# abstracts: oral presentations

# ASCB | EMBO 2019 meeting

Washington, DC • December 7-11

## Walter E. Washington Convention Center

Washington DC, USA | December 7-11

ascbembomeeting.org | **#ascbembo19**

## Keynote Lecture

K1

**Copying the Genome in Eukaryotic Cells: Insights into the Evolution of Origin Specification and its Relationship to Gene Silencing Mechanisms**

**B. Stillman**; Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Over many years, the primary goal has been to understand how chromosomes are duplicated during the cell division cycle to ensure faithful inheritance of genetic information in eukaryotic cells. Studies on replication of DNA from chromosomal origins of DNA replication in eukaryotic cells, primarily the yeast *S. cerevisiae*, lead to the discovery of an ATP-dependent protein machine, the Origin Recognition Complex (ORC) that is required to form a pre-Replicative Complex (pre-RC) at all origins of DNA replication prior to S phase. The assembly of pre-RCs licenses chromosomes for subsequent DNA replication during the S phase of the cell cycle. The regulated assembly of the pre-RC explains how each segment of the genome is copied only once per cell division cycle. pre-RC assembly and more recently, complete replication of origin containing DNA, have been reconstituted *in vitro* with purified proteins and structural studies have revealed the mechanisms of protein assembly on origin DNA. ORC and Cell Division Cycle 6 (Cdc6) protein bind to budding yeast origins in a sequence-specific manner. Chromatin licensing and DNA replication factor 1 (Cdt1) and replication helicase Mcm2-7 are recruited to the ORC-Cdc6 bound origins, forming an ORC-Cdc6-Cdt1-Mcm2-7 (OCCM) structure, which is an intermediate for assembling a pre-RC at all origins prior to the initiation of replication in S phase. The Cryo-EM structure of *S. cerevisiae* OCCM bound to origin DNA revealed that an α-helix within the Orc4 subunit of ORC that inserts into a major groove of origin DNA and that three loops within Orc2 and Cdc6 also interact with origin DNA. Interestingly, the sequence of the Orc4 insertion α-helix and DNA interacting loop within Orc2 have rapidly evolved within budding yeasts and is absent most eukaryotes. Multiple genetic studies have revealed how some budding yeasts have DNA sequence specific origins and how, in various mutants of Orc4, the repertoire of origins utilized in the genome has changed significantly, reminiscent of the origin distribution in most eukaryotes. Importantly, the evolution of the Orc4 α-helix and the Orc2 loop parallels changes in the Dicer (*Dcr*) and Argonaute (*Ago*) genes that participate in gene silencing by RNA interference, as well as the acquisition of *Sir4* and ORC-mediated, sequence-specific gene silencing. I will discuss the implications of these observations.

## Symposium 1: Beyond Figure 7: Integrating Modeling and Experiment in Cell Biology

S1

**Physics of Adherent Cells**

**M. L. Gardel**; University of Chicago, Chicago, IL.

Molecular-scale details of cytoplasmic machines that control cell physical behaviors have been revealed through modern tools of cell biology, biochemistry and biophysics. These advances have led to dizzying amounts of data that, alone, do little to provide insightful understanding of how cells control their shape, adhesivity, motion, and transport. A long term goal of mine is to establish these approaches for living cells, taking inspiration from traditions established in condensed matter physics. I will describe our efforts to establish predictive physical models of adherent cells from the organelle to tissue scales through quantitative imaging and biophysical methods. I will discuss on what we've learned from collaborating with theorists who use diverse approaches, from agent-based models to analytical theory. I will also discuss the use of in vitro model systems and cellular engineering approaches to design experiments that test models. Through this feedback between theory and experimentation, I will show how this approach allows us to establish control parameters of cytoplasmic machines.

S2

**Theory and Experiments in the Study of the Mitotic Spindle**

**I. Tolic**; RBI, Zagreb, CROATIA.

Cell biology is immensely complex. To understand how cells work, we scientists try to find patterns and propose hypotheses to identify underlying mechanisms. However, it is not always easy to make a coherent picture out of the massive amount of experimental data on biological systems, where main players have multiple interactions or act in redundant pathways. In such situations, where a hypothesis does not lead to a conclusion in a straightforward way, theory is a great tool. Theoretical modeling allows us to formulate our hypotheses to describe interactions between multiple players in a quantitative manner. Solving the model shows us the consequences of these hypotheses. A successful model should not only reproduce the basic features of the system, but also provide exciting predictions for what would happen under certain conditions, motivating new experiments. A lot is learned when a model based on generally accepted knowledge cannot explain the experiments of interest, as this indicates that the original hypothesis needs to be changed or new players need to be included. To illustrate these challenges in the interplay between theory and experiments, I will use examples from our work on the mechanics of the mitotic spindle.

S3

**Bottom-up Engineering of Protein Pattern Formation**

**P. Schwille**; Max-Planck-Institute für Biochemie, Martinsried, GERMANY.

Self-organized concentration patterns are crucial for the development and function of organisms on all levels. However, there is a large gap of knowledge on how different molecular features determine the emergence of a pattern, because it has so far not be possible to systematically design biological gradients from scratch. In my talk, I will highlight our present approaches towards modular engineering of protein pattern formation, based on *in vitro* reconstitution of purified proteins. I will discuss the great

impact that the exchange with theoretical groups has on our design of functional features and modules, through the *in silico* modelling of pattern-forming protein networks.

## Symposium 2: Attack of the Killer Bugs: The Cell Biology of Infectious Disease

S4

**Unravelling the Hallmarks of Apicomplexan Parasitism**

**S. Lourido**[1,2]; [1]Whitehead Institute for Biomedical Research - MIT, Cambridge, MA, [2]Biology Department, MIT, Cambridge, MA.

Apicomplexans have coevolved with their animal hosts for half a billion years, developing the complex adaptations that support their obligate parasitic lifecycle. This protist phylum includes deadly and prevalent human infections like malaria and toxoplasmosis. Our work seeks to uncover the cellular adaptations that enable this form of parasitism. Using *Toxoplasma gondii* as a model organism for the phylum, we have integrated a variety of systematic approaches to assess gene function, including CRISPR-based genetic screens, quantitative proteomics, and single-cell transcriptional profiling. Together these methods provide new perspectives on the critical molecular events that govern the invasion of host cells, the regulation of gliding motility, and the establishment of chronic infections. Evidence points to conserved functions throughout the phylum for several of the pathways we have uncovered, including essential steps in the erythrocytic cycle of *Plasmodium falciparum*, which causes the most lethal form of malaria in humans. Beyond uncovering novel avenues for therapeutic intervention, our results inform our understanding of eukaryotic diversity.

S5

**Characterization of the Intracellular Pathogen Response in *C. Elegans***

**E. Troemel**; University of California, San Diego, La Jolla, CA.

We study host/pathogen interactions in the nematode *C. elegans*, and focus in particular on the *C. elegans* response to infection by microsporidia, which are fungal-related, obligate intracellular pathogens. Microsporidia comprise a phylum of over 1400 species, some of which can infect the intestine and cause lethal diarrhea in humans, with all animals likely susceptible to infection by at least one species of microsporidia. Microsporidia are the most common cause of infection for *C. elegans* in the wild, and most microsporidia pathogens of *C. elegans* infect the intestine, where they restructure host tissue and also induce rapid gene expression changes in the host. Interestingly, microsporidia infection induces a common set of genes as the 3-gene RNA virus from Orsay, which is another natural intracellular pathogen of the *C. elegans* intestine. We have named this commonly induced set of genes the Intracellular Pathogen Response (IPR), and it appears to be distinct from previously described proteostasis pathways like the heat-shock response and the insulin signaling pathway. *C. elegans* mutants with constitutive expression of IPR genes have increased resistance to intracellular infection as well as increased proteostasis, which is dependent on ubiquitin ligase components. We have recently found that there are multiple pathways for inducing IPR gene expression. Induction of the IPR by virus is mediated by the dsRNA sensor RIG-I/DRH-1, while other triggers like microsporidia and proteotoxic stress induce IPR gene expression independently of RIG-I. Altogether our studies indicate there are several independent inputs and outputs of this novel proteostasis/immune pathway in *C. elegans*.

## Symposium 3: Decisions, Decisions: How Cells Choose Their Fates

S6

**Cellular Biographies: Reconstructing Developmental Trajectories**

**A. Schier**; Harvard University/University of Basel, Basel, SWITZERLAND.

Complex gene expression programs determine the biographies of cells: how a cell divides, becomes specialized and differentiates. I will describe our recent efforts to use single-cell RNA sequencing and CRISPR-Cas9 genome editing to generate new tools to reconstruct differentiation trajectories and lineage trees at very large scales. Using mesoderm and brain development as examples, I will discuss the opportunities and challenges for these technologies to reveal the developmental biology of cells (also see McKenna et al. Science 2016; Farrell et al. Science 2018; Raj et al. Nature Biotechnology 2018).

S7

**Time to Get Up: Awakening Stem Cells in the Brain**

**A. Brand**; The Gurdon Institute, University of Cambridge, Cambridge, UNITED KINGDOM.

Neural stem cells can generate new neurons in the brain in response to a range of stimuli, including exercise, nutrition and injury. In this way stem cells meet the needs of the organism during growth and in response to damage. A key control point is the decision between stem cell quiescence and proliferation. *Drosophila* neural stem cells enter quiescence in late embryogenesis and are reactivated post-embryonically in response to nutrition. We found that feeding induces the expression of insulin-like peptides within the brain itself. We showed that insulin signalling is essential for the stem cells to exit quiescence and resume proliferation. Insulin signalling can also promote proliferation in vertebrate neural stem cells, suggesting that the mechanisms controlling stem cell reactivation may be conserved. We are investigating the systemic and local signals that regulate neural stem cell quiescence and reactivation. Understanding the signals that instruct stem cells to produce new neurons at will raises the prospect of future therapies for brain repair after damage or neurodegenerative disease.

## Symposium 4: 21st Century Machinery: The Structure, Function, and Evolution of Protein Machines

S8

**The Kinetochore, An Intrinsically Divisive Molecular Machine**

**A. Musacchio**; Department of Mechanistic Cell Biology, Max-Plank Institute of Molecular Physiology, Germany, Dortmund, GERMANY.

Chromosome bi-orientation is the pre-condition for successful cell division, but how it is achieved on the molecular level in settings as diverse as mitosis and meiosis remains poorly understood. Kinetochores play a decisive role in promoting chromosome bi-orientation and in imparting fidelity to the chromosome segregation process. In addition to binding microtubules, they recognize and correct improper microtubule attachments, and make the timing of cell division contingent on completion of bi-orientation through the spindle assembly checkpoint. How are these different activities regulated and integrated within the kinetochore's structure? To answer this question, our laboratory took up the long-

term goal of reconstituting kinetochores and their functions *in vitro*, focusing on human kinetochores as model system. The reconstitution is challenging, because kinetochores consist collectively of ~35 core subunits, and several additional regulatory subunits, for a total of ~100 different polypeptides. The challenge is compounded by the embedding of kinetochores in the complex and incompletely understood environment of the centromere, a specialised chromatin domain that specifies the kinetochore assembly site. As a summary of our work so far, I will present three large reconstitutions, comprising two major stable kinetochore sub-complexes (each with molecular mass in excess of 1 MDa), and the signalling ensemble of the spindle assembly checkpoint. I will illustrate what organizational principles have emerged from this work, and how they are inspiring our current attempts to build the entire kinetochore and its functions *in vitro*. All three reconstitutions reflect stable interactions at thermodynamic equilibrium, and therefore cannot be considered “alive” by any means. The ultimate challenge for future *in vitro* work on the kinetochore, and a more general challenge for any *in vitro* reconstitution, is to ignite the energy-dissipating reactions that subtend to regulatory switches. We would like to build particles that, like their cellular counterparts, sense bi-orientation (or lack thereof) and turn the checkpoint on or off depending on context. This will require the addition of microtubules and of enzymes, most notably mitotic kinases and phosphatases, whose opposing regulation determines, at any given time, appropriate context-dependent signalling outcomes.

S9

**Mechanisms of Centriole Assembly**

**P. Gönczy**, N. Banterle, G. Hatzopoulos, A. P. Nievergelt, T. Kükenshöner, T. Favez, T. Hübscher, G. E. Fantner, O. Hantschel; Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne, SWITZERLAND.

Understanding the organizing principles driving the assembly of cellular organelles and of protein machines is a fundamental pursuit in biology. The centriole is a microtubule-based organelle that is essential for the formation of flagella, cilia and centrosomes. As such, the centriole is critical notably for cell motility, sensing of the environment and cell architecture. The centriole has a signature nine-fold symmetrical arrangement of microtubules and is organized around a likewise symmetrical cartwheel that is critical at the onset of organelle biogenesis. The cartwheel comprises a stack of ring-containing elements that each accommodates nine homodimers of SAS-6 proteins. Although important advances have been made in recent years, understanding of centriole assembly remains incomplete, partly due to the lack of cell-free assays to probe the dynamics of the process at nanometric scale.To fill this gap, we have developed a high-speed atomic force microscopy (AFM) to reconstitute and monitor the assembly dynamics of SAS-6 proteins with utmost spatial and temporal resolution. This novel assay enabled us to better understand the mechanisms imparting the signature nine-fold radial symmetry of the organelle. Moreover, in order to develop tools to dissect and modulate centriole assembly, we have identified monobodies that recognize SAS-6 proteins with high affinity. Our analysis established that some of these monobodies prevent the assembly of ring-containing elements in vitro and of the centriole organelle in cellulo. Together, these approaches provide novel insights into the mechanisms governing the assembly of the fundamental centriole organelle.

