genetic depletion of adipocyte creatine metabolism inhibits diet-induced thermogenesis and drives obesity.

L. Kazak[1], G.Z. Lu[1], E.T. Chouchani[1], B.M. Spiegelman[1];
[1]Cancer Biology, Dana-Farber Cancer Institute, Boston, MA

The increase in obesity worldwide has led to a surge in the prevalence of metabolic disease. The capacity of thermogenic fat to dissipate chemical energy as heat offers tremendous potential to combat obesity. Adipose tissue thermogenesis is best understood to be mediated by Uncoupling Protein 1 (UCP1). Although UCP1 is required to defend body temperature upon acute exposure to environmental cold, thermal homeostasis is maintained in Ucp1-deficient animals via alternative thermogenic pathways upon gradual cold exposure. Moreover, Ucp1-deficient mice are protected from obesity at sub-thermoneutral temperatures, underscoring the powerful anti-obesogenic properties of UCP1-independent thermogenic pathways.
Diet-induced thermogenesis is an important homeostatic mechanism that limits weight gain in response to caloric excess and contributes to the relative stability of body weight in most individuals. We previously demonstrated that creatine enhances energy expenditure through stimulation of mitochondrial ATP turnover, but the physiological role and importance of creatine energetics in adipose tissue has not been explored. Here, we have inactivated the first and rate-limiting enzyme of creatine biosynthesis, glycine amidinotransferase (GATM), selectively in fat (Adipo-Gatm KO). Adipo-Gatm KO mice are prone to diet-induced obesity due to the suppression of elevated energy expenditure that occurs in response to high calorie feeding. This is paralleled by a blunted capacity for β3-adrenergic activation of metabolic rate. These results provide strong in vivo genetic support for a role of GATM and creatine metabolism in energy expenditure, diet-induced thermogenesis, and defense against diet-induced obesity.
These data reinforce the importance of creatine metabolism and mitochondrial energetics in supporting adipose tissue thermogenesis to combat obesity.

M221

Molecular mechanisms of Scavenger Receptor SR-BI regulation: linking HDL binding to cholesterol transport.

P.E. Marques[1], S. Nyegaard[1], R.F. Collins[1], W.S. Trimble[1], S. Grinstein[1];
[1]Cell Biology, The Hospital for Sick Children, Toronto, ON

Cholesterol is delivered to cells by LDL (low-density lipoprotein) and removed from them by HDL (high-density lipoprotein). Excess cholesterol removed from peripheral tissues by HDL is transported to the liver for conversion into bile acids that are excreted with gastrointestinal contents, a process known as reverse cholesterol transport. HDL also delivers cholesterol to adrenocortical cells for steroid hormone synthesis. Delivery of cholesterol from HDL to these tissues is mediated by the scavenger receptor SR-BI. To better understand the molecular mechanism whereby SR-BI removes cholesterol from HDL before releasing the depleted lipoprotein, we analyzed the subcellular distribution of the receptor, its mobility in the membrane and the dynamics of its internalization. To this end, we developed a receptor-specific short-chain antibody (ScFv). Immunostaining using ScFv covalently labeled with fluorescent probes confirmed that SR-BI is highly expressed on the surface of adrenocortical and liver cells. Monitoring SR-BI in live cells revealed that, strikingly, this receptor was retained at the surface of cells for extended periods, while other receptors and the bulk of the PM were being constantly and rapidly internalized. Failure to internalize did not result from attachment of SR-BI to cytoskeletal structures, since single-

particle tracking indicated that the receptors move freely in the plane of the membrane. Accordingly, removal of the PDZ-binding domain proposed to anchor SR-BI to the cytoskeleton did not affect its retention on the membrane. Quantitative analysis of ScFv binding showed that SR-BI exists on the surface of adrenocortical cells as large multimers consisting of an average 10 receptor molecules. These large clusters fail to enter clathrin coated pits, caveolae or GEEC/CLICs, accounting for the inordinate retention of the receptors at the surface. Importantly, SR-BI was able to remove cholesterol from HDL while at the cell surface, without requiring internalization. Cholesterol transported from the lipoprotein to the PM via SR-BI was delivered to the endoplasmic reticulum and eventually to lipid droplets. In this regard, HDL-associated cholesterol is handled very differently from that bound to LDL, which was rapidly internalized and routed through the endocytic pathway. We suggest that the surface retention of SR-BI is attributable to its ability to multimerize and that this feature plays an important role in its function.

M222

Mitochondrial Calcium Uniporter controls AMPK activity and lipid metabolism.

D. Tomar[1,2], F. Jaña[1], Z. Dong[1,2], N. Nemani[1,2], S. Santhanam[1,2], A. Tripathi[1,2], E. Carvalho[1,2], S. Rajan[1,2], D.S. Wijesinghe[3], R.S. Ahima[4], M. Madesh[1,2];
[1]Center for Translational Medicine, Temple University, Philadelphia, PA, [2]Department of Medical Genetics Molecular Biochemistry, Temple University, Philadelphia, PA, [3]Department of Surgery, Virginia Commonwealth University, Richmond, VA, [4]Division of Endocrinology, Diabetes and Metabolism, John Hopkins University, Baltimore, MD

Mitochondrial calcium dynamics promote the buildup of reducing equivalents that fuel oxidative phosphorylation and ATP production for cellular metabolism. Mitochondria sequester cytosolic calcium into the matrix through the Mitochondrial Calcium Uniporter (MCU). Although MCU plays an integral role in regulating mitochondrial bioenergetics, its function in in-vivo energy homeostasis remains elusive. We found that the tissue-specific deletion of the Mcu gene in mouse liver exhibited loss of mitochondrial calcium uptake, depletes matrix calcium, reduced glucose and fatty acid coupled mitochondrial oxygen consumption and leads to the accumulation of lipids. Loss of MCU results in hepatic lipidome remodeling with increased diglyceride and triglyceride lipid species in the mouse model. MCU-knockout *Danio rerio* generated by targeted disruption of Mcu gene using CRISPR/Cas9 approach, recapitulate the lipid accumulation phenotype. Mouse hepatic lipid accumulation is associated with decreased phosphorylation of AMPK. This indicates that besides beta-oxidation of fatty acids, AMPK phosphorylation regulated lipid synthesis is also controlled by MCU. Deletion of MCU in liver promoted extramitochondrial calcium-dependent protein phosphatase-4 (PP4) activity that dephosphorylates AMPK. Treating the hepatic MCU-knockout mice with Metformin restored AMPK-dependent lipid clearance in the liver by an MCU-independent mechanism. MCU appears to be key in a regulatory circuit involving mitochondrial calcium-dependent activation of AMPK to control hepatic lipid metabolism.

M223

Regulation of adipose tissue metabolism via non-proteolytic ubiquitination and mitochondria retrograde signaling.

M.D. Cardamone[1], C.T. Cederquist[1], B. Tanasa[2,3], J. Huang[1], K. Mahdaviani[4], C. Lentucci[1], W. Li[2,5], M.G. Rosenfeld[2], M. Liesa[4,6], V. Perissi[1];
[1]Biochemistry, Boston University, Boston, MA, [2]Medicine, UCSD, La Jolla, CA, [3]Pediatrics, Stanford University, San Francisco, CA, [4]Medicine, Boston University, Boston, MA, [5]Biochemistry and Molecular Biology, UTHealth McGovern Medical School, Houston, TX, [6]Medicine, UCLA, Los Angeles, CA

Key players in the regulation of the balance between lipid storage and utilization in the adipose tissue are the mitochondria, with mitochondrial dysfunctions being associated with defects in lipid and glucose metabolism and with the development insulin resistance and type 2 diabetes (T2D). Because most mitochondrial proteins are encoded in the nuclear genome, regulation of mitochondrial biogenesis, health and functionality depend on anterograde and retrograde signaling pathways for mitonuclear communication. While there is a good understanding of the molecular players involved in the nuclear regulation of mitochondrial functions, little is know about mitochondria-to-nucleus communication strategies, particularly in mammalian cells.
Here, we have identified a novel mediator of the mitochondria retrograde signaling pathway in the transcriptional cofactor G-Protein Suppressor 2 (GPS2). Our results reveals that GPS2 translocates from the outer mitochondrial membrane to the nucleus in a regulated manner to promote the activation of the nuclear-encoded mitochondrial gene program through the establishment of a chromatin environment permissive for RNA polymerase II (POL2)-mediated transcriptional activation. We will present in vitro data characterizing GPS2 role in regulating mitochondrial biogenesis during adipocyte differentiation and report about the in vivo physiological relevance of this newly described pathway in the brown adipose tissue of GPS2-AKO mice. These results will be discussed in the context of previous work characterizing GPS2 functions in regulating the insulin signaling cascade and pro-inflammatory pathways via inhibition of the enzymatic activity of the ubiquitin conjugating enzyme Ubc13.
Together, our findings suggest that the modulation of non-proteolytic ubiquitination, through the opposing actions of GPS2 and Ubc13, represents a critical and unappreciated hub for the coordinated regulation of metabolic pathways controlling lipid storage and energy expenditure.

M224

Aerobic Respiration Enhanced by Mitochondrial Fusion Remodels Vacuolar Liquid-ordered Membrane Domain to Induce Micro-lipophagy for Cell Survival during Glucose Restriction.

A.Y. Seo[1], F. Sarkleti[2], C.A. Larabell[3], J. Lippincott-Schwartz[1];
[1]Janelia Research Campus, HHMI, Ashburn, VA, [2]Institute of Molecular Biosciences, University of Graz, Graz, Australia, [3]Department of Anatomy, University of California San Francisco, San Francisco, CA

Cells undergoing glucose restriction start to catabolize fat molecules to produce ATP through mitochondrial oxidative phosphorylation (OXPHOS). At the same time, autophagy (a conserved lysosome-mediated self-eating process) is trigged to support cellular lipid catabolic activity via redistributing stored fatty acids in lipid-droplets (LDs) to fat-metabolizing organelles such as mitochondria. However, how mitochondria and LD-specific autophagy (i.e. micro-lipophagy) interplay in starved cells for their long-term survival remains unknown. Here, we demonstrate that a regulatory feedback mechanism exists between mitochondria and micro-lipophagy in budding yeasts. We find that in response to acute glucose restriction, cells rapidly induce mitochondrial OXPHOS, followed by the upregulation of genes that control mitochondrial fusion activity. This leads to mitochondrial tubulation

and prevents loss of mitochondrial DNA and respiratory capacity by protecting the organelles from being targeted to bulk autophagy during starvation. Our analysis reveals that mitochondrial respiratory activity is the key trigger for remodeling vacuolar membrane to form a micro-scale liquid-ordered (Lo) membrane domain. We show that vacuolar Lo domain forms by the redistribution of Atg14p (a component of class III phosphatidylinositol-3-kinase (PI3K) complex I) to the vacuole surface upon the induction of mitochondrial OXPHOS. Through stabilizing other PI3K components (e.g., Atg6p) on the vacuole, Atg14p further differentiates the Lo domains to recruit LDs onto the vacuole surface for triggering micro-lipophagy. Cells lacking mitochondrial fusion activity or incapable of boosting aerobic respiration due to genetic/pharmacological stresses fail to develop vacuolar Lo domains, completely block micro-lipophagy induction, and display significantly diminished ATP production with shortened lifespans during glucose starvation. Thus, our data suggests that mitochondrial fusion plays a key role in micro-lipophagy by modulating mitochondrial aerobic respiration and vacuolar Lo domain differentiation in starved cells. This additional interplay between mitochondria and LD-specific autophagy may allow cells to operate a precise regulation of LD-stored lipid recycling in response to cellular energy demand and would help drive effective cell survival program during metabolic stress.

M225

Regulated assembly of a macromolecular complex of IMPDH during T cell activation.

K.C. Duong-Ly[1], S.A. Anthony[1], A.L. Burrell[2], M.C. Johnson[2], Y. Kuo[3], J.C. Simonet[1], P. Michener[4], J. Soboloff[5], G. Rall[6], A. Andrews[3], J.M. Kollman[2], J.R. Peterson[1];
[1]Cancer Biology, Fox Chase Cancer Center, Philadelphia, PA, [2]Biochemistry, University of Washington, Seattle, WA, [3]Cancer Epigenetics, Fox Chase Cancer Center, Philadelphia, PA, [4]Biochemistry Molecular Biology, Drexel University College of Medicine, Philadelphia, PA, [5]Felt Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA, [6]Blood Cell Development and Function, Fox Chase Cancer Center, Philadelphia, PA

Activation of naive T cells triggers dramatic alterations in T cell metabolism in preparation for cell proliferation and differentiation. The de novo nucleotide biosynthetic enzyme inosine monophosphate dehydrogenase (IMPDH), for example, is highly upregulated following T cell activation and inhibitors of IMPDH potently suppress T cell expansion and are used as immunosuppressive agents clinically. In treated patients, IMPDH assembles into micron-scale filamentous structures but the physiological significance of these large assemblies is unknown. We have discovered that IMPDH in naïve T cells in mice assembles into similar filaments in response to a viral challenge, and we have reconstituted this process with primary cells in vitro. Filament assembly, but not increased IMPDH expression, is abolished in T cells from mice deficient in the calcium sensor STIM1 but not STIM2-deficient mice, implicating signaling downstream of the T cell receptor in regulating IMPDH assembly. To understand the function of these assemblies we reconstituted IMPDH polymers in vitro. Electron microscopy demonstrates that recombinant human IMPDH2 assembles into linear chains of an octameric subunit with substantial conformational plasticity. Octamer conformation is regulated by binding of substrates as well as allosteric binding of purine nucleotides. The effect of IMPDH2 assembly and conformational states on catalytic activity will be presented. Overall this work identifies for the first time a native context in which IMPDH filaments occur and utilizes both in vivo and in vitro approaches to understand their regulation and physiological function in T cell metabolism.

M226

Prolyl dihydroxylation of extra-ribosomal Rps23/uS12 regulates hypoxic adaptation in fission yeast.

S.J. Clasen[1], W. Shao[1], H. Gu[1], P.J. Espenshade[1];
[1]Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD

Low-oxygen adaptation is transcriptionally controlled by the Sterol regulatory element-binding protein Sre1N in fission yeast. Sre1N is inactive in the presence of oxygen through direct binding to the prolyl-3,4-dihydroxylase Ofd1. We show that under hypoxic conditions, Ofd1 is sequestered from Sre1N by the nuclear import adaptor Nro1 and the Ofd1 substrate Rps23/uS12. Ofd1, Nro1, and Rps23 form a complex in the cytosol that traffics to the nucleus to deliver dihydroxylated Rps23 for assembly into the small ribosomal subunit. In cells lacking Nro1, we find that Rps23 is predominantly unmodified or monohydroxylated using SILAC and targeted mass spectrometry. Low oxygen stabilizes the Ofd1-Nro1-Rps23 complex by inhibiting Ofd1-dependent dihydroxylation of Rps23. This sequestration model for Sre1N regulation by Ofd1 is further supported by our discovery of a shared Ofd1-binding sequence in Sre1N, Nro1, and Rps23, and by our ability to control Sre1N activation by reducing or elevating Rps23 levels. Since ribosomal protein expression is tightly correlated to the growth state of the cell, this allows nutrients to control Ofd1 availability and thus Sre1N activity. Ofd1 is one of several hydroxylases that modify ribosomal proteins. Our work provides a paradigm for how ribosomal oxidases use their substrates to regulate extra-ribosomal signaling networks to coordinate pro-growth pathways in the cell.

M227

Mutation of dgat2 uncouples lipolysis and lipoprotein synthesis in the zebrafish embryonic digestive organ resulting in excess ectopic lipid droplets.

M.H. Wilson[1], J.H. Thierer[1,2], S.A. Farber[1,2];
[1]Embryology, Carnegie Institution for Science, Baltimore, MD, [2]Biology, The Johns Hopkins University , Baltimore, MD

During zebrafish development, maternally-deposited yolk is the source of nutrients for embryogenesis prior to digestive system maturation. The yolk syncytial layer (YSL) is the cytoplasmic layer surrounding the yolk mass that metabolizes, re-packages and exports yolk nutrients to the developing embryo. Export of lipid from the YSL is via triglyceride-rich lipoproteins. We show that blocking lipoprotein production, by inhibiting the activity of microsomal triglyceride transfer protein (MTP), results in aberrant accumulation of lipid droplets in the YSL. The dense packing of lipid droplets refracts light such that the yolk takes on a dark appearance. Paradoxically, we have also found that zebrafish embryos with a nonsense mutation in exon 2 of diacylglycerol acyltransferase 2 (dgat2), an ER resident enzyme that synthesizes triacylglycerol from diacylglycerol and fatty acyl-CoA, also exhibit dark yolks indicative of excess lipid. mRNA in situ hybridization indicates dgat2 is expressed in the YSL, as well as in the intestine and liver at later developmental stages. Unlike DGAT2-null mice, which are lipopenic and die soon after birth, zebrafish dgat2 mutants are viable and fertile. Similar to inhibition of MTP, dgat2 mutant embryos have visible accumulation of lipid droplets in the YSL. These droplets vary in size, stain positively with oil-red-O and some can even persist after yolk depletion. Biochemical analyses reveal that the YSL still has remaining dgat activity and lipid droplets are rich in triglycerides and cholesterol esters. Residual dgat activity cannot be explained by the expression of other known dgat isoenzymes (e.g. dgat1a and dgat1b are not expressed in the YSL). Dgat2 mutant embryos synthesize fewer and smaller beta-lipoproteins, and transmission electron microscopy indicates the ER in the YSL of dgat2 mutants is abnormally swollen and electron dense, suggesting possible accumulation of lipid within the leaflets of the ER membrane.

Experiments are currently underway to identify the source of the remaining dgat activity and determine why YSL triglyceride export is attenuated. These data suggest that blocking triglyceride export uncouples the rates of yolk degradation and lipid export, forcing the YSL to pack the accumulating lipid species into lipid droplets, likely to prevent lipotoxicity. Future work will be aimed at understanding whether the embryo, YSL and the yolk degradation machinery communicate to ensure the correct provision of nutrients to the body to promote proper embryonic development.

M228

Metabolic control of beta-cell survival.

N.N. Danial[1];
[1]Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

Islet inflammation is central to the loss of insulin-producing beta-cells in diabetes. Recent studies have indicated that, beyond the immune cell component of islet inflammation, beta-cell intrinsic pathways modulate the outcome of islet inflammation. However, the molecular determinants of these beta-cell intrinsic pathways are not known. We have found that increased beta-cell glucose flux can protect beta-cells from death induced by inflammatory cytokines. This is critically dependent on the specific mechanism by which the beta-cell glucose phosphorylating enzyme glucokinase (GK, Hexokinase IV) is activated. Specifically, activating GK through mimicking the phosphorylation of the BCL-2 family protein BAD, an endogenous GK activator protein, which targets GK near its active site and preserves GK's native affinity for glucose, is beta-cell protective. In contrast, pharmacologic or genetic activation of GK through an allosteric mechanism that drastically changes GK's affinity for glucose exacerbates beta-cell demise under inflammation stress. These observations prompted interrogation of beta-cell metabolome in response to these two independent modes of GK activation to molecularly define the protective versus toxic components of glucose metabolism in islet inflammation. Untargeted metabolomics analyses of human islets coupled with functional studies indicate that GK activation through its allosteric site versus phospho-BAD mimicry is associated with profoundly different metabolic signatures in the presence of inflammatory cytokines. These include significant distinctions in amino acid metabolism and the extent of oxidative stress. The mechanistic underpinnings of protective versus toxic glucose signaling in beta cells and their translational utility for enhancing functional beta-cell mass in diabetes will be discussed.