S10

**Condensin-based Chromosome Organization: New Insights From in Vitro Assays**

**T. Hirano**; Chromosome Dynamics Laboratory, RIKEN, Wako, Saitama, JAPAN.

Condensins are large protein complexes that play a central role in mitotic chromosome assembly and segregation in eukaryotic cells. Many eukaryotic species have two types of condensins, condensin I and condensin II, that share the same pair of SMC (structural maintenance of chromosomes) ATPase subunits and have distinct sets of non-SMC regulatory subunits. Despite recent progress, it remains obscure how condensins might work at a mechanistic level. My laboratory uses a pair of powerful in vitro assays for dissecting the pathway of mitotic chromatid assembly and the mechanisms of action of condensins. In the first assay, we use *Xenopus* egg cell-free extracts, and test the ability of recombinant condensin complexes to assemble mitotic chromosomes in the extract. A panel of mutant complexes is tested to examine the role of individual subunits and the SMC ATPase cycle. The use of unconventional substrates (e.g., mouse sperm nuclei) enables us to further expand the potential of this experimental system. In the second assay, we reconstitute mitotic chromosomes with a simple substrate and a minimum set of purified factors. This assay allows us to precisely dissect the functional interplay among histones, topoisomerase II and condensin I in this process. In the current presentation, I will discuss emerging lines of evidence that condensins are highly sophisticated protein machines that utilize multiple mechanisms including loop extrusionand dynamic protein-protein interactions.

## Symposium 5: What Blueprints Tell Us: How Genomics Informs Cell Biology

S11

**Centromeres: Scanning Genomes for Signs of Conflict**

**H. Malik**; HHMI & Fred Hutchinson Cancer Research Center, Seattle, WA.

Genetic conflicts take place between different genomes (e.g., host-virus interactions, mitochondrial conflicts with nuclear genomes) or between components of the same genome (e.g., chromosomal competition at centromeric regions). Understanding these "molecular arms races" and how they drive recurrent genetic innovation, can reveal important perspectives into both evolutionary and cellular biology. Studying a variety of ‘arms-races’ allows an explicitly broad evolutionary perspective onto the study of rapid evolution in genes involved in essential cellular processes such as cytoskeleton or chromosome segregation. Based on the unexpected discovery of rapid evolution of centromeric proteins in plants and animals, we first proposed the ‘centromere-drive’ model, in which centromeric DNA elements act as selfish genetic elements to exploit asymmetries in female meiosis (in which only one of four meiotic products is chosen) for their own transmission, even at great cost to host fitness. Several aspects of the original ‘centromere-drive’ have now been elegantly demonstrated by other labs, but the question of what drives rapid evolution of centromeric proteins remained unsolved. We employed a novel strategy using a gene-swap strategy in Drosophila, in which centromeric histones are reverted to an ancestral state, to study the causes and consequences of this rapid evolution in vivo. Furthermore, centromeric histone duplications that lead to gametic specialization further highlight the inherent differences in centromeric function in somatic versus germline cells in animals. Genetic conflicts at centromeres can also explain dramatic turnover in centromeric proteins, with evolutionarily young genes becoming essential for centromeric function whereas evolutionary old, essential genes can be

rendered dispensable. Finally, genetic conflicts during meiosis may provide a basis of postzygotic reproductive isolation between recently diverged species.

S12

**From Phenotypes to Pathways: Global Analysis of Cellular Networks Using Systematic Yeast Genetics and Single Cell Image Analysis**

**B. Andrews**; University of Toronto, Toronto, ON, CANADA.

A powerful method to study the genotype-to-phenotype relationship is the systematic assessment of mutant phenotypes using high-content screening and automated image analysis. We have developed a combined experimental-computational pipeline for analysis of the effect of genetic perturbations on subcellular compartments in yeast. Our approach involves using systematic genetic methods to introduce markers of various subcellular compartments into yeast mutant arrays, in order to identify comprehensive lists of genes involved in subcellular morphology. We describe a general computational pipeline for single cell image analysis to quantify penetrance of perturbations affecting the morphology of 18 sub-cellular compartments. To develop the pipeline, we first used four fluorescent markers that enable observation of the morphology of the dynamic subcellular structures associated with endocytosis, from the formation of early endocytic sites at the plasma membrane to the final destination compartment, the vacuole/lysosome. We examined ~5300 unique yeast genes, including both essential and non-essential genes, for roles in endocytic compartment morphology. Using our single cell image analysis, we identified 17 mutant phenotypes and ~1600 genes - a remarkable 30% of the yeast genome - whose perturbation affects at least one endocytic compartment. Numerous mutants were associated with multiple phenotypes, indicating that morphological pleiotropy is often seen within the endocytic pathway. Morphological profiles based on the 17 aberrant phenotypes were highly correlated for functionally related genes, enabling prediction of gene function. Incomplete penetrance was prevalent, and single cell analysis enabled exploration of the mechanisms underlying cellular heterogeneity, which include replicative age, organelle inheritance, and stress response.

## Symposium 6: Getting from Here to There: Individual and Collective Cell Migrations

S13

**Dendritic Cell Migration At Various Scales**

**A. Lennon-Duménil**; Institut Curie, France, Paris, FRANCE.

Dendritic cells are immune sentinels in charge of patrolling tissues and present the antigens they capture to T lymphocytes to initiate adaptive immune responses. Their immune-surveillance function heavily relies on cell migration. Here, I will discuss the findings that we have made studying the migration of dendritic cells at various scales. This includes the roles played by distinct subcellular actin pools and nucleators in (1) defining the migration mode and type of trajectories that dendritic cells harbor in different tissues, and (2) allowing their response to environmental cues such as physical confinement or hydraulic resistance. I will further show our results highlighting that dendritic cell migration and endocytic functions are coordinated, which facilitates their capacity to sample the environment and transmit the information collected to T lymphocytes. Finally, I will describe how dendritic cell migration can shape their identity and fate *in vivo*.

S14

**Collective Cell Movements: Cellular and Molecular Dynamics At the Leading Edge**

G. Scepanovic, **R. Fernandez-Gonzalez**; University of Toronto, Toronto, ON, CANADA.

Embryos repair wounds rapidly, with no inflammation or scarring, in a process driven by collective cell movements. Upon wounding, the cells adjacent to the wound polarize actin and the molecular motor non-muscle myosin II. Actomyosin polarization results in the assembly of a contractile cable at the wound edge that coordinates cell movements. Our lab is interested in (1) how does the actomyosin cable coordinate cell movements around the wound, and (2) what are the signals that trigger cytoskeletal polarization in the cells immediately adjacent to embryonic wounds. We recently showed that a heterogeneous actomyosin distribution at the wound edge is critical to coordinate cell movements. Non-uniform contractility at the wound margin generates mechanical signals (stress and strain), relayed by mechanically gated ion channels, that regulate myosin dynamics to drive rapid wound closure. In addition, we found that production of reactive oxygen species (ROS) upon wounding triggers cell polarization, in a process that involves oxidation of the tyrosine kinase Src. We are currently investigating other downstream targets of oxidative stress during embryonic wound repair. We found that the serine/threonine kinase p38 MAP kinase was activated in the cells immediately adjacent to embryonic wounds in *Drosophila*. Genetic or pharmacologic disruption of p38 resulted in an overexpansion of wounds and a reduction in the speed of cell migration that delayed wound repair. Strikingly, cytoskeletal polarization and force generation at the wound edge were not affected by p38 inhibition. Inhibition of the serine/threonine kinase TOR, a downstream effector of p38 that regulates protein synthesis and cell growth, reproduced the phenotypes obtained when p38 was disrupted, suggesting that p38 acts through TOR during embryonic wound repair. Together, our data indicate that p38, TOR signaling and cell growth may play unexpected roles in embryonic wound closure.

S15

**Mechanosensation of Tight Junctions by Zo-1 Phase Separation and Flow**

**C. Heisenberg**; IST Austria, Klosterneuburg, AUSTRIA.

Cell-cell junctions respond to mechanical forces by changing their organization and function. Tension-dependent conformational changes of junctional proteins are thought to underlie this junctional mechanosensitivity. Here we show that in the gastrulating zebrafish embryo, tight junctions (TJ) mechanosensitivity is mediated by actomyosin-driven flow of phase separated Zonula occludens-1 (ZO-1) clusters. We found that ZO-1 junctional accumulation at the contact between the Enveloping Layer (EVL) and the Yolk Syncytial Layer (YSL) closely scales with actomyosin tension. Actomyosin tension triggers ZO-1 junctional accumulation by driving retrograde actomyosin flow within the YSL that transport non-junctional ZO-1 clusters towards the TJ. Non-junctional ZO-1 clusters form by phase separation, and their effective formation is dependent on the actin binding region (ABR) within the C-terminus of ZO-1. If the non-junctional ZO-1 pool is absent, TJ lose their mechanosensitivity, and, consequently, EVL-YSL movement is impaired. Thus, phase separation and flow of nonjunctional ZO-1 confer mechanosensitivity to TJ.

## Symposium 7: Google Maps of the Cell: Controlling Intracellular Traffic Flow and Direction

S16
**Building Memories: Cell Biological Mechanisms Underpinning Synapse Assembly and Function**
**D. Colón-Ramos**; Yale University, New Haven, CT.

Synapses are cellular junctions between excitable cells. When, where, and how synapses form underpin the architecture of the nervous system, and behaviors. Synapses are both precisely assembled during development and flexible during learning and memory. How can synapses be both precise and malleable to facilitate both the assembly and function of the brain? How do fundamental cell biological properties of synapses underpin emergent properties of the nervous system, like memories and behaviors? In this lecture I will discuss recent data from my lab and the field on the use of model organisms, like C. elegans, to reduce systems level questions like development and behavior to cell biological questions at the neuronal synapse.

S17
**Slippery Cargo Takes Multiple Routes: Identifying Bottlenecks in Lipid Transport and Storage**
**E. Ikonen**; University of Helsinki, Helsinki, FINLAND.

Cell organization and membrane-related functions depend on the correct compartmentalization of lipids. Our research aims at uncovering key mechanisms that govern the trafficking and storage of major lipid species in human cells and elucidating disturbances in these processes that lead to human lipid storage diseases. We studied how lipid droplets are normally generated from the endoplasmic reticulum by using CRISPR-based endogenous protein tagging in combination with live cell imaging and correlative light electron microscopy (Salo et al., Dev Cell 2019). We also investigated this process by re-localizing lipid storage sites in ER subdomains and by manipulating specific proteins implicated in lipid droplet formation, regulation of ER morphology or lipid delivery. We recently developed a system for rapid inducible degradation of proteins of interest with minimal basal degradation in human cells (Li et al., Nat Methods 2019). This strategy is powerful e.g. for studying rapidly adaptive processes, such as lipid transport, that may harness compensatory routes.

## Symposium 8: D'Arcy Thompson at 100: Controlling Cell Shape and Function

S18
**Signals, Forces, and Cells: Decoding Tissue Morphogenesis**
**J. Zallen**; HHMI/Sloan Kettering Institute, New York, NY.

A major challenge in developmental biology is to understand how tissue shape and structure are generated by processes that occur on a cellular and molecular scale. A conserved feature of multicellular animals is a body axis that is elongated from head to tail. In Drosophila, this structure is actively generated in the embryo by the coordinated movements of hundreds of cells. We discovered that these movements are oriented by subcellular asymmetries in the localization and activity of proteins that regulate contractile and adhesive forces within cells. We also identified a positional code that

systematically orients cell movements throughout the embryo. This spatial guidance is provided by an ancient family of Toll-related receptors that are widely used for pathogen recognition by the innate immune system. Using live imaging and automated cell tracking, we found that these molecular asymmetries produce dynamic rosette structures that form and resolve directionally, driving tissue elongation. Planar polarized actomyosin contractility and rosettes have now been shown to promote tissue elongation in flies, chicks, frogs, and mice, and form a general mechanism linking cellular asymmetry to tissue organization. We are currently using genetic, cell biological, biophysical, and live imaging approaches to understand how genes encode the forces that shape tissues, and how these forces modulate cell behavior.

S19

**How Cell Shape Arises - the Minimal, Self-propagating Systems that Create Rod Shaped Cells and Determine Their Width.**

**E. Garner**; Harvard University, Cambridge, MA.

It is not known how cells grow in reproducible shapes with defined dimensions. The simplest advanced shape (containing 2 axes) is a rod, a geometry seen in all kingdoms. We study rod shape formation in bacteria, as only a handful of proteins are required to build these shapes. Bacterial shape is determined by the cell wall, a single cross-linked macromolecule, and to elongate in these defined shapes, new material must be added into this structure in a spatially controlled manner. Two distinct enzymatic systems mediate rod-shaped growth: The Rod complex moves around the cell circumference, while class A penicillin-binding proteins (aPBPs) do not.In order for biological systems to construct long-range shape, small proteins must be able to sense and respond to the much larger cellular geometry. Here this is accomplished by MreB, an actin homolog that polymerizes into inwardly curved filaments that bind to the membrane. MreB filaments are pulled around the cell by the activity of the associated cell wall synthesis enzymes. We find that a key feature of MreB is that it acts as short axis sensor, pointing along the greatest inward membrane curvature, constraining filaments and associated enzymes to move around the rod width. MreB motions are circumferentially oriented in rods, yet isotropic in spheres.We next wanted to understand MreB creates rod shape and determines their width. Rod width is not determined by properties within MreB filaments, as previously proposed, rather it depends on the balance between the two systems: the Rod system reduces diameter, while aPBPs increase it. As Rod complex levels increase and cells get thinner, and we see an increased density of moving MreB filaments and a higher fraction of directionally moving enzymes. This increased circumferential synthesis increases the amount of oriented material within the cell wall, which increases its structural anisotropy: in response to internal turgor pressure, cells stretch less across their width (reinforcing rod-shape) instead, stretching more along their length (causing rod-shaped growth).Thus, the orienting of MreB filaments to the greatest curvature, coupled with cell-wall reinforcing synthesis, creates a geometric and mechanical feedback loop that reinforces existing rod shape. This local feedback also allows the creation of rod shape from spheres, as oriented MreB motion arises in small bulges on the sphere surface attract oriented MreB motion, causing that bulge to rapidly elongate into an emerging rod.

## MAC Mentoring Keynote

A1
**It's Personal**
**D. Asai;** Senior Director for Science Education, Howard Hughes Medical Institute, Bethesda, MD.

Who gets to participate in science? The "mismatch effect" plays an outsized and poorly understood role in our nation's debate about race, access, inclusion, and excellence. I plan to examine the idea of "mismatch" and offer my personal views on how mismatch is being misused.

## E.E. Just Award Lecture

A2
**Regenerative Engineering of Complex Musculoskeletal Tissues**
**C. Laurencin**; University of Connecticut School of Medicine, Farmington, CT.

We define the new field of Regenerative Engineering as the Convergence of Advanced Materials Science, Stem Cell Science, Physics, Developmental Biology, and Clinical Translation. Work in the area of musculoskeletal tissue regeneration has focused on a number of technologies. Polymeric nanofiber systems create the prospect for biomimetics that recapitulate connective tissue ultrastructure allowing for the design of biomechanically functional matrices, or next generation matrices that create a niche for stem cell activity. Polymer and polymer-ceramic systems can be utilized for the regeneration of bone. Through the use of inducerons, small molecules fostering induction, the design of regeneration-inducing materials can be realized. Hybrid matrices possessing micro and nano architecture can create advantageous systems for regeneration, while the use of classic principles of materials science and engineering can lead to the development of three dimensional systems suitable for functional regeneration of tissues of the knee. Advances in stem cells science present new possibilities for disease treatment, while discoveries in developmental biology in concert with other advances in science offer fascinating new directions. Through convergence of a number of technologies, we believe the prospect of engaging future grand challenges is possible.

## Keith R. Porter Lecture

A3
**Speed, Direction And Persistence**
**J. A. Theriot**; University of Washington, Seattle, WA.

How is a cell created from its molecular constituents? Proteins are typically only a few nanometers in size; other individual cellular components such as lipid molecules can be even smaller. Without a blueprint or an architect, these tiny molecular parts organize themselves in a dynamic and self-correcting manner to form precise cellular structures that may span tens or hundreds of micrometers, a scaling of four or five orders of magnitude. Furthermore, cell organization is extremely dynamic; a cell with a given complement of molecular components can assume a mind-boggling variety of shapes with internal structural variations, and can dramatically alter its shape and movement behavior in response to changes in its environment with astonishing speed. Although the large-scale structure and mechanics

of the cell must be somehow encoded in the physical properties of its constituents, a functional living cell cannot (yet) be reconstituted simply by mixing together the appropriate components. Understanding the proper arrangement of macromolecules in cells and the large-scale coordination of their functions requires the discovery of organizational principles and mechanisms that work at a cellular scale. The research of my group explores the mechanics and dynamics of cell self-organization and movement in a variety of cells ranging from bacteria to human neutrophils. By studying diverse questions in diverse biological systems, using both bottom-up approaches (biochemical reconstitution, single-molecule force measurements, mathematical modeling) and top-down approaches (genetic and pharmacological perturbations in live cells, quantitative video-based analysis of cell movement, shape, and mechanical coupling), we aim to develop a broad conceptual understanding of the organizational rules and physical principles that give rise to large-scale cell structure and coordinated movement. One specific biological system where this approach has borne fruit is the study of force generation and motility driven by assembly of dendritically branched actin networks, which drive complex forms of motility in one dimension (comet tails associated with bacterial pathogens such as *Listeria monocytogenes*), two dimensions (flat lamellipodia of fish epidermal keratocytes), and three dimensions (protrusions of human neutrophils in collagen matrices). For these systems, we have elucidated the principles that translate microscopic parameters such as the association rate of actin monomers onto the ends of growing filaments into large-scale properties of cell movement including speed, direction and persistence.

## Bruce Alberts Award for Excellence in Science Education

A4

**"Show Me The Data" Building And Supporting A Community Of Biology Education Researchers To Maximize Student Learning**

**M. Wenderoth**; University of Washington, Seattle, WA.

To build strong and productive academic networks that advance knowledge, people need to meet face-to-face in a welcoming environment. In 2009, that environment did not exist for those conducting research on how students learned in college biology classes. Instead, these researchers were scattered across the societies that represented the 17 different subdisciplines of Biology. Diversity is the basis of success in biological systems but this diversity was a barrier to growth of Biology Education Research. There was a journal, CBE-LSE, for publishing our work, but we lacked a venue to showcase our recent findings. Therefore, three faculty wrote a NSF grant to fund an initial meeting of 25 individuals to discuss forming a new society. The group created the vision, goals, name, (Society for the Advancement of Biology Education Research: SABER) and motto ("Show Me the Data") for the society. Since then SABER has held annual meetings every year and attendance has grown from 130 to 425. But the hallmark of SABER is the rigorous research conducted by its members. This research focuses on collecting data to determine how teaching interventions improve academic performance and increase retention of a diversity of students. As an example of this type of work, I will discuss the meta-analysis of active learning studies that Scott Freeman lead and I was a co-author on. However, if these research findings are not implemented in the classroom, our research will be for naught. Therefore, I will describe my current effort at the UW to encourage and support faculty in STEM to implement evidence-based teaching methods. This NSF funded effort, CAUSE: Consortium for the Advancement of Undergraduate STEM Education enrolls three faculty from seven different STEM departments each year for three years.

Each faculty cohort receives feedback on their teaching by analyzing class videos with PORTAAL, a classroom observation tool and on differential student academic performance by analyzing exam scores. With this type of feedback, faculty make data informed decisions about their teaching methods.

## EMBO Gold Medal

A5
**Proteomes In 3D**
**P. Picotti**; ETH Zurich, Zurich, SWITZERLAND.

Protein structural changes induced by external perturbations or internal cues can profoundly influence protein activity and thus modulate cellular physiology. In my talk, I will present a recently developed structural proteomics method that enables analysis of protein structural changes on a proteome-wide scale and directly in complex biological extracts. The approach, termed LiP-MS, relies on the generation of mass spectrometric fingerprints for specific structural states of a given protein. LiP-MS can detect subtle alterations in secondary structure content, larger scale movements such as domain motions, and more pronounced transitions such as the switch between folded and unfolded states. Further, it can pinpoint regions involved in the structural transitions with an average resolution of ten amino acids. I will describe how we are applying this approach to study the molecular bases of protein aggregation diseases and to the identification of protein-small molecule interactions (e.g drug targets). I will also propose that monitoring protein structural states on a proteome-wide scale can serve as a new powerful readout to pinpoint altered protein functional states and the (de)regulation of biochemical pathways. Last, I will discuss the power and limitations of the new approach.

A6
**Data Science Approaches To Biological Systems**
**M. Madan Babu**; MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM.

Over the last decades, we have witnessed an explosion in the quantity and diversity of information describing biological systems. My group is interested in understanding the molecular basis of life through data driven approaches. To this end, we develop computational methods to integrate, mine, and analyse biological data to uncover general principles, generate experimentally testable hypotheses and make specific biological discoveries. We also carry out experimental work to generate large-scale data for our research and to test the principles that we have discovered. In the first part of my talk, I will discuss our work on G-protein coupled receptors, which regulate virtually every aspect of human physiology and are a major drug target. Specifically, I will discuss our work on activation and selectivity in the GPCR signalling system. I will then present our recent work integrating population-level data of millions of polymorphisms with atomic-level data of GPCR structures to investigate GPCR pharmacogenomics. I will highlight how mapping polymorphism data onto structures of molecular machines can provide mechanistic insights into biochemical and phenotypic variation in biological systems. In the second part of my talk, I will present our work on disordered protein regions, which constitute over 40% of eukaryotic proteomes. Specifically, I will discuss how disordered regions can increase phenotypic complexity and contribute to diseases such as cancer. I will present IDR-Screen, which is a high throughput experimental approach to discover functional disordered regions from large

libraries of sequences. I will emphasise how machine learning on these data can help us learn rules that make disordered regions functional. This can be exploited for synthetic biology and to interpret impact of mutations in disordered segments. We are living in an exciting time with access to unprecedented amounts of biological information. This allows us to investigate fundamental questions of a different magnitude and kind that is complementary to conventional approaches. I will conclude my presentation by highlighting that the time is right for a data-science based approach to unravel the complexities of living systems with direct implications not only for understanding basic biology but also for biotechnological applications, medicine, human health and society.

## E.B. Wilson Medal Presentation and Address

A7

**Excitable Networks In Directed Cell Migration**

**P. Devreotes**; Johns Hopkins University School of Medicine, Baltimore, MD.

**Excitable Networks in Directed Cell Migration**Directed cell migration is critical for an extensive range of physiological events. Duringdevelopment, concerted cellular movements bring form to the embryo and, in the adult, migrationis critical for immune response, wound healing, stem cell homing, and neuronal wiring. Whenthese orchestrated movements occur improperly or are subverted, disease results. The molecularcomponents involved in cell migration are remarkably conserved between the social amoeba,*Dictyostelium* and mammalian cells. It is generally believed that cytoskeletal activities driverandom cell migration whereas signal transduction events initiated by receptors regulate thecytoskeleton to guide cells. However, using amoebae, neutrophils, and mammary epithelial cells,we found that the cytoskeletal network, involving SCAR/WAVE, Arp 2/3 and actin-bindingproteins, is capable of generating only rapid oscillations and undulations of the cell boundary. Thesignal transduction network, comprised of multiple pathways including Ras GTPases, multiplephosphoinositides, and Rac GTPases, is required to generate the sustained protrusions ofmigrating cells. The signal transduction network is excitable, exhibiting wave propagation,refractoriness and maximal response to suprathreshold stimuli, even in the absence of thecytoskeleton. We suggest that cell motility results from coupling of ‘pacemaker’ signaltransduction and ‘idling motor’ cytoskeletal networks. We have been able to exploit the excitablenature of the signaling network to force cells to assume different morphologies and modes ofmigration from amoeboid to keratinocyte-like to oscillatory. The application of these concepts tothe diverse migratory profiles exhibited by different cells and the ability of cells to detect andintegrate extracellular cues is discussed.

## Subgroup A: Biological Timing: Molecular Clocks and Timers, from Systems to Synthetic Biology

SG1

**Synchronization of the Cell Cycle in *Drosophila* Embryos**

**S. Di Talia**; Duke University, Durham, NC.

Embryonic development requires a high degree of spatiotemporal coordination. I will describe my lab efforts to understand the synchronization of the cell cycle in early Drosophila embryos . I will show how synchronization of the cell cycle in Drosophila embryo is linked to precise nuclear positioning, which is in turn driven by cytoplasmic flows generated by actomyosin cortical contractions. I will also discuss our work on size control of the Histone Locus Body, a phase separated droplet regulating histone biogenesis, in Drosophila embryos.

SG2

**How to Set and Rewind the Clock of Centriole Formation: Autonomous Oscillators that Control Organelle Biogenesis.**

M. Mofatteh, **M. G. Aydogan**, F. Y. Zhou, S. Saurya, M. A. Boemo, J. W. Raff; University of Oxford, Oxford, UNITED KINGDOM.