Minisymposium 23: The Life of a Microtubule: Birth, Dynamics and Function

M229

The role of GCPs 4, 5, and 6 in microtubule nucleation.

D. Farache[1], L. Emorine[1], L. Haren[1], A. Merdes[1];
[1]Centre Biologie Developpement, University Toulouse III / CNRS, Toulouse, France

A large number of spindle microtubules need to be nucleated at the onset of mitosis. These microtubules are composed of alpha/beta-tubulin dimers, arranged in a three-start helix, with a tubular wall of 13 protofilaments. The precise geometry of 13-protofilament-microtubules is pre-defined by multi-protein complexes of gamma-tubulin that act as nucleators for microtubule assembly. To serve as structural templates, gamma-tubulin molecules are bound to gamma-tubulin complex proteins (GCPs) 2 and 3 that assemble into small gamma-tubulin complexes (gamma-TuSCs). Gamma-TuSCs themselves have the potential to associate laterally into helical structures, matching the geometry of 13-protofilament microtubules. The lateral assembly of gamma-TuSCs is directly supported by proteins

containing a conserved sequence motif termed “CM1”, and by proteins related to MOZART1, all of which binding to the N-termini of GCPs. Whereas the lateral assembly of gamma-TuSCs yields helical filaments of various size in vitro, the size of gamma-tubulin complexes is limited to approximately 2 Megadalton in cells, yielding helices of one turn with a small zone of overlap, resembling rings by electron microscopy, and coining the name of “gamma-tubulin ring complexes” (gamma-TuRCs). In most eukaryotes, the formation of gamma-TuRCs involves additional GCPs, 4, 5, and 6. Whereas older models propose a “cap” function of GCPs 4, 5, 6 at the basis of gamma-TuRCs, we present direct evidence for a model in which these proteins integrate into the helical wall of gamma-TuRCs, binding laterally to gamma-TuSCs. Our evidence is based on biophysical, biochemical, and cell biological data, involving the construction of chimeric GCPs, sucrose gradient analysis, immunofluorescence, and FLIM-FRET. Moreover, we show by co-immunoprecipitation from cell extracts that GCP4, 5, and 6 form a sub-complex within gamma-TuRCs that resists high salt-treatment. Reconstitution experiments show that this sub-complex can re-form larger complexes with gamma-TuSCs when the salt concentration is reduced. Complementation experiments with a depleted extract, containing only gamma-TuSCs, allows the reassembly of complexes from GCPs 4, 5, 6 that are able to nucleate microtubules. Our results suggest that GCPs 4, 5, 6 initiate the association of gamma-TuSCs into gamma-TuRCs, and maintain gamma-TuRC stability.

M230

XMAP215 is a microtubule nucleation factor that functions synergistically with the gamma-tubulin ring complex.

S. Petry[1], A. Thawani[2], R. Kadzik[1];
[1]Molecular Biology, Princeton University, Princeton, NJ, [2]Chemical and Biological Engineering, Princetno University, Princeton, NJ

Assembly of cytoskeletal structures is required for cells to perform functions such as transport and cell division. While significant advances have been made in understanding how microtubules (MT) are organized in cell, early steps in generation of MTs is still poorly understood. For several decades, γ-TuRC has been accepted as the universal MT nucleator of the cell. Although there is evidence γ-TuRC might not be the sole MT nucleator, identification of other MT nucleation factors has proven difficult. Here, we report that XMAP215, the well-characterized MT polymerase in the cell, is required for MT nucleation in meiotic Xenopus egg extracts. While XMAP215 and γ-TuRC possess minimal MT nucleation activity individually, together these factors synergistically stimulate MT nucleation. We found that the tubulin binding residues in XMAP215, required for polymerization, are required for MT nucleation and for the interaction between XMAP215 and γ-TuRC in extract. In sum, XMAP215 is a novel MT nucleation factor that cooperates with γ-TuRC to generate the MT cytoskeleton of the cell.

M231

A two-step mechanism for inactivation of MTOC function at the centrosome.

J. Magescas[1], J.C. Zonka[1], J.L. Feldman[1];
[1]Department of Biology, Stanford, Satnford, CA

During mitosis, the centrosome acts as a microtubule organizing center (MTOC), creating a radial array of microtubules to separate chromosomes between daughter cells. Microtubules are organized by complexes within the centrosome’s pericentriolar material (PCM), which is steadily recruited to the centrosome, culminating in a peak in MTOC activity in metaphase. After metaphase, PCM is removed from the centrosome thereby attenuating MTOC function. This inactivation of MTOC function at the

centrosome is likely required for the timely exit from mitosis and for subsequent cell cycle events such as centriole duplication and the reassignment of MTOC function to other structures. While a large body of work has revealed the phosphorylation-dependent steps required for MTOC assembly at the centrosome, little is known about the mechanisms reversing this process at the end of mitosis. We are using C. elegans as a model to characterize MTOC inactivation at the centrosome. In C. elegans, the PCM is comprised of two main proteins, SPD-2/CEP192 and SPD-5, which localize the microtubule nucleating complex ɣ-TuRC to the centrosome. Pairwise analysis of SPD-2, SPD-5, and ɣ-TuRC components revealed that the PCM proteins decrease at different rates: SPD-2 decreases at a faster rate than SPD-5 and ɣ-TuRC, leaving a "cage" of SPD-5/ɣ-TuRC around the centrosome. Following the gradual removal of SPD-2, the SPD-5/ɣ-TuRC cage fragments into smaller "packets" that are still associated with microtubules. The packets move towards the cell cortex and first lose their association with ɣ-TuRC and microtubules, leaving only SPD-5 foci in the cytoplasm that eventually disappear. These data strongly suggest that PCM disassembly is a two-step process, beginning with the initial removal of SPD-2 from the centrosome, and followed by the fragmentation of the remaining PCM. We used pharmacological and genetic manipulations to determine the mechanisms underlying either step in disassembly. Using inhibitors of serine/threonine phosphatases, we observed a stabilization of the PCM during anaphase, implicating this phosphatase family in the process of disassembly. Because of the cortical directionality of the packets and their association with microtubules, we hypothesized that packet formation relies on cortical microtubule pulling forces. Using RNAi to perturb these cortical forces we observed a loss of the packets and PCM disassembly was instead accomplished through a much slower, steady removal of components. Based on these observations we propose that PCM disassembly is initiated in anaphase through a phosphatase-dependent removal of SPD-2. SPD-2 removal weakens the PCM that is then torn apart by cortical pulling forces, ultimately inactivating MTOC function at the centrosome.

M232

EB1 and EB3 regulate microtubule minus end organization and Golgi morphology.

C. Yang[1], J. Wu[1], C. Heus [2], I. Grigoriev[1], N. Liv[2], Y. Yao[3], I. Smal[3], E. Meijering[3], J. Klumperman[2], .Z. Qi[4], A. Akhmanova[1];
[1]Cell Biology, Department of Biology, Utrecht University, Utrecht, Netherlands, [2]Department of Cell Biology, University Medical Center Utrecht, Utrecht, Netherlands, [3]Departments of Medical Informatics and Radiology, Erasmus University Medical Center, Rotterdam, Netherlands, [4]Division of Life Science, The Hong Kong University of Science and Technology, Hong Kong, China

End binding proteins (EBs) are key regulators of growing microtubule plus ends. Recent work demonstrated that microtubule minus ends can also grow in cells, and that minus end dynamics are important for shaping non-centrosomal microtubule networks. However, the role of the EBs in controlling the organization of microtubule minus ends is poorly understood. Here, by using CRISPR/Cas9 technology, we have generated cell lines that lack EB2, EB3 and the C-terminal partner binding part of EB1. These cell lines show only mild defects in mitosis and microtubule plus end dynamics, but, surprisingly, they display severely perturbed microtubule minus end organization. In the studied cell lines, microtubule minus ends that are not attached to the centrosome are stabilized by CAMSAP2, and many of them are tethered to the Golgi apparatus. Disruption of EB1 and EB3 leads to shortening of CAMSAP2-decorated microtubule minus ends, their detachment from the Golgi and their relocalization to the cell periphery. EB1 or EB3 are recruited to the Golgi through an interaction with myomegalin, and our data suggest that the weak binding of Golgi-associated EB1 and EB3 to stable microtubule lattices contributes to microtubule tethering to Golgi membranes and counteracts compaction of Golgi stacks. Furthermore, we find that disruption of EB1 and EB3 affects vesicle transport, cell motility on 2D substrates and cell invasion in 3D matrices. Our results demonstrate that

EB proteins control diverse aspects of interphase cell architecture and have an unexpectedly profound impact on the organization of microtubule minus ends.

M233

Carboxy-terminal tail of β-tubulin regulates dynamic instability.