In order for a cell to divide and produce two daughters who can go on to divide again, it must accomplish sufficient biosynthesis to provide the components essential for each of its progeny. Such metabolic processes must be completed before division occurs - so the accurate timing of organelle biogenesis is crucial for cell physiology. To investigate the physical and molecular principles that regulate this, we have recently established a non-membranous organelle, the centriole, as a model system in syncytial *Drosophila melanogaster* embryos. Employing a set of in-house, imaging-based biophysical assays, we found that Polo-like kinase 4 (Plk4) localises to the base of growing centrioles in an oscillatory fashion that appears to determine the time of centriole formation during the cell cycle. In contrast to the current paradigm of how the cell cycle works (i.e. the “ratchet" model), we discovered that Plk4 could continue to oscillate and drive cycles of centriole formation even in the absence of a robust Cdk/Cyclin cell-cycle oscillator. Our efforts in mathematical modelling and biochemical perturbation experiments indicate that these oscillations are generated in a circuit where Plk4 binds and multiply phosphorylates its receptor Asterless (Asl) at the centriole, which in turn renders the receptor unable to bind further Plk4. The ability to initiate the oscillation at the next cycle, therefore, seems to require a non-phosphorylated pool of the receptor. This may be feasible by a “re-setting” factor/event that operates out-of-phase with the Plk4 oscillations (e.g., a phosphatase) and functions to replenish the necessary “active” pool of the receptor. Here we will present our strategy and work so far, that helps us explore this factor, as well as the preliminary data that allow narrowing the list of candidates.

SG3

**E2F-dependent Genetic Oscillators Control Endoreplication**

**M. Kim**, N. Moon; McGill University, Montreal, QC, CANADA.

Polyploidy is an integral part of development and is associated with cellular stress, aging and pathological conditions such as cancer. Across many organisms, the endoreplication cycle, comprised of

successive alternations of G and S phases without cell division, is commonly employed to produce polyploid cells. To maintain the endocycle, oscillation of Cyclin E (CycE)/Cdk2 activity is required, where low Cdk2 activity during G phase allows pre-replicative complex (pre-RC) formation and high Cdk2 activity triggers origin firing. Previous studies have identified E2F1 as one of the core molecular machineries that drives the biphasic G-S oscillations. In this study, we provide mechanistic insight on how endoreplication cycles are maintained by E2F proteins. Specifically, we show that the biphasic oscillation of E2F1 and CycE requires the recently described alternate isoform of E2F1, E2F1b, which is necessary for development of polyploid tissues in Drosophila. The impact of E2F1b deregulation was analyzed using the Fluorescent Ubiquitination-based Cell Cycle Indicator (FUCCI) in actively endoreplicating larval salivary glands. Genetic experiments revealed that E2F1b regulates the circuitry of timely S phase entry and exit during endoreplication by activating a subset of E2F-target genes. E2F1b regulates the Drosophila ortholog of CDK inhibitor Dacapo (Dap) to precisely time S phase entry by controlling the CycE/Cdk2 activity threshold. Dap-mediated inhibition of CycE/Cdk2 allows G phase cells to accumulate CycE and pre-RC components, such as Double Parked/Cdt1, prior to entering S phase. Upon entry to S phase, E2F1b-dependent burst of Proliferating Cell Nuclear Antigen (PCNA) expression establishes a negative feedback loop through the PIP box-mediated degradation of E2F1. This mechanism is critical for ensuring proper downregulation of E2F1 target genes and S phase exit. Overall, our study uncovered a network of E2F-dependent genetic oscillators that are critical for the periodic transition between G and S phases during endoreplication.

SG4

**Programming Bacteria in Time and Space**

**L. You**; Duke University, Durham, NC.

Microbes are by far the most dominant forms of life on earth. In every imaginable habitat, they form complex communities that carry out diverse functions. Microbial communities drive the geochemical cycling of diverse chemicals and through these activities shape the earth's climate and environment. They are also intimately tied to human physiology and health. Members of each microbial community may compete for resources, collaborate to process the resources, or to cope with stress. They communicate with each other by producing and responding to signaling molecules. And they innovate by exchanging genetic materials. These interactions raise fundamental questions regarding the evolutionary and ecological forces that shape microbial consortia. Our lab has adopted a combination of quantitative biology and synthetic biology to explore these questions. We engineer gene circuits to program dynamics of one or more Escherichia coli bacterial populations, and use them to examine questions in cellular signal processing, evolution, ecology, and development. Analysis of these systems have provided insights into bacterial tolerance to antibiotics, developmental pattern formation and scaling, as well as strategies to use bacteria to fabricate functional materials.

SG5

**Rhythmic Replication in Cyanobacteria**

**M. Rust**, Y. Liao; University of Chicago - Chicago, IL, Chicago, IL.

Circadian rhythms are biological oscillations that organize gene expression, metabolism, and physiology across all kingdoms of life to match the demands of the day-night cycle. In many cases, the mechanisms by which these rhythms support the health of the organism are unclear, even in cyanobacteria, the

organisms with the simplest known circadian clock. Here, we interrogate the role of the clock in controlling initiation of DNA replication, an essential cellular process that also has the property that, once initiated, requires permissive conditions over a sustained window of time to allow successful completion. Using direct imaging of nucleotide incorporation in single cells, as well as live cell imaging of components of the replication fork, we identify a role for the circadian rhythm in ensuring that initiation occurs at appropriate times and that replication can successfully complete. We speculate that under natural conditions where the threat of DNA damage may be prevalent, rhythmic control of replication may be a key function of the circadian clock.

SG6

**Localization of *frequency* mRNA in Biomolecular Condensates Contributes to Period Length Determination in the *Neurospora crassa* Circadian Clock**

**B. Bartholomai**[1], A. Gladfelter[2], J. Loros[1], J. Dunlap[1]; [1]Geisel School of Medicine at Dartmouth, Hanover, NH, [2]University of North Carolina, Chapel Hill, NC.

Circadian rhythms in animals and fungi are generated by a transcription - translation negative feedback loop. In *Neurospora crassa,* the WCC, a complex of WHITE COLLAR - 1 (WC-1) and WC-2, is the major transcription factor on the positive arm of the clock, while a multi-protein complex nucleated by FREQUENCY (FRQ) acts on WCC to repress its function. This feedback loop requires approximately 24-hours for a complete cycle, and has been extensively studied at the tissue, organismal, and population level for several decades using biochemical and genetic techniques. Spatiotemporal dynamics of core clock molecules at the cellular level, however, have remained elusive. In particular we are interested in the mechanism underlying the approximately 4 hour delay between peaks of *frq* mRNA accumulation and expression of FRQ protein, a delay that contributes to the long 24 hr circadian period length. An additional spur arose from recent extensive circadian transcriptomics and proteomics data that revealed unexpectedly large temporal delays between transcription and translation: a quarter of the proteome is circadianly regulated with a median delay between RNA peak and protein peak of ~10 hrs. We became curious as to how mRNA could be protected from degradation long enough to facilitate these temporal delays. We have identified a novel RNA binding protein that undergoes liquid-liquid phase separation, interacts with *frq* mRNA, and plays a role in the observed perinuclear enrichment of *frq* mRNA. The RNA-binding protein shows heterogeneous distribution throughout the cytoplasm and displays dynamic liquid like behavior in live-cell imaging. In knockouts lacking the protein, the heterogeneous perinuclear localization of the *frq* transcript is lost and a significant lengthening of the circadian period is observed. We hypothesize that sequestration of clock related transcripts through phase separation could facilitate local translation and/or protection of transcripts. Additionally, sequestration of circadian mRNA in biomolecular condensates could contribute to temporal delays between mRNA expression and protein accumulation, subsequently affecting the overall temporal mechanism of the clock.

SG7

**Distinguishing Dormant From Dead with Yeast Spores**

T. Maire, T. Allertz, M. Betjes, **H. Youk**; Delft University of Technology, Delft, NETHERLANDS.

How a ceased life can resume and why it is not dead are fundamental questions. Dormant microbial spores, whose lives have completely halted and thus appear dead, are well-suited for addressing these questions at the cellular level. We uncovered quantifiable, varied capacities among dormant yeast-

spores for waking-up (germinating) that also forecast their lifespans, thus revealing how dormancy progressively transitions to death. Different glucose concentrations germinate distinct percentages of spores, with low glucose concentrations priming un-germinated spores to accelerate their germinations days later. A GFP-inducible synthetic circuit shows that inducing transcription and translation in dormant yeast-spores is possible without nutrients. Dormant yeast-spores with higher GFP-expressing (gene-expressing) abilities are more likely and require less glucose to germinate. Spores' gene-expressing abilities progressively decrease over time without nutrients, eventually increasing glucose concentration required for germination beyond saturating levels, thus prohibiting germination (dying). Spores' deaths are predictable days-to-months in advance, with higher gene-expressing abilities now meaning longer lifespans. Our findings redefine dormancy as a measurable spectrum between replicative life and death, and motivate a rethinking of gene-regulations in dormant spores and cells.

SG8

**Quantitative Evidence for a Cargo-sensitive Checkpoint in Clathrin-mediated Endocytosis**

**R. T. A. Pedersen**, J. E. Hassinger, P. Marchando, D. G. Drubin; University of California, Berkeley, Berkeley, CA.

Clathrin-mediated endocytosis (CME) involves the coordinated assembly of over 50 different proteins that together reshape a portion of the plasma membrane into an internalized vesicle. A detailed timeline of the arrival of a large number of the proteins involved in CME has been traced and the locations and functions of these proteins at endocytic sites have been uncovered in studies of the budding yeast *Saccharomyces cerevisiae*. However, questions remain about how progression through the endocytic pathway is regulated and how collection of cargo is coordinated with vesicle internalization. To address these questions, we used two-color total internal reflection fluorescence microscopy to systematically analyze the dynamic recruitment and turnover of more than a dozen proteins involved in CME in *S. cerevisiae*.We found significant variability in protein lifetime and abundance at endocytic sites, revising the view of the field that CME in budding yeast is extremely regular. We exploited this variability to gain new insights into the mechanism of CME. While the lifetimes of early arriving CME proteins were positively correlated with one another, they had little to no correlation with the behavior of later-arriving CME proteins such as actin. Conversely, lifetimes of late-arriving CME proteins are positively correlated with the lifetime and abundance of endocytic actin. These data are quantitative evidence for a mechanistic transition point in the CME pathway, with events before the transition point having little bearing on events after it.We demonstrate that the rate of maturation through the transition point is accelerated by the presence of CME cargos, suggesting that the transition represents a cargo checkpoint. Many membrane-associated CME cargos are delivered to the plasma membrane through exocytosis. CME site maturation is faster in subcellular locations where exocytosis, and hence CME cargo, is naturally concentrated. When exocytic vesicles are redirected to subcellular locations where exocytosis does not normally occur, maturation of nearby CME sites through the checkpoint accelerates. This spatial variation in CME site maturation rate explains the long-standing observation that “actin patches” (late CME sites) are polarized in *S. cerevisiae.* Mechanisms of CME cargo sensing are currently unclear but present an exciting avenue for future research.

SG9

**Stochastic Activation and Bistability in a Rab GTPase Regulatory Network**

U. Bezeljak[1], H. Loya[2], T. Saunders[3], **M. Loose**[1]; [1]Institute of Science and Technology Austria, Klosterneuburg, AUSTRIA, [2]IIT, Bombay, INDIA, [3]National University Singapore, Singapore, SINGAPORE.

**Rab GTPases are the central regulators of intracellular traffic. Their function relies on their conformational change triggered by nucleotide exchange and hydrolysis. While this switch is well understood for an individual protein, how Rab GTPases collectively transition between different states is not known. Here, we combine in vitro reconstitution experiments with stochastic reaction-diffusion modeling to study the non-equilibrium properties of the minimal Rab5 activation network. We find that collective switching of Rab5 relies on a positive feedback and is triggered by intrinsic noise due to low amounts of Rab5[GDP] preexisting on the membrane. We show that this inactive population is not only the source of stochastic behavior of this biochemical circuit, but can act as a control parameter for Rab5 activation.**

SG10

**An Increase in the Duration of An Extended G1 Period Enables Stochastic Competition between Terminal Cell Differentiation and Cell-cycle Re-entry**

M. L. Zhao, A. Rabiee, K. M. Kovary, B. Taylor, Z. Bahrami-Nejad, **M. N. Teruel**; Stanford University, Stanford, CA.

Terminal cell differentiation is essential for developing and maintaining tissues in all multi-cellular organisms and typically requires that proliferating progenitor cells exit the cell cycle. However, the relationship and timing between the underlying opposing processes, proliferation and terminal differentiation, is not yet understood. Using adipogenesis as a model system for terminal cell differentiation, we delineate the timing between cell-cycle exit and terminal differentiation by live-cell imaging of cell-cycle reporters and expression of PPARG, a master regulator of differentiation. We show that, during terminal cell differentiation, the levels of PPARG and of the CDK inhibitor p21 are coupled, and both gradually increase after mitosis. Expression of p21 regulates the duration of a variable extended G1 phase that allows some cells sufficient time to reach a PPARG threshold for differentiation instead of entering the next cell cycle when PPARG is again suppressed. By way of regulating the duration of an extended G1 phase during terminal differentiation, progenitor cells can stochastically control the number of final cell divisions and thus, the total number of differentiated cells.

SG11

**Signaling Dynamics in the Control of Vertebrate Mesoderm Segmentation**

**K. Sonnen**; Hubrecht Laboratorium, Utrecht, NETHERLANDS.

How information is transmitted between cells to govern development and tissue homeostasis in time and space remains a central question in biology. In particular, the role of signaling dynamics in this control is still largely unknown. During embryonic development timing of mesoderm segmentation is coordinated by a molecular clock, which is composed of signaling oscillations of the Notch, Wnt and FGF pathways in mice. While gain- and loss-of-function experiments have traditionally been applied to study the function of signaling pathways in developing organisms, manipulation of the dynamics *per se* has been difficult to achieve. To be able to functionally investigate signaling oscillations, we established a

microfluidic system, which allows entrainment of endogenous signaling oscillations in the developing PSM to periodic application of pathway modulators. Combined with real-time imaging of dynamic signaling reporters this approach enables the functional dissection of complex dynamic signaling networks in a multicellular context. Here, I will present our findings on the control of mouse segmentation by the interaction between multiple dynamic signalling pathways.

SG12

**Lighting Up Single-cell Transcriptional Dynamics in the Vertebrate Segmentation Clock**

**H. G. Garcia**[1], E. Eck[1], D. Soroldoni[2], A. Oates[2]; [1]UC Berkeley, Berkeley, CA, [2]École Polytechnique Fédérale de Lausanne, Lausanne, SWITZERLAND.

An abiding mystery in nature is how a single cell develops into a multicellular organism. One of the great achievements of genetics has been the identification of the regulatory molecules that underlie these developmental programs. However, the accumulation of regulatory molecules and network connections has been mostly descriptive and has not been matched by a concomitant effort aimed at predictively understanding cellular decision-making in developing embryos. Indeed, we remain incapable of predicting the development of animal morphology from knowledge of regulatory network diagrams. This limitation stems in great part from the fact that technologies to visualize animal development based on fixed embryos and slowly maturing fluorescent proteins give incomplete, often static, information about the protein and transcriptional dynamics that underlie developmental processes.To establish a predictive understanding of the gene regulatory networks that govern vertebrate body plans, we focus on the segmentation clock in zebrafish, in which the number of body segments is determined by an oscillatory gene network. We report on the implementation of the MS2 system to visualize transcriptional initiation at the single-cell level in living zebrafish embryos as development takes place. Specifically, we quantify the transcriptional dynamics of the *her1* gene, one of the main components of the segmentation clock, and show how tissue-level oscillations in Her1 protein arise from coordinated bursts in *her1* transcriptional activity. The new data afforded by these techniques will inform the development of theoretical models that predict how molecular interactions lead to oscillations with a prescribed period and amplitude. These predictions will be tested through “proof by synthesis” via the creation of synthetic oscillators with engineered dynamical properties that will be used to generate embryos with altered body plans. We envision that these iterations of model and experiment will set the stage for a new paradigm in synthetic biology that enables the rational design of multicellular organisms.

SG13

**Sequential Nuclear Protein Titration as a Timer in Early Vertebrate Development**

T. Nguyen, E. Costa, A. Amodeo, **M. Wühr**; Princeton University, Princeton, NJ.

The large frog oocyte contains an equally large nucleus, thereby conserving the typical 1/10 nucleocytoplasmic (NC) volume ratio. However, upon fertilization the NC ratio drops to ~1/1,000,000. During the following 12 rapid early cleavage divisions in which transcription is silenced, the NC-ratio exponentially increases to a value similar to the oocyte. Nearly 40 years ago, Newport and Kirschner proposed that a cytoplasmic factor is titrated onto the exponentially increasing DNA to initiate the maternal-zygotic transition. Since then, several proteins have been suggested as candidates for this model, but many questions remain. To obtain an unbiased overview of which proteins titrate from the

cytoplasm to the nucleus, we developed a proteomics assay that quantifies nuclear composition during early frog development for thousands of proteins, we observe that the maternally deposited nuclear proteins sequentially enter embryonic nuclei at distinguishable times. Many key regulators of embryonic transcription, including transcription factors and RNA polymerases enter the nucleus sequentially with some entering at lower and others requiring higher NC ratios. This suggests a novel timing mechanism for ordering their targets' downstream transcripts. We hypothesize that the differential timing of nuclear entry results from differential binding strength to either importins or DNA. We aim to quantify the affinities of nuclear proteins to importins and to DNA on a proteome-wide scale to test these hypotheses. Overall, we have discovered that nuclear composition changes reproducibly as a function of increasing NC-ratios. We propose that the embryos utilize this differential nuclear composition to govern transcriptional output and ultimately cellular decision making.

SG14

**Investigating the Coordination of Global Transcriptional Scaling with Cell Size and Growth**

**M. P. Swaffer**[1], G. Marinov[1], H. Zheng[2], W. Greenleaf[1], R. Reyes[2], J. Skotheim[1]; [1]Stanford University, Stanford, CA, [2]McGill University, Montreal, QC, CANADA.

Cell size varies widely amongst cell types or environmental conditions, and during the life cycle of a proliferating cell its size will increase, on average, twofold. However, how the basic biosynthetic process of the cell, such as transcription, adapt to account for changes in volume has remained poorly understood. More specifically, it was first described in the 1970s that as cell increase in size the rate of RNA-synthesis per gene increases in proportion to cell size - this size-scaling of RNA-synthesis ensures RNA and protein concentrations are kept constant and is likely critical for maintaining biochemical reaction rates and setting the cellular growth rate. Despite the apparent importance of this phenomenon, the molecular basis of this size-scaling control has remained unexamined.Using a combination of ChIP-seq, chromatin-proteomics, and super-resolution (PALM) single-molecule imaging, we have determined that the RNA polymerases occupancy per gene increases across the genome as cell size increases. Furthermore, this increase is not correlated with any major change in histone occupancy or chromatin accessibility. Consistent with this, most histone modifications measured are unchanged with the exception of those downstream of the elongating polymerase.Nearly all transcription factors examined, including the major subunits of the RNAPII Pre-Initiation complex (PIC), increase their fractional occupancy on the genome in proportion to size, concomitant with the increased polymerase occupancy. Given that we have also observed that the amount of polymerase and transcription-factor molecules per cell is strongly correlated with cell size, we considered whether a critical subunit of PIC was limiting for transcription such that as the amount of this factor increases so would global transcription rates. To test this idea, we have measured polymerase occupancy after local perturbation of the nuclear concentrations to PIC components. Based on these data we propose a working model where global transcription rates are set via the combinatorial amount of multiple different PIC subunits - each of which is partially limiting.

## Subgroup B: Building the Cell

SG15

**Decoding the Variance in Intracellular Organization of the Undifferentiated hiPS Cell**

**M. P. Viana**, S. M. Rafelski, .. Allen Institute for Cell Science; Allen Institute for Cell Science, Seattle, WA.

The Allen Institute for Cell Science is developing a state space of structural signatures of the undifferentiated human induced pluripotent stem cell (hiPSC) to understand the principles by which cells organize and transition between states (cellular morphogenesis). To do this we take advantage of the ~35 endogenous fluorescently tagged hiPSC lines in the Allen Cell Collection (www.allencell.org), each expressing a monoallelic EGFP-tagged protein representing a particular organelle or structure. We develop image-based assays and segmentation algorithms for quantitative analyses, taking advantage of thousands of replicate high resolution 3D images for each structure. We are investigating biological sources of cellular variation to identify the basis functions of a dimensionally-reduced, interpretable parameter space that represents integrated intracellular organization. We prototyped an analysis workflow based on the nucleus, using lamin B1, as our first key cellular structure. We used the Allen Cell Structure Segmenter to create accurate 3D segmentations of the nuclei in multi-hour 3D timelapse movies. We fit these extracted nuclear shapes using spherical harmonic functions. We analyzed the contributions of the spherical harmonic coefficients to the variations in nuclear shapes and found that nuclear shape could be well described by five coefficients representing three distinct shape modes. Each nuclear shape mode represented a different source of biological variation in hiPS cell colonies and occurred on a distinct timescale. The mode represented nuclear volume, which increases throughout interphase, occurring over a one day timescale. The second mode represented how flat (vs. round) a nucleus appeared in the apical-basal axis (Z-direction). This nuclear ‘flatness’ was linked to differences in cell packing in distinct regions of the colony, i.e., more densely packed regions were composed of taller cells with rounder nuclei, when compared to the less densely packed, shorter cells with flatter nuclei that were often found in the center of colonies. Individual nuclei exhibited little variation in flatness over a multi-hour time period, consistent with the longer timescale of cell packing within colonies that occurs over several days. The third mode represented how ‘squeezed’ a nucleus appeared in the XY plane; this arose from constant interactions between neighboring cells occurring at timescales of minutes or less. We are now applying these analyses to develop biophysical models of nuclear shape and colony dynamics. This general analysis framework will be extended to cell shape and to each of the key intracellular structures in an integrative fashion.

SG16

**Mapping the Spatial Organization of Genomes through Data Integration**

**F. Alber**, N. Hua, A. Yildirim, L. Boninsegna, Y. Zhan; University of California Los Angeles, Los Angeles, CA.

The spatial organization of the genome plays a key role in the regulation of gene expression and cell differentiation. One of our goals is to unite genomics with microscopic data to acquire a more complete insight about the basic principles governing chromosome folding and the partition of genomic regions into nuclear bodies, which drive the spatial organization of genomes. An accurate description of the 3D structure and dynamics of entire genomes requires a combination of complementary data and methods. As part of a joint analysis project we have developed computational methods for integrating varied

experimental data sources to produce quantitative models of the nuclear organization. Here, we will present an overview of our efforts and perform a comparative structure analysis of genomes in three different human cells. We will analyze the spatial partition of chromatin into functional subnuclear compartments and discuss cell-type specific structural features. Our analysis allows insights into the guiding principles of genome organization and its functional relevance.

SG17

**Numa Is Required for the Formation of a Single Nucleus After Mitosis**

**A. Serra-Marques**[1], R. Houtekamer[1,2], D. C. Hintzen[1,3], S. Dumont[1]; [1]University of California, San Francisco, San Francisco, CA, [2]University of Utrecht, Utrecht, NETHERLANDS, [3]The Netherlands Cancer Institute, Amsterdam, NETHERLANDS.

Eukaryotic cells pack their genome into a single nucleus at the end of mitosis, and failure to do so leads to DNA damage and loss of genomic integrity. However, how mammalian cells form a single and robust nucleus remains poorly understood. NuMA is a long coiled-coil protein essential for spindle structural stability and chromosome segregation. Impaired NuMA function results in nuclear fragmentation, and whether this is due to impaired mitotic function or to a direct role in nuclear formation remains unclear. Here, we show that NuMA plays a *bona fide* role in the formation of a single nucleus after mitosis, independent of its spindle role. We further demonstrate that NuMA is essential for forming a round, smooth nucleus, suggesting a structural role. We find that its coiled-coil is essential to the formation of a single nucleus, and to its stable incorporation in its nuclear structure. Finally, we show that the cell actively regulates NuMA's chromosome binding: NuMA’s C-terminus binds chromosomes at interphase but its long coiled-coil prevents it from binding chromosomes at mitosis, keeping its action on distinct cellular structures compartmentalized. Together, our findings indicate that NuMA plays key, long-range structural roles throughout the cell cycle, keeping both the mitotic spindle and the nucleus intact.

SG18

**Molecular Determinants of Pseudopod Morphology**

**D. Mullins**, K. Cheng; University of California, San Francisco, San Francisco, CA.

The shape of many animal cells is defined by the actin cytoskeleton and the plasma membrane; and dynamic shape changes are often driven by protrusion of actin-filled pseudopods. These pseudopods generally resolve into a combination of one- and two-dimensional elements (filopodia and lamellipodia). The laminar nature of lamellipodia does not depend on interaction with an external surface, but likely reflects an underlying linear arrangement of actin regulatory molecules. “Filopodia” describes a variety of linear structures that protrude by different mechanisms, including the dynamic reorganization of growing filaments within a pre-existing lamellipod. A key protein required for the emergence of filopodia from lamellipodial networks is the actin polymerase VASP, whose clustering promotes the formation of linear actin bundles. We find that VASP clusters arise from small, pre-existing clusters of the VASP binding partner, lamellipodin, at the growing edge of a lamellipodial actin network. Growth of VASP/lamellipodin clusters exhibits some features of liquid demixing/phase separation, including a requirement for multivalent interactions and droplet-like fission and fusion. Surprisingly, we also observe that cluster growth is limited by a previously undescribed, size-dependent instability.