C.P. Fees[1], J.K. Moore[1];
[1]Cell and Development , University of Colorado Anschutz Medical Campus , Aurora, CO

According to a widely accepted model, during microtubule growth GTP-bound tubulin heterodimers assemble into the growing microtubule end, and once within lattice, undergo nucleotide hydrolysis and corresponding conformational change. How nucleotide status governs assembly activity is an area of intense investigation, particularly the mechanisms that regulate conformational changes in tubulin. The negatively-charged carboxy-terminal tail (CTT) domains of the α- and β-tubulins are highly variable across species and tubulin isotypes, and are targets of post translational modifications, therefore, they are an excellent intrinsic candidate for regulating dynamic instability in a context-specific manner. Here we investigate the role of CTTs using a combination of in vitro studies with purified proteins and in vivo studies of budding yeast mutants to demonstrate that the β-tubulin CTT is a key regulator of dynamic instability. We show that β-CTT inhibits polymerization and microtubule nucleation while promoting microtubule disassembly. Although microtubules assemble faster in the absence of β-CTT, we find that the ends of these microtubules are highly unstable and tend to immediately depolymerize when free tubulin is depleted. We propose a novel mechanism in which the β-tubulin CTT acts by promoting the selective assembly of GTP-tubulin heterodimers into the microtubule end, thereby regulating subunit-polymer incorporation and dynamic instability. Consistent with this model, our complementary experiments in cells expressing mutant β-tubulin that lacks the CTT exhibit more stable microtubules and resist depolymerization by nocodazole, suggesting that β-CTT normally promotes microtubule dynamics. Furthermore, experiments with mutants that alter the composition of β-CTT suggest that the nucleotide sensing mechanism of β-CTT depends on negatively-charged amino acids in the CTT. The canonical model of microtubule growth requires assembly competent heterodimers to have a higher affinity for the growing end than heterodimers which have already undergone nucleotide hydrolysis. Our model adds a new dimension to this by establishing the CTT as a regulator linking nucleotide exchange and polymer assembly.

M234

Mechanisms to localize and regulate katanin activity.

G.M. Burkart[1], R.V. Dixit[1];
[1]Biology, Washington University in St.Louis, St. Louis, MO

The microtubule severing protein katanin, a heterodimer of a p60 catalytic subunit and a p80 regulatory subunit, regulates the organization and turnover of the microtubule cytoskeleton by the localized destruction of microtubule polymers. In higher plants, katanin activity is essential for the formation of linearly organized cortical microtubule arrays, which determine the axis of cell expansion. Live imaging studies have shown that even though p60 binds to the sidewalls of cortical microtubules, severing activity is restricted to microtubule crossover and nucleation sites. We have hypothesized that both targeting and protective mechanisms are needed to precisely localize katanin severing activity. We are using genetic and biochemical approaches to investigate whether p80 plays a role in targeting p60 to particular microtubule locations. There are four p80 orthologs in *Arabidopsis thaliana*, and we found that all interact with p60 with p80-4 consistently shows the strongest interaction in yeast-two-hybrid

studies. In addition, we discovered that severing activity is regulated by phosphorylation of three serine residues in the N-terminal domain of p60. Mutating all three residues to aspartate to mimic phosphorylation completely inhibits severing activity *in vitro*, whereas single site phosphomimetic mutants did not alter severing activity compared to wild-type p60. Furthermore, mutating these residues to alanine did not alter severing activity of p60. Both the phosphomimetic and phosphonull mutants of p60 interact normally with all four p80 subunits and we are currently investigating whether microtubule binding and/or ATPase activity is affected in these p60 mutants. Using *in vitro* severing assays, we found that the microtubule bundling protein MAP65-1, a plant homolog of human PRC1, is a potent inhibitor of katanin-mediated microtubule severing. At low MAP65-1 concentrations, severing is inhibited at bundled microtubule segments and the severing rate of non-bundled microtubules is reduced. At higher MAP65-1 concentrations, severing is nearly completely inhibited, even on non-bundled microtubules, thus providing a mechanism to protect the sidewalls of linearly ordered cortical microtubules against katanin attack. Our ongoing biochemical and cell biological experiments seek to determine whether inhibition of p60 by MAP65-1 requires microtubule bundling activity and whether it involves sterically blocking p60 binding to microtubules. Together, these experiments are advancing us toward our ultimate goal of reconstituting targeted microtubule severing *in vitro*.

M235

Katanin spiral and ring structures shed light on power stroke for microtubule severing.

E.A. Zehr[1], A. Szyk[1], G. Piszczek[2], E. Szczesna[1], X. Zuo[3], A. Roll-Mecak[1];
[1]Cell Biology and Biophysics , NIH, Bethesda, MD, [2]Lung and Blood Institute, NIH, Bethesda, MD, [3]X-Ray Science Division, Argonne National Laboratory, Argonne, IL

Microtubule-severing enzymes katanin, spastin and fidgetin are AAA ATPases important for the biogenesis and maintenance of complex microtubule arrays in axons, spindles and cilia. They generate internal breaks in microtubules thus modulating their dynamics and organization. They are found throughout the animal kingdom, plants and protozoa, and mutations in microtubule-severing enzymes cause severe neurodegenerative and neurodevelopmental disorders. Because of a lack of 3D structures, the mechanism of microtubule severing remains poorly understood 25 years after its discovery. Here we report the first X-ray structure of the monomeric AAA katanin module and cryo-EM reconstructions of the hexamer in two conformations at 4.4Å and 6Å resolution. These reveal an unexpected asymmetric arrangement of the AAA domains mediated by structural elements unique to severing enzymes that are critical for their function. Our reconstructions show that katanin cycles between open spiral and closed ring conformations, depending on the ATP occupancy of a gating protomer that tenses or relaxes inter-protomer interfaces and closes a 40Å wide gate in the katanin hexamer. Cycling of the hexamer between these conformations would provide the power stroke for microtubule severing.

M236

Structure, biochemistry, and activity of a CLASP family TOG.

S. Majumdar[1], L.M. Rice[1];
[1]Biophysics, UT Southwestern Medical Center, Dallas, TX

Microtubule (MT) dynamics are tightly regulated by associated proteins. Two conserved regulatory factors, XMAP215 family polymerases and CLASP family rescue factors, use tubulin-binding TOG domains to control MT dynamics. In S. cerevisiae, the polymerase STU2 and rescue factor STU1 produce different effects on MT dynamics but have similar architectures: two TOG domains followed by a basic region and a dimerization domain. The minimal functional unit for a MT polymerase appears to be two

flexibly-linked TOGs that preferentially bind unpolymerized αβ-tubulin (curved conformation) plus a lattice-binding basic region. The minimal requirement for a rescue factor has not been defined. Our working hypothesis is that the divergent functions of the two families arise from differences in the structural and biochemical properties of CLASP TOGs and/or how they are linked. To provide a structural context for subsequent biochemical and functional studies, I determined the structure of the TOG2 domain from Stu1. The overall structure of this domain resembles that of another CLASP TOG and is characteristically distinct from that of polymerase TOGs in a way that suggests a change in the conformation-selectivity of TOG-tubulin interactions. The structure of Stu1:TOG2 also reveals that part of the sequence that would normally link TOG1 to TOG2 forms a helix that docks onto the TOG2 domain in a novel way, indicating that the Stu1 TOGs are more rigidly coupled than the polymerase TOGs. Also in contrast to the polymerase TOGs, Stu1:TOG2 shows appreciable affinity for both curved/unpolymerized αβ -tubulin and straight/polymerized αβ -tubulin. STU1:TOG1, on the other hand, does not show appreciable affinity for either polymerized or unpolymerized αβ-tubulin. These interactions with curved and straight αβ-tubulin both depend on the same conserved positions that mediate tubulin binding in polymerase TOGs. To begin investigating conformation-selectivity of interactions between Stu1:TOG2 and αβ -tubulin, I measured the effects of the domain on MT polymerization dynamics in vitro. These assays revealed that Stu1:TOG2 by itself – without a basic region or a dimerization element - suppresses catastrophe and promotes rescue. These results expand our understanding of TOG structure, biochemistry, and mechanism. They also indicate that in contrast to polymerases where two TOGs are required, a single CLASP-family TOG is sufficient to suppress catastrophe and promote rescue.

M237

Ndc80 complex as an intrinsic regulator of molecular friction at mitotic kinetochores.

V.M. Demidov[1], S.K. Tripathy[1], F.I. Ataullakhanov[2], E.L. Grishchuk[1];
[1]Physiology, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, [2]Center for Theoretical Problems of Physicochemical Pharmacology, Moscow, Russia

The microtubule wall–binding Ndc80 complex serves as the primary molecular glue connecting mitotic kinetochores to spindle microtubules. During metaphase chromosome oscillations, Ndc80 is thought to form mobile diffusive bonds that enable sister kinetochores to glide repeatedly toward the plus- and minus-ends of the microtubules without detaching. To investigate the biophysical underpinning of this striking behavior, we adopted a highly sensitive dual-trap three-bead assay employing ultrafast force-clamp spectroscopy in vitro. Microtubule-wall gliding of single Ndc80 molecules was examined under controlled forces, imitating the forces and motions the Ndc80 experiences during metaphase. Unexpectedly, we find that the character of Ndc80 motility and its velocity depend strongly on the direction of force. When pulled toward the microtubule minus-end, Ndc80 obeys the typical prediction for a diffusive MAP: it moves smoothly with little molecular friction, in correspondence with the relatively fast rate of its unloaded diffusion. However, Ndc80 strongly resists forces pulling it toward the microtubule plus-end, exhibiting frequent pauses that are indicative of catch-bond-like behavior. Strikingly, the strongly-bound Ndc80 retains the ability to glide continuously on the microtubule lattice, translocating almost 10-times slower than under the same force acting in the opposite direction. These results strongly suggest that Ndc80 has the ability to serve as an intrinsic direction-specific regulator of molecular friction at human kinetochore. We propose that low internal friction at the force-generating, leading sister kinetochore maximizes the useful work available from the microtubule depolymerization motor to transport the chromosome. The increased friction at the trailing kinetochore should help to prevent its slipping from the microtubule plus-ends, as this kinetochore is dragged passively in this direction by its sister.

M238

Profilin Directly Enhances Microtubule Growth Through Residues Mutated in Amyotrophic Lateral Sclerosis.

J.L. Henty-Ridilla[1], M.A. Juanes[2], B.L. Goode[2];
[1]Cell and Developmental Biology, SUNY Upstate Medical University, Syracuse, NY, [2]Cell Biology, Brandeis University, Waltham, MA

Profilin is one of the most abundant and central actin regulatory proteins in eukaryotic cells. Profilin also decorates the sides of microtubules and indirectly influences cellular microtubule dynamics through interactions with Formins. Here, we investigated whether Profilin has direct regulatory effects on microtubule dynamics. We show that human Profilin-1 binds to microtubules in vitro and enhances the growth rate of microtubules several-fold. These microtubule effects are conserved in budding yeast and *Drosophila* Profilin homologs, and are unaffected by mutations in its canonical actin monomer– or poly-*L*-proline–binding sites. Instead, microtubule regulation depends on several residues mutated in patients with amyotrophic lateral sclerosis (ALS). The enhanced microtubule dynamics elicited by Profilin are attenuated by increasing concentrations of actin monomers. This suggests a competitive relationship between microtubules and actin for Profilin binding and this agrees with the close proximity of the known actin and microtubule binding surfaces. Consistent with these biochemical results, a two-fold increase in expression of wildtype Profilin accelerates the growth rate of microtubules in cells, and cells expressing similar levels of each of the ALS-associated Profilin mutants did not. These results demonstrate Profilin directly interacts with and enhances the growth rate of microtubules in vitro and in cells, and indicate that Profilin coordinates cellular actin and microtubule dynamics. Further, cells harboring ALS-linked Profilin mutations may ultimately leads to the motor neuron degeneration through defective microtubule regulation.

Minisymposium 24: Membrane Shaping by Fusion and Fission

M239

FUSEXINS, a family of sexual, somatic and viral cell fusion proteins.

C. Valansi[1], D. Moi[2], E. Leikina[3], E. Matveev[1], M. Graña[4], L.V. Chernomordik[3], H. Romero[5], P.S. Aguilar[2], B. Podbilewicz[1];
[1]Biology, Technion- Israel Institute of Technology, Haifa, Israel, [2]Laboratorio de Biología Celular de Membranas, Instituto de Investigaciones Biotecnologicas "Dr. Rodolfo A. Ugalde" (IIB), Buenos Aires, Argentina, [3]Section on Membrane Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, [4]Unidad de Bioinformática, Institut Pasteur Montevideo, Montevideo, Uruguay, [5]Ecología y Evolución, Universidad de la República, Montevideo, Uruguay

Cell-cell fusion is essential for fertilization and sculpting of bones, muscles, epidermis and placenta in different organisms. However, the proteins that mediate membrane fusion between cells have not yet been well-characterized and in some cases are still unknown. HAP2(GCS1) are membrane glycoproteins essential for gamete fusion in plants, *Chlamydomonas*, *Plasmodium*, *Tetrahymena*, and *Dictyostelium*. To investigate whether HAP2 is not only essential but also sufficient for cell-cell fusion, we expressed the *Arabidopsis* sperm HAP2 in cultured baby hamster kidney cells that normally do not fuse. We found that HAP2 expression in these heterologous cells results in the formation of multinucleate cells by cell-to-cell fusion [1]. Genetic and cell biological analyses showed that HAP2 has to be present in only one of the fusion partners (usually the male gamete) [2,3]. However, we found that when expressed in mammalian cells, *Arabidopsis* HAP2 is sufficient to fuse them only when expressed in both fusing cells [1]. Thus, HAP2-

mediated plasma membrane merger occurs via a bilateral zippering mechanism reminiscent to intracellular fusions mediated by SNAREs, atlastins and mitofusins. Furthermore, expression of HAP2 on the surface of pseudotyped vesicular stomatitis virus results in virus-cell fusion only to cells that also express HAP2. Thus, we propose that, in addition to sperm HAP2, a HAP2-like protein is needed in the egg for gamete fusion. Structural modeling of the HAP2 protein family predicts that it is homologous to class II viral fusion proteins (e.g. Zika, rubella and dengue) and the somatic cell fusogen EFF-1 from C. elegans [1-3]. Moreover, the recently solved crystal structure of *Chlamydomonas*' HAP2 demonstrates structural homology with EFF-1 and class II viral fusion proteins [3]. We name this superfamily FUSEXINS: FUSion proteins essential for sexual reproduction and EXoplasmic merger of plasma membranes. Fusexins mediate enveloped virus entry into cells, sexual reproduction, and somatic cell fusion. These proteins share the same structure and function but use distinct mechanisms to merge membranes. We hypothesize that modern fusexins have existed since the dawn of eukaryotic cells.

[1] Valansi, C. et al. J Cell Biol (2017)[2] Pinello, J. F. et al. Curr Biol (2017)[3] Fedry, J. et al. Cell (2017)

M240

Adhesion between membranes is a barrier to membrane fusion.

S. Son[1], E.M. Schmid[1], M.D. Vahey[1], M.H. Bakalar[1], K. Chan[1], D.A. Fletcher[1];
[1]Bioengineering, University of California, Berkeley, Berkeley, CA

Membrane fusion is a critical step in tissue formation, intracellular vesicle trafficking, and viral entry. Typically, fusion is preceded by protein-mediated adhesion between the two membranes that specifies which membranes should interact. Though the energetics of bare membrane fusion have been extensively studied, whether and how proteins on membranes affect the energetics of fusion is not well understood. Here we experimentally test the effect of adhesive and non-adhesive membrane proteins on fusion and find that the energy required to clear proteins from membrane contact sites presents an energy barrier comparable to – and in addition to – the energy barrier of bare membrane fusion. We use in vitro reconstitution of fusogenic membranes decorated with proteins together with a force-mediated fusion assay to systematically investigate the energetics associated with protein clearance from membrane contact sites prior to membrane fusion. Fusion is monitored by lipid mixing while controlled forces are applied between two supported lipid bilayers. We show that clearance of membrane proteins at physiological densities presents a significant energy barrier that can suppress membrane fusion depending on the concentration and size of proteins, as well as their affinity for each other. Consistent with this result, Sticks-and-Stones (Sns) and Dumbfounded (Duf), cell adhesion proteins involved in Drosophila myoblast fusion, are shown to increase the energy barrier to membrane fusion in vitro. Our findings suggest that a force-mediated clearance step, possibly generated by conformational changes of a fusogen or activity of the actin cytoskeleton, may be required to form close contacts between membranes so that fusion may proceed.

M241

Redox-regulated C-terminus of Mitofusins reside within the mitochondrial intermembrane space.

H.M. McBride[1];
[1]Neurology and Neurosurgery, McGill University, Montreal, QC

Mitochondrial fusion occurs in many eukaryotes including animals, plants, and fungi. It is essential for cellular homeostasis, and yet, the underlying mechanisms remain elusive. Comparative analyses and

phylogenetic reconstructions revealed that fungal Fzo1 and animal Mitofusin proteins are highly diverged from one another and lack strong sequence similarity. Bioinformatic analysis showed that fungal Fzo1 proteins exhibit two predicted transmembrane domains whereas metazoan mitofusins contain only a single transmembrane domain. This prediction contradicts the current models suggesting both animal and fungal proteins share one topology. This newly predicted topology of Mfn1 and Mfn2 was demonstrated biochemically, confirming that the C-terminal, redox-sensitive cysteine residues reside within the intermembrane space (IMS). Functional experiments established that redox-mediated disulfide modifications within the IMS domain are key modulators of reversible Mfn oligomerization that drives fusion. Together, these results lead to a revised understanding of Mfns as single-spanning outer membrane proteins with an Nout-Cin orientation, providing functional insight into the IMS contribution to redox-regulated fusion events.

M242

Mitofusin 1-mediated mitochondrial membrane fusion.

J. Hu[1];
[1]National Laboratory of Biomacromolecules, Institute of Biophysics, CAS, Beijing, China

Mitochondria undergo constant fusion and fission to maintain proper functions. The fusion of outer mitochondrial membrane is mediated by a class of dynamin-like GTPases, including mitofusins (MFNs) in mammals and Fzo1p in yeast. MFN forms a nucleotide-dependent dimer and continuously hydrolyzes GTP to achieve membrane tethering. However, the exact mechanism of fusion is poorly understood. Here, we determined the structures of the minimal GTPase domain (MGD) of human MFN1 including the predicted GTPase and the distal part of the C-terminal tail (CT). The structures revealed that a helix bundle (HB) formed by three helices extending from the GTPase and one extending from the CT closely attaches to the GTPase domain. We show that the nucleotide-binding pocket is shallow and narrow, rendering weak hydrolysis, and that association of HB affects GTPase activity. MFN1 forms a dimer when GTP or GDP/BeF_3^-, but not GDP or other analogues, is added. The proposed model was supported by Förster Resonance Energy Transfer (FRET) experiments and mitochondria morphology rescue assays in MFN1-deleted cells. These findings provide important insight into MFN-mediated membrane fusion and an explanation for how Charcot-Marie-Tooth neuropathy type 2A-causing mutations compromise MFN actions.

M243

Structural Basis of Mitochondrial Receptor Binding and GTP-Driven Conformational Constriction by Dynamin-Related Protein 1.