SG19

**Coordination of Protein Homeostasis with Cell-volume in Budding Yeast**

**K. Claude**, D. Bureik, K. M. Schmoller; Helmholtz Zentrum München, München, GERMANY.

In proliferating cells, protein homeostasis is tightly coordinated with cell-volume and most proteins are kept at a constant, cell-volume independent concentration. While well suited for most proteins, a constant concentration imposes a problem for DNA-binding proteins such as histones, which on the contrary are required at a constant amount. Indeed, using live-cell fluorescence microscopy, we find that in budding yeast the histone concentration decreases with increasing cell-volume. This raises the question of how cells achieve to produce histones in proportion to DNA content even though total protein production increases with cell-volume. To identify the cell-volume dependence of histone transcript concentrations, we controlled cell-volume through inducible expression of the G1/S inhibitor Whi5. Using RT-qPCR, we find that for control genes the mRNA concentration relative to total RNA stays constant with increasing cell-volume. In contrast, the relative concentration of histone mRNA decreases in inverse proportion to cell volume. Next, we asked whether histone promoters are sufficient to establish the cell-volume-dependence of histone homeostasis. Using RT-qPCR and flow cytometry, we find that histone promoters driving expression of a fluorescent reporter result in a decrease of reporter mRNA concentration with increasing cell-volume, as opposed to the *ACT1* promoter, which leads to a constant, cell-volume independent, mRNA concentration. In conclusion, we find that histone transcription is limited by the DNA template, which is in contrast to the production of other proteins such as Act1, where transcription is limited by the transcriptional machinery. In addition, such a mechanism also explains the differential ploidy dependence of transcription driven by histone and control promoters. Thus, our work identifies a novel regulatory mechanism that enables cells to couple histone production to ploidy rather than cell size.

SG20

**Mitochondrial Volume Fraction Controls Translation of Nuclear-encoded Mitochondrial Proteins**

**T. Tsuboi**[1], M. Viana[2], F. Xu[1], S. Rafelski[2], B. Zid[1]; [1]University of California, San Diego, La Jolla, CA, [2]University of California, Irvine, Irvine, CA.

Mitochondria are dynamic in their size and morphology, yet must also precisely control their protein composition according to cellular energy demand. We have found that cells are able to use mitochondrial morphology to post-transcriptionally coordinate protein expression with metabolic demands through enhanced mRNA localization to the mitochondria. As yeast switch to respiratory metabolism they increase their mitochondrial volume fraction, this drives the localization of nuclear-encoded mitochondrial mRNAs to the surface of the mitochondria. Through artificial tethering experiments, we show that this mitochondrial localization is necessary and sufficient to increase protein production to levels required during respiratory growth. Furthermore, we find that this mRNA sensitivity to mitochondrial volume fraction is driven by the speed of translation of the ORF. This points to a mechanism by which organelle volume fraction provides feedback to regulate organelle-specific gene expression through mRNA localization while potentially circumventing the need to directly coordinate with the nuclear genome.

SG21

**How Cell Shape Shapes Cells**

A. Weems, M. Driscoll, P. Roudot, E. Welf, **G. Danuser, 75390**; UT Southwestern, Dallas, TX.

Cell signaling controls cell shape. However, how much does cell shape also control signaling. There are numerous well-understood physical and chemical scenarios, including variation in surface-to-volume ratio, diffusion trapping, and nonlinearity of molecular interactions in narrow spaces, that put shape potentially in control of signaling. Nonetheless, most signal transduction cartoons exclude shape as a decisive factor. Supported by the development of high-resolution 3D light-sheet microscopy, enabling the measurement of shape and signals at a length scale where the two variables may be coupled, and by novel computer vision approaches to relate shape and signals, our lab systematically tests how much shape matters for signaling. We are particularly interested in scenarios in which shape may control oncogenic signals. In Mohan et al. (PMID 31063759) we described such a scenario: Elevated Rac1 signaling in melanoma promotes the formation of extended lamellipodia, which isolate signaling microdomains that sequester and inactivate Merlin (encoded by the tumor suppressor NF2). Hence, cells become non-responsive to major classes of melanoma-targeting drug treatments, as well as proliferative in metastatic colonies. Similarly, we find that bleb formation also drive oncogenic signals, via multiple complementary mechanisms. In melanoma, inhibition of bleb formation both attenuates proliferative signaling associated with oncogenesis and triggers cell death via apoptosis. Restricted to the concave surface areas between blebs we find a pronounced assembly of septin networks that have been described in other works as organizing scaffolds for signaling pathways. Indeed, pharmacological inhibition of septins greatly reduces the viability of melanoma lines in the same manner bleb inhibition does. To causally couple bleb formation to septin recruitment we performed quantitative 3D time-lapse imaging and statistical event tracking and we show that bleb inhibition leads to septin mislocalization and detachment from the membrane. Proteomic analysis reveals that septin networks bind components of a variety of signaling pathways, especially NRAS. Both bleb and septin inhibition greatly attenuates NRAS/PI3K signaling. Thus, we propose that bleb-dense morphologies produce a second class of cell membrane topography, besides lamellipodia, that drive oncogenic signals, in this case via provision of a geometry that promotes septin assembly. As high-density membrane blebbing occurs in many different cell types (leukocytes, neural crest cells, etc), it will be interesting to consider whether bleb-associated signaling plays a universal mode of cell signal transduction.

SG22

**Agent-Based Models Predict How Context Impacts Cell Population Dynamics**

**N. Bagheri**[1,2,3], J. Yu[1]; [1]Chemical & Biological Engineering, Northwestern University, Evanston, IL, [2]Biology & Chemical Engineering, University of Washington, Seattle, WA, [3]Allen Institute for Cell Science, Seattle, WA.

Computational models are essential tools that can be used to guide such biological intuition. My lab employs both data-driven (machine learning) models and agent-based models to help explain biological observations and to uncover design principles that drive individual cellular decisions and cell populations. I am particularly interested in uncovering the multiscale nature of cellular signaling—how "the whole is greater than the sum of its parts"—and in predicting cell population dynamics from the composition of simpler biological modules. In this talk, I describe how we have developed an agent-

based model of the tumor microenvironment by integrating intracellular signaling with intercellular dynamics. In piecing together the language of cell signaling, we step closer to predicting, and subsequently controlling, rich complex biological function.

## Subgroup C: Cell Biology Meets the Hippo Pathway

SG23
**Growth Control and Hippo Signaling in the Drosophila Abdomen**
**N. Tapon**; Francis Crick Institute, London, UNITED KINGDOM.

To unravel the mechanisms of tissue size determination, we have established quantitative biology tools to study the growth of the Drosophila abdominal histoblasts (HBs), the precursor cells of the adult abdominal epidermis. HBs are organised in nests located in the abdominal segments, surrounded by larval epithelial cells (LECs). HBs are quiescent throughout larval development, then proliferate extensively during abdominal morphogenesis at the pupal stage, replacing the surrounding LECs which undergo apoptosis. To cover the entire abdominal surface, the HBs undergo eight cell cycles (three synchronous cleavage divisions followed by five asynchronous cycles), then cease proliferating at 31-33hr after puparium formation and differentiate. A major advantage of this system is its amenability to long-term live imaging, allowing us to visualise proliferation/growth of the HBs and death of the LECs. I will present our ongoing work on the role and regulation of Hippo signalling in this system.

SG24
**Hippo Pathway in Mammalian Kidney Homeostasis and Disease**
A. Reginensi[1], J. Wrana[2], B. Humphreys[3], **H. McNeill**[3,2]; [1]Lunenfeld Tanenbaum Research Institute, Toronto, ON, CANADA, [2]Lunenfeld-Tanenbaum Research Institute, Toronto, ON, CANADA, [3]Washington University School of Medicine, St. Louis, MO.

Alterations of the Hippo signaling pathway have been found in patients with sarcomatoid renal cell carcinoma, a highly aggressive kidney disease with rapid progression, and poor prognosis. It is unclear whether deregulated Hippo pathway is a primary cause of sarcomatoid cancers. To investigate whether conditional (kidney-specific) Hippo knockout in adults leads to renal cancer development and/or sarcomatoid dedifferentiation, we conditionally inactivated both *Lats1* and *Lats2* from the renal proximal tubule epithelial cells. Mosaic deletion of *Lats1 and Lats2* in the adult proximal tubules results in cell-autonomous sarcomatoid renal cell carcinoma (54%) as early as 8 weeks after gene deletion and lung metastasis. RNA sequencing of tumors compared to control renal cortices revealed massive transcriptional changes with 2292 genes significantly upregulated (FC>2) and 2261 genes significantly downregulated (FC<0.5). To visualize the cellular onset and progression of renal defects, we conducted histological examination at different time points after induction of *Lats1/2* deletion. Mutant kidneys displayed tubular atrophy with glomerular cysts. Loss of proximal tubule epithelial markers, and de novo expression of the mesenchymal marker Vimentin, indicated that kidney epithelial cells had undergone an epithelial to mesenchymal transition (EMT). These studies demonstrate that loss of *Lats1/2* rapidly leads to aggressive, metastatic sarcomatoid renal cell carcinoma

SG25

**Yap Is Required for Load-induced Gene Expression Changes in the Tendon**

M. Grinstein[1], L. Gaut[2], L. O'Connor[1], H. Dingwall[3], T. Capellini[3], D. Duprez[2,4], **J. L. Galloway**[1,4,5]; [1]Harvard Medical School, Massachusetts General Hospital, Boston, MA, [2]Institut Biologie Paris Seine, Sorbonne University, Paris, FRANCE, [3]Harvard University, Cambridge, MA, [4]co-corresponding, Boston, MA, [5]Harvard Stem Cell Institute, Cambridge, MA.

Tendons are essential for the transfer of force from the muscles to the bones of the body, enabling movement. Changes in the physical forces applied to the tendon affect its composition: absent or excessive loading conditions can disrupt normal tendon homeostasis and result in abnormal matrix deposition and degenerative conditions. An understanding of the molecular mediators of this process would have significant implications for how we approach tendon disease and rehabilitation. Although the Hippo pathway regulates mechanotransduction in other cell types, there is currently no understanding of the Hippo pathway in tendon biology. We propose that Yap has an important function in mechanotransduction in the adult tendon by integrating cues from the loading environment to regulate cell behaviors and gene expression. Therefore, we sought to define the function of Yap in adult tendons using gain and loss of function studies in mice. Voluntary exercise in control wild type mice caused increased expression of *Yap* and the Hippo kinase, *Lats1* in the Achilles tendon. In addition, we also observed increased expression of the tendon markers, *Scx*, *Col1*, and *Tnmd*, compared with non-running controls. In Yap conditional knockout (CKO) mice, we found expression of *Scx*, *Col1*, and *Tnmd* were decreased in the Achilles tendons compared to controls, but there were no changes in tendon cell number. Expression of these genes were further decreased upon exercise in Yap CKOs compared to non-running Yap CKO mice. These results indicate that Yap is required for maintaining tendon gene expression and for the induction of gene expression following exercise. To test the effect of Yap activation, we used Yap constitutive gain-of-function mice and found that activation of Yap increased expression of *Scx* and *Col1a2* compared with controls. Interestingly, this effect synergistically increased with exercise where expression of *Scx*, *Sox9*, *Tnmd*, *Col1a2* and *Col2a1* were markedly increased. Experiments in 3D cell constructs demonstrated that loss of mechanical tension reduced Yap nuclear localization as well as the expression of *Scx* and Yap target genes, *Cyr61* and *Ctgf*, whereas mechanical stimulation increased expression *Scx*, *Cyr61* and *Ctgf*. Chemical inhibition of Yap blocked activation of loading induced gene expression, demonstrating a direct upstream requirement for Yap signaling in mechanically induced gene expression. Together, these results *in vivo* and *in vitro* demonstrate that Yap is required for tendon cell mechanotransduction and is a key regulator of tendon maintenance and response to exercise.

SG26

**Yap and Taz Limit Cytoskeletal and Focal Adhesion Maturation to Enable Persistent Cell Motility**

**J. D. Boerckel**; University of Pennsylvania, Philadelphia, PA.

Cell migration initiates by traction generation through reciprocal actomyosin tension and focal adhesion reinforcement, but continued motility requires adaptive cytoskeletal remodeling and adhesion release. Here, we asked whether de novo gene expression contributes to this cytoskeletal feedback. We found that global inhibition of transcription or translation does not impair initial cell polarization or migration initiation, but causes eventual migratory arrest through excessive cytoskeletal tension and over-

maturation of focal adhesions, tethering cells to their matrix. The transcriptional coactivators YAP and TAZ mediate this feedback response, modulating cell mechanics by limiting cytoskeletal and focal adhesion maturation to enable persistent cell motility and 3D vasculogenesis. Motile arrest after YAP/TAZ ablation was partially rescued by depletion of the YAP/TAZ-dependent myosin phosphatase regulator, NUAK2, or by inhibition of Rho-ROCK-myosin II. Together, these data establish a transcriptional feedback axis necessary to maintain a responsive cytoskeletal equilibrium and persistent migration.