R. Kalia[1,2], R.Y. Wang[1], A. Yusuf[1], P.V. Thomas[1], D.A. Agard[1], J.M. Shaw[2], A. Frost[1,2];
[1]Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA, [2]Biochemistry, University of Utah, Salt Lake City, UT

Mitochondrial inheritance, genome maintenance, and metabolic adaptation all depend on organelle fission by Dynamin-Related Protein 1 (DRP1) and its mitochondrial receptors. DRP1 receptors include the paralogs Mitochondrial Dynamics 49 and 51 (MID49/MID51) and Mitochondrial Fission Factor (MFF), but the mechanisms by which these proteins recruit DRP1 and regulate its activities are unknown. Here we present a cryoEM structure of human, full-length DRP1 bound to MID49 and an analysis of structure- and disease-based mutations. We report that GTP binding allosterically induces a remarkable elongation and rotation of the G-domain, Bundle-Signaling Element (BSE) and connecting hinge loops of DRP1. In this nucleotide-bound conformation, a distributed network of multivalent interactions promotes DRP1

copolymerization into a linear filament with MID49, MID51 or both. Subsequent GTP hydrolysis and exchange within the filament leads to receptor dissociation, shortening through disassembly, and concomitant curling of DRP1 oligomers into closed rings. The dimensions of the closed DRP1 rings are consistent with DRP1-constricted mitochondrial tubules observed in human cells. These structures are the first views of full-length, receptor- and nucleotide-bound dynamin-family GTPases and—in comparison with nucleotide-free crystal structures—teach us how these molecular machines perform mechanical work through nucleotide-driven allostery.

M244

Novel roles for Dynamin2 (Dnm2) during ER scission and autophagy.

A. Martorell Riera[1], M. Iriondo Martinez[1], S. Itskanov[1], A.M. Van Der Bliek[1,2,3];
[1]Department of Biological Chemistry, University of California Los Angeles, Los Angeles, CA, [2]Molecular Biology Institute, University of California Los Angeles, Los Angeles, CA, [3]Johnson Comprehensive Cancer Center, University of California Los Angeles, Los Angeles, CA

The endoplasmic reticulum (ER) is a closed network of tubes and membranous sacs. Live cell imaging shows that this network is very dynamic. ER tubules routinely branch, elongate and fuse with other tubules or sacs. Elongation occurs along microtubules and fusion is mediated by the dynamin-related GTPase Atlastin. Frequent fusion events must be balanced by similarly frequent fission events or else the ER will form a continually tightening network that eventually collapses into a densely woven fabric. Mechanisms for ER fission are, however, as of yet unknown. Here, we show that Dynamin-2 (Dnm2), which severs endocytic vesicles from the plasma membrane, can also be recruited to the ER under stress-inducing conditions, coincident with the severing of ER membrane. Dnm2 knockout cells have more tightly woven ER networks and they show significant delays in ER fragmentation induced by calcium ionophores. These results suggest that Dnm2 contributes to ER scission. We also observe increased colocalization of Dnm2 with Endophilin B1 (EndoB1) and other ER markers when cells are treated with calcium ionopohores or with autophagy inducing chemicals. Increased interactions are detected with immunofluorescence and proximity ligation assays for endogenous proteins and with transiently transfected fluorescent proteins. EndoB1 is a BAR domain and SH3 domain containing protein, closely related to Endophilin A1, which interacts with Dnm2 at the plasma membrane during clathrin-independent endocytosis. Although EndoB1 interacts with Dnm2 when autophagy is induced, these proteins unexpectedly have opposite effects on the progression of autophagy. Loss of EndoB1 through CRISPR knockout inhibits autophagy, but this inhibition is fully suppressed in EndoB1 - Dnm2 double knockout cells. These results suggest that Dnm2 can interfere with autophagy but this interference is relieved through binding interactions with EndoB1. We have thus uncovered novel interactions between Dnm2 and ER membrane proteins that control ER scission and autophagy.

M245

BAR scaffolds drive membrane fission by locally concentrating intrinsically disordered domains.

W.T. Snead[1], W.F. Zeno[1], G.K. Kago[1], E.M. Lafer[2], J.C. Stachowiak[1];
[1]Biomedical Engineering, The University of Texas at Austin, Austin, TX, [2]Biochemistry and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX

Crescent-shaped BAR domains (bin-amphiphysin-rvs), found in many membrane traffic adaptors, serve an important role in shaping cellular membranes. The major function of BAR domains is to assemble into rigid, cylindrical scaffolds on membrane surfaces. For example, the N-terminal BAR (N-BAR) domain of amphiphysin has been shown to form membrane tubules and stabilize them against division into smaller

vesicles through the process of membrane fission. However, these conclusions were reached by studying the N-BAR domain alone, in the absence of amphiphysin's long, intrinsically disordered C-terminal domain. In contrast, here we show that full-length amphiphysin, containing both the structured N-BAR domain and the disordered C-terminus, is a highly potent driver of membrane fission. How can inclusion of the disordered C-terminus so dramatically impact the membrane remodeling ability of amphiphysin? Recent work from our group showed that when disordered domains become concentrated on membrane surfaces, steric pressure arising from protein collisions provides the necessary force to drive membrane fission (Snead et al, PNAS 2017). Building on these findings, our present experiments suggest that assembly of amphiphysin's N-BAR domain into scaffolds heavily concentrates amphiphysin's disordered C-terminal domain at membrane surfaces, generating sufficient steric pressure to drive membrane fission. This collaboration between structured scaffolding domains and disordered pressure generators provides a highly efficient, yet previously unknown mechanism of membrane fission. This model makes two key predictions. First, the essential, fission-driving feature of BAR domains is their ability to assemble into scaffolds, regardless of their curvature. Second, adding an arbitrary disordered domain to a BAR scaffold should promote fission. To test these predictions, we fused a bulky, disordered domain from another membrane traffic adaptor, AP180, to an inverse BAR (I-BAR) domain. I-BAR domains form scaffolds similar to N-BAR, but drive membrane curvature in the opposite direction, and should therefore be inhibitory to fission. However, we find that the I-BAR-AP180 fusion protein is indeed a potent driver of membrane fission, suggesting that steric pressure among disordered domains is sufficient to overcome the structure-based curvature preference of BAR scaffolds. Our results expand understanding of the functions of protein scaffolds, suggesting that they partner with disordered domains to amplify steric pressure at presumptive sites of membrane fission.

M246

ESCRT membrane scission revealed by optical tweezers.

J. Schöneberg [1,2,3], S. Yan[1,2,4], A.H. Bahrami[3], M. Righini[5], I. Lee[1,2], M.R. Pavlin[2,6], L. Carlson[1,2], D.H. Goldman[1,2,4], G. Hummer[3,7], C. Bustamante[1,2,4,5,6,8,9], J.H. Hurley[1,2,5,8];
[1]Molecular and Cell Biology, UC Berkeley, Berkeley, CA, [2]California Institute for Quantitative Biosciences, UC Berkeley, Berkeley, CA, [3]Theoretical Biophysics, Max Planck Institute of Biophysics, Frankfurt a. M., Germany, [4]Howard Hughes Medical Institute, UC Berkeley, Berkeley, CA, [5]Department of Chemistry, UC Berkeley, Berkeley, CA, [6]Biophysics Graduate Group, UC Berkeley, Berkeley, CA, [7]Institute of Biophysics, Goethe University, Frankfurt/M, Germany, [8]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA, [9]Department of Physics, UC Berkeley, Berkeley, CA

ESCRT proteins catalyze the function of membrane budding and scission from the inside of the cytosol to the outside. This process is fundamental in cell biology, comprising multivesicular endosome biogenesis, cytokinesis, viral budding (e.g. HIV, Ebola, Dengue) and other pathways. The mechanism underlying ESCRT-III-mediated membrane budding and scission remains elusive. We have encapsulated within giant unilamellar vesicles (GUVs) a minimal ESCRT module consisting of ESCRT-III subunits and the AAA+ ATPase Vps4. Using optical tweezers, membrane nanotubes reflecting the correct topology of scission can be pulled from these GUVs. Upon photo-uncaging of ATP, surprisingly large forces in the tens of piconewtons were recorded and tube scission could be observed. ESCRT subunit composition and concentration alter force generation and scission behavior. In combining confocal fluorescence microscopy and optical tweezers, the scission events can be observed in both force and fluorescence and studied in detail. For the first time, the biophysics of ESCRT membrane budding and scission are revealed.

M247

Resolving ESCRT-III spirals at the intercellular bridge of dividing cells using 3D STORM imaging.

I. Goliand[1], T. Dadosh[2], N. Elia[1];
[1]Life Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel, [2]Faculty of Chemistry, Chemical research support, Weizmann Institute of Science, Rehovot, Israel

The ESCRT machinery mediates membrane fission in a verity of processes in cells. According to the proposed mechanism, ESCRT-III proteins drive membrane fission by assembling into helical filaments on membranes. Yet, ESCRT-III filaments have never been directly visualized in a cellular process that utilizes this machinery for its function. Here we used 3D STORM imaging of endogenous ESCRT-III component IST1, to describe the structural organization of ESCRT-III during mammalian cytokinetic abscission. Using this approach, ESCRT-III ring and spiral assemblies were resolved at the intercellular tube of cells at different stages of abscission. Characterization of these structures reveals the structural remodeling that ESCRT-III filaments undergo during abscission. Structural analysis of ESCRT-III polymers in cells depleted of different ESCRT components highlights the contribution of these components to ESCRT-III spiral formation and remodeling. This work provides the first evidence that ESCRT-III proteins assemble into helical filaments in physiological context, indicating that the ESCRT-III machine indeed derives its contractile activity through spiral assemblies. Moreover, it provides new structural information on ESCRT-III filaments, which raise new mechanistic scenarios for ESCRT driven membrane constriction.

M248

Recruitment dynamics of ESCRT-III and Vps4 to endosomes and implications for reverse membrane budding.

M. Alonso Y Adell[1], S. Migliano[1], S. Upadhyayula[2], Y. Bykov[3], J.A. Briggs[3], T. Kirchhausen[2], D. Teis[1];
[1]Biocenter / Division of Cell Biology, Medical University of Innsbruck, Innsbruck, Austria, [2]Department of Cell Biology , Harvard Medical School , Boston, MA, [3]Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg, Germany

The ESCRT machinery mediates reverse membrane scission. By quantitative fluorescence lattice light-sheet microscopy, we have shown that ESCRT-III subunits polymerize rapidly on yeast endosomes, together with recruitment of at least two Vps4 hexamers. During their 3-45 second lifetimes, the ESCRT-III assemblies accumulated 75-200 Snf7 and 15-50 Vps24 molecules. Productive budding events required at least two additional Vps4 hexamers. Membrane budding was associated with continuous, stochastic exchange of Vps4 and ESCRT-III components, rather than steady growth of fixed assemblies, and depended on Vps4 ATPase activity. An all-or-none step led to final release of ESCRT-III and Vps4. Tomographic electron microscopy demonstrated that acute disruption of Vps4 recruitment stalled membrane budding. We propose a model in which multiple Vps4 hexamers (four or more) draw together several ESCRT-III filaments. This process induces cargo crowding and inward membrane buckling, followed by constriction of the nascent bud neck and ultimately ILV generation by vesicle fission.

Minisymposium 25: Visualization of Compartmentalized Signaling in Cancer

M249

The lateral mobility of membrane-tethered KRAS4b revealed spatiotemporal complexity of signaling.

D. Goswami[1,2,3,4], D. Chen[1,2,3,4], J. Columbus[1,2,3,4], T.J. Turbyville[1,2,3,4];
[1]Frederick National Laboratory for Cancer Research, Frederick, MD, [2]National Cancer Institute, Frederick, MD, [3]Cancer Research Technology Program, Frederick, MD, [4]Leidos Biomedical Research, Inc, Frederick, MD

RAS proteins function as GTP/GDP-dependent control switches to regulate signaling networks in cells and play a major role in human cancer. The major difference across the isoforms of RAS proteins (KRAS4a, KRAS4b, NRAS, and HRAS) lies within the C-terminus hypervariable region (HVR, aa 167-189) and its post-translational lipid modifications. One hypothesis is that the differential signaling behavior from RAS isoforms is controlled by the recruitment and organization of RAS into distinct membrane nano-domains. Association with these nano-domains is determined by RAS lipid-tethers and key residues in the HVR. However, very little is known about the molecular dynamics of membrane-tethered RAS molecules and how these dynamics influence the downstream signaling cascade within a living cell. Herein, the molecular mobility of membrane-tethered Ras variants was characterized by using single molecule tracking method that further estimated underlying mobility states. Detailed analysis of tracks revealed that KRAS4b exhibit confined mobility with three diffusive states in the active plasma membrane of living cells. This diffusion characteristic was unique to KRAS4b and influenced by both the hypervariable region and globular domain of the protein, compared to all the other RAS isoforms. Importantly, the occupancy of each diffusive states was altered for the oncogenic mutant of KRAS4b, implicating enhanced signaling activity. Our study begins to decipher the underlying principle of KRAS functionality in real time on the membrane of living cancer cells that will augment our ability to develop novel therapeutic strategies for targeting oncogenic KRAS4b.
Contract HHSN261200800001E - Funded by the National Cancer Institute

M250

Engineering Cell Sensing and Responses Using a GPCR-Coupled CRISPR-Cas System.

P.P. Dingal[1,2,3], N.H. Kipniss[1], L. Labanieh[1], Y. Gao[4], L.S. Qi[1,2,3];
[1]Bioengineering, Stanford University, Stanford, CA, [2]ChEM-H, Stanford University, Stanford, CA, [3]Chemical Systems Biology, Stanford University, Stanford, CA, [4]Cancer Biology Program, Stanford University, Stanford, CA

G-protein coupled receptors (GPCRs) are the largest and most diverse group of membrane receptors in eukaryotes, and detects a wide array of physiological cues in the human body. We describe a new molecular device that couples CRISPR-Cas9 programmed genome regulation to natural and synthetic extracellular signals via GPCRs. The design of our synthetic device, named CRISPR ChaCha, displays superior performance over an architecture proposed by the previously reported Tango system. Using a parsimonious mathematical model and gene-reporter assays, we find that CRISPR ChaCha can recruit and activate multiple Cas9 molecules for each GPCR molecule. We also characterize key molecular features that modulate CRISPR ChaCha performance. We adopt the design to diverse GPCRs that sense synthetic and natural ligands including chemokines, mitogens, and fatty acids, and observe efficient conversion of signals to customizable genetic programs in mammalian cells, including regulation of

endogenous genes. The new class of CRISPR-coupled GPCRs provides a robust and efficient platform for engineering cells with novel behaviors in response to the diverse GPCR ligand repertoire.

M251

Divergence in the temporal dynamics of Extracellular-signal regulated kinase (ERK) activity between subcellular compartments.

J.D. Keyes[1], A. Ganesan[2], J. Zhang[1,2];
[1]Pharmacology, University of California San Diego, San Diego, CA, [2]Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD

The ERK1/2 pathway plays critical roles in eukaryotic biology by transducing extracellular signals into cell-fate decisions. One conundrum is in understanding how disparate signals induce specific responses through a common, ERK-dependent kinase cascade. One major mechanism to control ERK-signaling to induce proper cellular responses is through precise regulation over the temporal dynamics of ERK activity. For example, in the pheochromocytoma PC-12 cell line, ERK exhibits transient activity in response to Epidermal Growth Factor (EGF), ultimately resulting in proliferation. On the other hand, PC-12 cells respond to Nerve growth factor (NGF) with sustained ERK activity and eventual differentiation into neuronal-like cells. An additional mechanism of control over ERK is through spatial regulation of activity; however, it has been difficult to accurately examine differences in activity among specific subcellular locations. To address this issue, we have expanded the toolbox of FRET-based ERK biosensors by creating a series of improved biosensors that are specifically targeted to various subcellular regions. Using these sensors, we have tested the hypothesis that a single extracellular signal can induce different temporal dynamics between subcellular compartments in PC-12 cells. We report that EGF induces transient ERK activity in the cytosol and nucleus, as expected based on previous reports. However, we have discovered that EGF simultaneously induces sustained ERK activity at the plasma membrane of PC-12 cells. Furthermore, we report that PKA and cAMP play an integral role in the differential regulation of ERK at the plasma membrane and the cytosol. Specifically, we observe that increasing basal cAMP induces a more transient activity at the plasma membrane without changing the temporal nature of ERK activity within the cytosol. Inhibiting PKA slows and minimizes the ERK response in the cytosol without affecting ERK activity at the plasma membrane. These spatially disparate results indicate that subpopulations of ERK are differentially regulated in a spatiotemporal manner and that crosstalk with other signaling pathways play an integral role in the regulation of these subpopulations, furthering our fundamental understanding of how this central kinase is regulated in cells.

M252

Information content of the single cell cortical travelling waves.

D. Xiong[1,2,3], S. XIAO[1,2], C. Tong[1,2], M. Wu[1,2,3];
[1] Department of Biological Sciences, National University of Singapore, Singapore, Singapore, [2]Centre for Bioimaging Sciences, National University of Singapore, Singapore, Singapore, [3]Mechanobiology Institute, National University of Singapore, Singapore, Singapore

Frequency-encoded signaling represents an appealing mechanism to integrate kinetic information in signaling networks. Using rat basophilic leukemia (RBL) cells as a model system (1,2), we discovered that the frequency of the cortical periodic travelling waves in these cells could encode phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) activity (3). Activating PI3K optogenetically or its chemical inhibition leads to the corresponding tuning of oscillation frequencies. We identified phosphoinositide phosphatases, SHIP1, that function as local inhibitor to generate sequential waves of phosphoinositides

underlying the cortical rhythmicity. In addition, we show that these frequencies could be converted to encode spatial information. At the onset of mitosis, interphase travelling waves turn into concentric target waves. Mitotic actin waves are mediated by activation of Cdc42 and require the biochemical signals of spindle assembly checkpoint. These waves temporally precede the clustering of the anaphase marker Anillin and spatially predict the future sites of cell divisions. Importantly, their appearances in metaphase indicate that they are likely the earliest spatial cues for division plane specification. Intriguingly, the frequencies and wavelengths of the mitotic waves are proportional to cell adhesion area, indicating that travelling waves could be employed by mitotic cells to encode size information. We will also discuss why these cortical spatial information could be necessary for the fidelity of cell division.

1. Wu, M., Wu, X. & De Camilli, P. Calcium oscillations-coupled conversion of actin travelling waves to standing oscillations. Proceedings of the National Academy of Sciences 110, 1339–1344 (2013).
2. Wu, Z., Su, M., Tong, C., Wu, M. & Liu, J. Collective membrane dynamics emerging from curvature-dependent spatial coupling. bioRxiv 164392 (2017).
3. Xiong, D. et al. Frequency and amplitude control of cortical oscillations by phosphoinositide waves. Nat Chem Biol 12, 159–166 (2016).

M253

Intersectin-s interaction with the Rab13 exchange factor DENND2B facilitates recycling of epidermal growth factor receptor.