SG27

**Regulation of Hippo Pathway Transcription Factor TEAD in Cancer Biology**

**H. Park**; Yonsei University, Seoul, KOREA, REPUBLIC OF.

The Hippo-YAP/TAZ Signaling Pathway have emerged as key regulator of organ size and tissue homeostasis, and their dysregulation contributes to human cancer. YAP/TAZ are transcription co-activators that regulate gene expression primarily through interaction with the TEA domain DNA-binding family of transcription factors (TEAD). The current paradigm for regulation of this pathway centers on phosphorylation-dependent nucleocytoplasmic shuttling ofYAP/TAZ through a complex network of upstream components. However, unlike other transcription factors, such as SMAD, NF-kB, NFAT and STAT, the regulation of TEAD nucleocytoplasmic shuttling has been largely overlooked. Here we show that environmental stress promotes TEAD cytoplasmic translocation via p38 MAPK in a Hippo-independent manner. Importantly, stress-induced TEAD inhibition predominates YAP-activating signals and selectively suppresses YAP-driven cancer cell growth. Our data reveal a mechanism governing TEAD nucleocytoplasmic shuttling and show that TEAD localization is a critical determinant of Hippo signaling output.

SG28

**Phase Separation of YAP Reorganizes Genome Topology for Long-term Yap Target Gene Expression**

**D. Cai**[1,2], D. Feliciano[1], P. Dong[2], N. Porat-Shliom[1], Z. Liu[2], J. Lippincott-Schwartz[2]; [1]National Institutes of Health, Bethesda, MD, [2]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA.

Yes-associated Protein (YAP) is a transcriptional co-activator that regulates cell proliferation and survival by binding to a selective set of enhancers for potent target gene activation, but how YAP coordinates these transcriptional responses is unknown. Here, we demonstrate that YAP forms liquid-like condensates in the nucleus in response to macromolecular crowding. Formed within seconds of hyperosmotic stress, YAP condensates compartmentalized YAP's transcription factor TEAD1 along with other YAP-related transcription co-activators, including TAZ, and subsequently induced transcription of YAP-specific proliferation genes. Super-resolution imaging using Assay for Transposase Accessible Chromatin with photoactivated localization microscopy (ATAC-PALM) revealed that YAP nuclear condensates were areas enriched in accessible chromatin domains organized as super-enhancers. Initially devoid of RNA Polymerase II (Pol II), the accessible chromatin domains later acquired RNA Pol II, producing newly transcribed RNA. Removal of YAP's intrinsically-disordered transcription activation domain (TAD) prevented YAP condensate formation and diminished downstream YAP signaling. Thus, dynamic changes in genome organization and gene activation during YAP reprogramming is mediated by liquid-liquid phase separation.

SG29

**Cellular Dynamics Driven by the Hippo Pathway**

J. Park[1], **C. G. Hansen**[1,2]; [1]University of Edinburgh Centre for Inflammation Research, Edinburgh, UNITED KINGDOM, [2]University of Edinburgh, Institute for Regeneration and Repair, Edinburgh, UNITED KINGDOM.

Tumours are fuelled by many different inputs. One of particular interest is the mechanical stress within tumours, which is higher than in the surrounding healthy tissue. This mechanical stress promote tumour growth and also makes the tumour more challenging to treat. So far how the underlying cellular processes driving the heterogeneous tumour, and the underlying processes fuelling these events are not fully understood. We combine genome editing, transciptomics, biochemistry and label free imaging techniques, that allow us to explore precisely how, at single cell level, cells response to this heterogeneous tumour environment. We will present work that reveal fundamental insights into how the Hippo pathway drives the cellular response to specific mechanical stress.

SG30

**Cell Density Regulates Cardiomyocyte Proliferation through the Hippo Pathway**

**A. Neininger**, D. T. Burnette; Vanderbilt University, Nashville, TN.

Adult mammalian cardiomyocytes have a low to negligible proliferative capacity, thus limiting the regenerative potential of the myocardium after a myocardial infarct. It has recently been shown that human induced pluripotent stem cell-derived cardiomyocytes have a basal proliferative capacity, but the factors regulating this proliferative capacity have not been fully elucidated. Furthermore, the relationship of density-dependent proliferation and the Hippo pathway in cardiomyocytes has not been shown. We show that cell density regulates cardiomyocyte proliferation through the Hippo pathway, and identify a unique combination of two small molecules to inactivate the Hippo pathway and thus induce proliferation of human cardiomyocytes in culture. Specifically, we show that a dense syncytium of cardiomyocytes can regain cell cycle activity when cell density is reduced, suggesting a role for density-dependent proliferation in regulating the cardiomyocyte cell cycle. Low cardiomyocyte density also increased nuclear YAP accumulation, a transcriptional coactivator inhibited by the Hippo pathway. Similarly, expression of YAP/TAZ target genes increased at low density conditions, suggesting a role of the Hippo pathway in regulating this proliferative capacity. Indeed, two small molecule inhibitors of the Hippo pathway increased cardiomyocyte proliferation, and when combined, further increased proliferation at lower concentrations. Taken together, these results further characterize the role of density-dependent proliferation and the Hippo pathway in regulating the normally low proliferation rate of human cardiomyocytes, and introduce a new potentially therapeutic combination of small molecule drugs which induce cardiomyocyte proliferation in various conditions.

SG31

**Cell Density Regulates Golgi Secretory Trafficking Via Hippo and GOLPH3**

T. T. T. Tran[1], H. C. Dippold[1], S. L. Makowski[1], M. D. Buschman[1], H. Tanaka[2], K. Guan[1], **S. J. Field**[1]; [1]University of California, San Diego, La Jolla, CA, [2]The University of Tokyo, Tokyo, JAPAN.

Little is known about cellular signaling pathways that regulate the Golgi in response to extracellular cues. Here we report the discovery that cell crowding leads to impaired Golgi-to-plasma membrane

trafficking along with changes in Golgi shape. We observe this regulation in a wide variety of mammalian cell types and conclude that regulation of the Golgi is a common feature of the cellular response to cell crowding. Furthermore, we find that this regulation is fully explained by the observation that the interaction between GOLPH3 and MYO18A is impaired in crowded cells. To identify the relevant signaling pathway, we perform unbiased immunoprecipitation/mass spectrometry, identifying a phosphatase complex that interacts with GOLPH3 and serves to promote the interaction between GOLPH3 and MYO18A in a fashion that is regulated by cell density. We further identify the Hippo proteins, MST1 and MST2, to act upstream of the phosphatase to regulate its activity at the Golgi. Thus, we have identified a signaling pathway from MST1/2 to GOLPH3/MYO18A that serves to regulate Golgi function in response to cell crowding.

SG32

**Effects of Age-dependent Changes in Cell Size on Endothelial Cell Growth through Yap1**

**A. Mammoto**, T. Mammoto; Medical College of Wisconsin, Milwaukee, WI.

Introduction:The aging population is rapidly growing. Aging is associated with impaired angiogenesis - the growth of new blood capillaries- and contributes to the increased susceptibility to various diseases. Thus, in order to develop more efficient therapies for aging-associated diseases, we need to understand the mechanisms by which aging impairs angiogenesis. In addition to soluble angiogenic factors, biophysical factors such as changes in cell size and geometry control endothelial cell (EC) growth and differentiation. A Hippo signaling transducer, Yes-associated protein (YAP1), acts as a mechanosensitive transcriptional co-activator and controls angiogenesis and organ regeneration. Methods: We use silver nitrate staining and immunocytochemical analysis to measure the EC size in blood vessels ex vivo and in vitro. To directly examine the effects of cell size on YAP1 activity and EC proliferation and senescence, we use the microcontact printing system and culture young vs. aged ECs on each island. Results: We have found that pulmonary artery ECs in aged mice are significantly larger than those in young mice. Aged human adipose tissue ECs are also larger than those in young adults. A major focal adhesion protein, paxillin, distributes at the distal ends of actin stress fibers in young ECs, while paxillin is localized along the actin fibers in the cytoplasm in aged ECs. The levels of YAP1 decrease and EC senescence is induced in aged ECs. The activity of CDC42, which controls actin cytoskeleton structures, is also higher in aged ECs compared to that in young ECs. To analyze whether age-related changes in EC size control YAP1 activity and EC senescence, we culture ECs on the microcontact-printed substrates consisting of square fibronectin-coated single-cell sized islands. When we culture aged human adipose ECs on large islands of size comparable to aged EC, YAP1 is excluded from the nucleus and EC proliferation is attenuated. Reduction of the aged EC size by culturing on smaller islands restores YAP1 nuclear localization, decreases Cdc42 activity, and inhibits EC senescence. Stimulation of YAP1 or inhibition of Cdc42 activity in aged ECs also restores blood vessel formation. These results suggest that age-dependent increases in EC size stimulate aged EC senescence through CDC42-YAP1 signaling. Conclusion: Modulation of EC size and/or Cdc42 and YAP1 activity will reverse age-related decline in angiogenesis and will lead to the development of promising strategies for age-dependent diseases.

SG33

**$\beta_H$-spectrin Recruits PP2A to Crumbs to Regulate Crosstalk with the Hippo/Warts Pathway in *Drosophila*.**

K. Browder[1], S. Lee[2], E. Klipfell[3], **C. Thomas**[3]; [1]Genentech, South San Francisco, CA, [2]National Institute of Aging (NIH/NIA/IRP), Baltimore, MD, [3]Pennsylvania State University, University Park, PA.

Spectrin is a large F-actin crosslinking protein that most famously forms 2D networks in association with the plasma membrane of red blood cells. There it confers cell shape and strength during the rigors of circulation. In NON-erythroid tissues, spectrin has additional roles in the endomembrane system. We have previously shown that the apically-polarized $\beta_H$-spectrin, encoded by the *karst* locus in *Drosophila*, is required for the stability of several apical proteins, through the promotion of endosomal recycling to the plasma membrane - so called, 'dynamic protein stabilization'. The apical protein determinant Crumbs recruits $\beta_H$ to the apical membrane and is itself trafficked in a $\beta_H$-dependent manner. $\beta_H$ binds to the Hippo/Warts pathway (HWP) regulator Expanded, which mediates Crumbs crosstalk to the HWP.Here we report that a yeast 2-hybrid (Y2H) screen identified the PP2A substrate-specificity subunit Waldorf (a PP2A-PR72/B'' isoform) as a binding partner of $\beta_H$-spectrin. Waldorf binds to $\beta_H$ *via* a short conserved sequence in the globular segment 33 of $\beta_H$. Genetic interaction and molecular epistasis experiments strongly suggest that PP2A with the Waldorf specificity-subunit bound to it ($PP2A^{Waldorf}$) acts as a negative regulator of Crumbs by acting to displace aPKC from Crumbs. Consistent with this notion, mutant versions of Crumbs lacking target residues for aPKC in the FERM-domain binding site do not respond to changes in Waldorf levels. The 'output' of Crumbs that appears to be most sensitive to the levels of Waldorf is crosstalk with the HWP and not apical polarity *per se*. Thus knockdown/overexpression of Waldorf results in the under/overgrowth of wing tissue, and Waldorf has a genetic interaction with Yorkie, but apicobasal polarity is not lost.Waldorf also modulates protein trafficking in a similar way to $\beta_H$ and its previously reported partner Annexin B9: Knockdown of Waldorf leads to an increase in Rab7-positive and acidic compartments, suggesting that $PP2A^{Waldorf}$ also normally acts by suppressing lysosomal trafficking, most likely in favour of recycling pathways.Our results support a model in which Crumbs recruits $\beta_H$ in a complex with the HWP activator Expanded, and $PP2A^{Waldorf}$ bound to $\beta_H$ acts in a homeostatic fashion to limit Crumbs activation of the HWP by displacing aPKC complex. This in turn limits the amount of growth suppression caused by Crumbs-dependent HWP activation.

SG34

**Basal Body Assembly and Hippo Signaling Are Linked Via the Sas4 Protein**

**M. D. Ruehle**, C. G. Pearson; University of Colorado Anschutz Medical Campus, Aurora, CO.

Basal bodies (BBs) are macromolecular complexes required for the formation and cortical positioning of cilia. Both BB assembly and DNA replication are tightly coordinated with the cell cycle to ensure their accurate segregation and propagation to daughter cells. However, it is unclear how this coordination is achieved. Here we show that the *Tetrahymena* Sas4/CPAP protein is enriched at assembling BBs, localizing to the core BB structure and to the base of BB-appendage microtubules. Sas4 deletion shows that it is necessary for BB assembly and cortical microtubule organization, but, unlike other core BB proteins, Sas4 regulates cell division furrow positioning and DNA segregation. The Hippo signaling pathway similarly regulates division furrow position in *Tetrahymena*, and Hippo molecules localize to BBs and BB-appendages. We show that Sas4 loss disrupts these binding sites, mislocalizing the Hippo

activator, Mob1. These data implicate Sas4 as a mediator of Hippo activity by promoting microtubule scaffolds for Mob1 localization to the cell cortex. Thus, Sas4 links BB assembly with an ancient signaling pathway known to promote the accurate and symmetric segregation of the genome.

SG35
**Acinus Supports Atg1-mediated Phosphorylation of Yorkie to Restrict Cell Growth**
**N. Nandi**, L. Tyra, H. Kramer; University of Texas Southwestern Med Ctr, DALLAS, TX.