M.S. Ioannou[1], G. Kulasekaran[1], M. Fotouhi[1], J.J. Morein[1], C. Han[1], S. Tse[1], N. Nossova[1], T. Han[1], E. Mannard[1], P.S. McPherson[1];
[1]Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, QC

The small GTPase Rab13 functions in exocytic vesicle trafficking and alterations in Rab13 activity have been observed in numerous human cancers. We previously identified DENND2B as the guanine nucleotide exchange factor for Rab13. Localized activation of Rab13 by DENND2B at the plasma membrane promotes vesicle fusion and the dynamic remodeling of the leading edge, driving cancer cell migration *in vitro* and growth and metastasis *in vivo*. Epidermal growth factor (EGF) activates the EGF receptor (EGFR) and stimulates its internalization and trafficking to lysosomes for degradation. However, a percentage of EGFR undergoes ligand-independent endocytosis and is rapidly recycled back to the plasma membrane. Importantly, enhanced EGFR recycling is commonly associated with cancer. Intersectin-s is a multi-domain adaptor protein required for the internalization of EGFR. Here, we discover that intersectin-s binds DENND2B promoting EGFR recycling to the cell surface. Intriguingly, upon EGF treatment, DENND2B is phosphorylated by protein kinase D and dissociates from intersectin-s. As EGF targets EGFR predominantly for degradation, we propose that DENND2B through its interaction with intersectin-s couples EGF-independent EGFR internalization with its recycling back to the plasma membrane. Enhanced activity of this process could contribute to the invasive and growth capacity of tumours in vivo. Our study thus reveals a novel mechanism controlling the fate of internalized EGFR with important implications for cancer.

M254

Engineered Allosteric Regulation of Protein Kinases by Light.

M. Shaaya[1], V. Huyot[1], V. Natarajan[1], A.V. Karginov[1];
[1]Cellular and Molecular Pharmacology, University of Illinois at Chicago, Chicago, IL

A single protein kinase can initiate very distinct physiological and pathological processes depending on the timing and the location of its activation in the cell. However, interrogation of kinase-mediated

processes remains challenging due to the paucity of tools that precisely regulate kinases in space and time. To overcome existing limitations, we developed a novel approach for light-mediated regulation of kinases. We engineered a light-regulated allosteric switch (LightR), a small domain based on Vivid (VVD) photoreceptor, that undergoes conformational changes upon illumination with blue light. Insertion of LightR at a specific location in the catalytic domain of Src kinase enables light-mediated control of its activity. In the dark, engineered LightR-Src is inactive. Illumination of cells with blue light leads to robust activation of the kinase. Activation of LightR-Src is specific, fast, and reversible. This allows us to mimic transient activation of the kinase in living cells. The level of activation can be controlled by changing light intensity. Specific modifications of the LightR domain allow us to modulate the activation/inactivation kinetics and the level of activity of LightR-Src, enabling additional "tuning" of the engineered kinase and its optimization for different applications. Activation of LightR-Src in living cells induces robust cell spreading and protrusive activity, which are known Src-induced phenotypes described in previous studies. Importantly, the morphological changes stop as soon as the kinase is inactivated, demonstrating reversibility of the manipulated signaling. Repeated activation/inactivation of LightR-Src induces cycles of morphodynamic activity of the cells, showing the capability of this tool to mimic oscillation of kinase signaling in living cells. Localized illumination of cells induces local formation of protrusions demonstrating spatial regulation of LightR-Src. Insertion of LightR domain at a homologous position in Abl kinase also enabled light-mediated regulation of its activity. These results, and the high structural similarity among catalytic domains of kinases, suggest broad applicability of this tool for regulation of different kinases. Furthermore, this approach could be potentially employed to control the activity of other enzymes in time and space.

M255

High resolution in vivo imaging of cancer cell extravasation.

T. Liu[1], V. Singh[1], E. Betzig[1], D.Q. Matus[2], B.L. Martin[2];
[1]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA, [2]Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY

The extravasation of circulating tumor cells (CTCs) is a critical process during the spread of cancer, but also one of the least understood aspects of the metastatic cascade due to the difficulty of visualizing the process in vivo. Genetic and in vitro data support a model in which CTCs co-opt mechanisms involved in leukocyte extravasation, but direct visualization of the process in CTCs is lacking. At sites of inflammation, leukocytes initially weakly adhere to activated endothelium through microvilli attachments mediated by selectin adhesion molecules. The weak adhesion, combined with force of blood flow, causes the leukocytes to roll along the endothelial lining of the blood vessel. Leukocytes will then firmly attach to the endothelium through integrin mediated adhesion and begin active migration along the endothelium. Finally, leukocytes undergo transendothelial migration to leave the blood vessel. We utilized a zebrafish xenograft system to observe human breast cancer cell extravasation in vivo at single-cell, subcellular resolution, using adaptive optics lattice light sheet microscopy. Time-lapse imaging of CTC extravasation captured all three critical leukocyte behaviors in CTCs, including microvilli mediated endothelial rolling, lamellipodia based active migration, and transendothelial migration. Our results provide direct visual evidence that CTCs utilize leukocyte-like behaviors during the extravasation phase of metastasis, and provide a framework for further understanding essential components of the metastatic cascade.

M256

Oscillatory HIF-1α induction promotes proliferation of hypoxic cells through a lactate dependent quorum autophagy response.

.. Kshitiz[1,2], J. Afzal[2], H. Chang[1], Y. Suhail[2], M. Hubbi[2], C. Dang[2,3], A. Levchenko[1];
[1]Biomedical Engineering, Yale University, New Haven, CT, [2]Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD, [3]Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA

Response to hypoxia is a highly regulated process controlling multiple cell and tissue level functions. However, we still know little about dynamic single cell responses to hypoxic conditions. Here, using fluorescent reporters of hypoxia response factor-1 alpha (HIF-1a) activity, we show that hypoxic responses in individual cells can be highly dynamic and variable across the population. These responses fall into three classes, including oscillatory activity. We identify a molecular mechanism that can account for all three response classes, demonstrating that the oscillations of HIF-1a activity and abundance are controlled by the reactive oxygen species-dependent chaperone-mediated autophagy in a subset of respiring cells. Furthermore, we find that the oscillatory response is modulated by the abundance of extracellular lactate in a quorum sensing-like mechanism. We show that oscillatory HIF-1a activity can help overcome hypoxia-mediated inhibition of cell division, suggesting a mechanism for aggressive behavior in a subset of hypoxic tumor cells.

M257

Endocytosis and PTEN in Tumor Suppression.

M. Lee[1], A. Naguib[1], L.C. Trotman[1], N. El-Amine[1];
[1]Cancer Center, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

PTEN has been thought to function at the plasma membrane, where receptor tyrosine kinases are activated. However the majority of PTEN is cytosolic, which implied that at any given time only a fraction of the PTEN pool is actively suppressing PI 3-K signaling. We find that PTEN acts on discrete, internalized vesicles by binding the signature lipid of endosomes, PI(3)P, through the CBR motif in the C2 domain. Mutation of this motif (mCBR) abrogates PTEN function in cells while the enzyme retains full activity in vitro. Importantly, the function of the mCBR mutant of PTEN could be rescued and even enhanced by fusion to a FYVE domain, the canonical PI(3)P vesicle targeting domain. Consistent with this observation, we find strong colocalization between internalized EGF, FYVE probes and the PTEN C2 domain, revealing that PTEN action is under tight spatial and temporal control in the endocytic compartment. Our results have now introduced us to a series of novel upstream activators of PTEN and we will discuss how they can be used to suppress cancer.

M258

Plasma Membrane PI(4,5)P2 Threshold Regulates Cell Migration Speed and Morphology.

M. Beshay[1], N. Bawazir[1], J. Notino[1], C. Janetopoulos[1];
[1]Biological Sciences, University of the sciences in Philadelphia, philadelphia, PA

During gradient sensing, many signaling and cytoskeletal markers become asymmetrically distributed, and cells establish a polarity circuit. A similar excitable network was shown in cells during cytokinesis. Phosphoinositide-linked signals at the poles of dividing cells were activated in a manner equivalent to those found at the front of migrating cells. Signals at the furrow between two daughter cells were similar in nature to those found at the rear of migrating cells. To further demonstrate the importance of plasma membrane (PM) PI(4,5)P2 in maintaining cell morphology and breaking cell symmetry during

polarized morphologies, we have used the inducible rapamycin (iRAP) system. iRAP was used to recruit various enzymes to the PM that regulate PM PI(4,5)P2 levels. Cell migration and the localization of various signaling and cytoskeletal biosensors were then monitored and recorded when PI(4,5)P2 levels were depleted or elevated. We find that lowering PM (PI4,5)P2 leads to cell spreading and increased migration, while elevating PM PI(4,5)P2 inhibits cell excitability, contributes to cell rounding, and reduced rates of cell migration. Depletion of PM PI(4,5)P2 is excitatory and increases Ras and PI3 Kinase activity. Cells show a variety of phenotypes when PI(4,5)P2 levels are depleted, including oscillatory behavior and dramatic changes in cell morphology. Similar effects were observed in Dictyostelium cells lacking the PI5 Kinase (pik1), whose loss leads to dramatic decreases in PM PI(4,5)P2 levels. We also investigated the localization of ForminA, which has a putative PI(4,5)P2 motif. ForminA localizes in a reciprocal manner with that of a marker for Rac activity during cell oscillations, with ForminA moving to the cytosol and Rac moving to the PM during cell spreading. Interestingly, this Formin also localizes to the rear of cells undergoing chemotaxis and is found at the furrow of dividing cells. Our data suggests that the PM levels of PI(4,5)P2 are critical for altering cell speed and morphology, with lower levels contributing to excitable networks controlling cell protrusions. PM PI(4,5)P2 levels above a threshold support regulators that contribute to quiescent membrane activity and actomyosin contraction. Interestingly, we find that lowering PIP2 levels alone does not appear sufficient for triggering these excitable networks, and another component is likely activated. We propose that this unknown molecule becomes positively charged during the formation of protrusions, and plays a major role in the transient binding affinity of proteins that are lipid anchored and have positively charged binding motifs.