Acinus has emerged as a signaling node that integrates multiple cellular stress signals and manages stress by regulating basal levels of autophagy in a TOR-independent pathway (1,2,3). Here, we identified Acinus-supported Atg1/ULK1-mediated phosphorylation of Yorkie as an additional inhibitory input restricting growth independently of the Hippo/Warts pathway. Two serine residues in Yorkie, S74 and S97, are Atg1/ULK1 consensus target sites phosphorylated by ULK1 in vitro. In vivo, Atg1 gain-of-function caused elevated Yorkie phosphorylation and reduced expression of Yorkie target genes. Conversely, knocking down Atg1, or its activator Acinus, reduced Yorkie phosphorylation and raised expression of Yorkie target genes to increase tissue size. Yorkie phosphorylated by ATG1 can still translocate to the nucleus, but was unable to bind the transcription factor Scalloped and failed to promote transcription of pro-proliferation genes, thereby restricting cell growth. Unlike Atg1's role in autophagy, Atg1-mediated phosphorylation of Yorkie does not require Atg13. Atg1 is activated by starvation or other cellular stressors and therefore can impose temporary stress-induced constraints on the growth-promoting gene network under control of Hippo/Yorkie signaling.

1. Haberman et al (2010) Development 137:2157-66
2. Nandi et al (2014) J Cell Biol 207(2):253-68
3. Nandi et al (2017) eLife 6:e30760

## Subgroup D: Cellular Symmetry Breaking

SG36
**Courtship Is a Two-way Conversation: Yeast Mating as a Model of Cell-cell Communication**
**R. Clark-Cotton**, N. Henderson, D. J. Lew; Duke University, Durham, NC.

Many cells track chemical signals during feeding, fertilization, development, and immune responses, but the mechanisms of target location are elusive. The budding yeast, an established model of such cell-cell communication, tracks a pheromone signal to locate a mating partner. Pheromone binding to cell surface receptors triggers the concentration of polarity proteins at a small zone of the cell cortex (the "polarity patch"). The polarity patch is oriented toward the partner, and through its effectors, directs polarized growth toward that mate. In classical models of polarization, a cell first decodes the pheromone gradient and then polarizes toward the region of highest signal. However, studies of yeast in artificial gradients found that cells that polarize in the wrong direction can move the patch to align with the partner. As receptors and G proteins concentrate at the patch, local pheromone sensing and secretion may mediate communication between partners. A fundamentally different "exploratory polarization" paradigm for orientation in a gradient posits that polarity sites move around the cortex in a search process, and that when the patches of two partner cells are aligned, each cell senses a high

pheromone signal, causing the patches to stop moving. Unlike the artificial gradient studies, cells in mating mixes first assemble weak and transient polarity clusters that move erratically around the cell cortex, often engaging different partners before stably polarizing. It is unclear whether such polarity clusters are functionally important for partner identification. Here we ask whether the weak early clusters are engaged in exploratory polarization, only stabilizing when two clusters co-oriented, or if the cells perform a more classical global survey of the pheromone landscape, without need for the weak polarity clusters. To distinguish between these models, we performed live-cell fluorescence microscopy of mating mixes in which wildtype cells were paired with partners that either failed to make polarity clusters, or that made but could not stabilize clusters. Strikingly, we found that the wildtype cells displayed extended indecisive behavior and did not commit to such partners. Stabilizing the patches of polarity mutants with exogenous pheromone allowed wildtype cells to commit to them. We also confirmed that both sensing, signaling, and secretion markers were enriched at indecisive phase polarity clusters, thereby providing direct experimental evidence that local pheromone secretion promotes commitment to a partner, in support of the exploratory polarization model. We conclude that reciprocal communication between two partners' polarity sites is essential for successful commitment to a mating partner.

SG37

**Regulation of P Granule Substructure in Space and Time**

**A. Folkmann**, M. Cassani, G. Seydoux; Johns Hopkins University HHMI, Baltimore, MD.

Cytoplasmic RNA granules are important regulators of posttranscriptional control of gene expression in normal health and disease. RNA granules contain translation factors, helicases, decay enzymes, RNA binding proteins, and scaffold proteins. Liquid-liquid phase separation of proteins and RNA (LLPS) has emerged as a driving force for the formation of RNA granules. The P granules of *C. elegans* are a well-studied example of RNA granules. P granules exhibit liquid-like characteristics *in vivo* including fusion and rapid dissolution and condensation. Prior studies have documented that P granules are non-homogenous and consist of at least two phases including a gel-like shell defined by the intrinsically disordered protein MEG-3/4 and a liquid core containing the PGL family of RNA binding proteins. Additionally, in our preliminary analysis we have found that the intrinsically disordered protein MEG-1 forms a dynamic shell that envelops both the MEG-3/4 and PGL phases. The molecular details underlying the compartmentalization of P granules in living embryos remains largely ambiguous. Genetic studies have highlighted the kinases MBK-2/DYRK and PLK-1 as potential regulators of P granule dynamics *in vivo*. Using chemical genetics and phosphoproteomics we are currently interrogating how the MEG and PGL phases within the P granule are regulated in space and time.

SG38

**Biomolecular Condensates Orchestrating Centriole Biogenesis in Human Cells**

J. Ahn, J. Park, L. Zhang, T. Kim, R. Ghirlando, **K. S. Lee**; National Institutes of Health, Bethesda, MD.

The centrosome, a unique membrane-less organelle that serves as the main microtubule-organizing center in animal cells, plays a pivotal role in the orderly progression of the cell cycle. Since faulty assembly and duplication of the centrosome results in abnormal cell division, which then leads to various human disorders, elucidating the molecular mechanisms underlying centrosome assembly and function is likely a key step to understanding the etiology of centrosome-associated diseases.By

combining cell biology with biophysical and X-ray crystallographic methods, we showed that two pericentriolar scaffolds, Cep152 and Cep63, possess intrinsic activity of co-phase-separating into condensates and form a heterotetrameric complex that serves as a building block for generating a cylindrical self-assembly around a centriole. Remarkably, two short self-assembling motifs (one each from Cep63 and Cep152) cooperatively conferred physicochemical properties that allowed them to undergo density transition and self-assemble into a cylindrical architecture. Interestingly, the Cep152-Cep63 condensates exhibited a rapid turnover, underwent fusion, and carried out a significant degree of internal rearrangement within a condensate. A Cep152-Cep63 cylindrical architecture that self-assembled on a flat substrate displayed a decreased but still detectable level of dynamic turnover. Interestingly, Plk4, a key regulator of centriole biogenesis, also dynamically phase-separated from a Cep152-bound state around a centriole (i.e., ring state) into a low-nanoscale spherical condensate (i.e., dot state) upon autophosphorylating its cryptic polo-box domain. Additional *in vitro* and *in vivo* data suggest that the Plk4 condensate serves as an assembling body by amassing downstream procentriole assembly components such as STIL and Sas6 and facilitating Plk4-mediated centriole biogenesis. Thus, the formation of biomolecular condensates appears to be a fundamental step that not only promotes the self-assembly of a pericentriolar architecture but also triggers the process of centriole duplication.This work offers a novel paradigm for understanding how centrosomal scaffolds and their associated proteins dynamically self-organize into a new functional entity and provides a holistic view of the interplay between pericentriolar architecture and its client-induced downstream event.

SG39

**Defining the Earliest Cues Driving Apical-basal Polarity Establishment: the Tumour Suppressor Proteins Scribble and Dlg Direct Supermolecular Assembly and Positioning of Adherens Junctions**

T. Bonello, **M. Peifer**; University of North Carolina, Chapel Hill, NC.

Apical-basal polarity is a fundamental property of animal tissues. *Drosophila* embryos provide an outstanding model for defining mechanisms that initiate and maintain polarity. Polarity is initiated during cellularization when cadherin-based adherens junctions (AJs) are positioned at the future boundary of apical and basolateral domains. Subsequent polarity maintenance involves complementary and antagonistic interplay between apical and basal polarity complexes. We and others identified Bazooka (Baz)/Par3 as a key polarity cue required for AJ formation. Canoe/Afadin, which links AJs to actin, and its regulator Rap1, act upstream of Bazooka. In the current model, Rap1/Canoe, Baz and AJs initiate polarity, and then other protein complexes are recruited to elaborate on the polarity program. We found that this linear hierarchy is significantly oversimplified. The Scribble/Discs Large (Dlg) module is well-known for promoting basolateral identity during polarity maintenance and for assembly of septate junctions, the Drosophila equivalent of tight junctions. Here we report a surprising role for Scribble/Dlg in polarity initiation, placing it near the top of the network directing AJ positioning and supermolecular organization, as one of the earliest symmetry breaking cues. Scribble/Dlg are enriched early in nascent AJs, and are essential from the start for AJ and Baz positioning and supermolecular assembly. They also play a role in basal junction assembly. Mislocalized AJ proteins remain associated, indicating Scribble/Dlg are critical for supermolecular assembly of smaller cadherin-catenin complexes into mature spot junctions and positioning junctions apically. We test hypotheses for underlying mechanisms. Our data suggest that the Scribble/Dlg module plays multiple roles in polarity initiation. Polarity establishment requires Scribble's scaffolding function via its PDZ domains, a function dispensable for polarity maintenance. Further Scribble/Dlg direct Par-1 localization, and Par-1 is

required for a subset of polarity establishment events, suggesting Scribble/Dlg act via Par-1-dependent and independent mechanisms. Thus Scribble/Dlg are master scaffolds regulating assembly of distinct junctional complexes at different times and places-this forms part of our larger scale effort to define the full network of proteins and mechanisms directing apical-basal polarity.

SG40

**Chiral Bending of Filopodia**

**W. Li, 7610001**, B. Geiger, A. Bershadsky; Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, ISRAEL.

The mechanism underlying the left-right asymmetry in multicellular organisms is still poorly understood. Some evidence suggests that such asymmetry depends on the motile behaviour of individual cells. Indeed, different cell types, growing under isotropic conditions, demonstrate left-right chiral swirling of their actin cytoskeleton. This swirling depends on myosin-II driven actomyosin contractility and formin-mediated actin polymerization, and can be regulated by a number of actin-associated proteins [Tee et al, 2015]. The molecular events responsible for the swirling asymmetry, however, remain to be elucidated. Here, we show that filopodia growth in HeLaJW cells plated on the substrate covered by animal lectin (galectin-8) demonstrate local left-right asymmetry in their growth pattern. Specifically, those filopodia which bend during elongation do it in an asymmetric manner so that a fraction of “left-turning” filopodia was significantly higher than that of “right-turning”. Overexpression of myosin-X, a protein known to be involved in filopodia growth, significantly enhanced the fraction of bending filopodia (up to forty percent) on galectin-8-coated substrate. Over 80% of the ‘bending filopodia’ turned left. The asymmetry of filopodia turning was observed also in cells with inhibited myosin-II function and therefore depended mainly on actin polymerization and function of non-conventional myosins. Interference reflection microscopy (IRM) and TIRF microscopy showed that filopodia turning occurs only in filopodia with tips attached to the galectin-8-coated substrate. Moreover, the bending of filopodia was preceded by significant decrease in filopodia protrusion speed, suggesting that friction between filopodia tip and the substrate triggered the filopodia turning. Since filopodia growth is known to be driven by formin-dependent actin polymerization, we overexpressed the major formins, mDia1, mDia2, FMNL2, and DAAM1 in the cells, and found that the formin overexpressing cells produce less, yet longer and thicker filopodia as compared to controls. These filopodia, however, did not turn. Another method of stimulating filopodia formation and elongation is the knock-down of Arp2 or Arp3. We have found that filopodia induced by Arp2/3 depletion demonstrated myosin-X-dependent bending. This bending was also asymmetric with the preferential left turning, similarly to the filopodia induced by overexpression of myosin-X. Thus, integrin-independent filopodia adhesion to galectin-8 coated substrate revealed intrinsic asymmetry in actin-polymerization dependent and myosin-X -enhanced filopodia turning. Asymmetry of filopodia turning could be one of the factors determining left-right asymmetry in more complex multicellular systems.

SG41

**The Actin Nucleator Cyk-1/mdia Drives Chirality of Actomyosin Flows and Facilitates Left-right Symmetry Breaking in Early *C. Elegans* Embryo's**

**T. Middelkoop**[1], P. Quintero-Cadena[2], L. Pimpale[1], S. Yazdi[3], S. Grill[1]; [1]Biotec/TU Dresden, Dresden, GERMANY, [2]California Institute of Technology, Pasadena, CA, [3]Massachusetts Institute of Technology, Cambridge, MA.

The emergence of organismal left-right asymmetry has puzzled developmental biologists for decades. In recent years, actomyosin activity has proven to be instrumental in driving the chirality of various cells, tissues and organisms. While several myosin motors and actin nucleators of the Formin family can rotate helical actin filaments in vitro, little is known about how these activities can lead to the chirality of entire cellular actin networks in vivo. Recently, it was shown that the *C. elegans* actomyosin cortex generates active torques that drive chiral cortical flows and organismal left-right symmetry breaking. These chiral flows are dependent on the RhoA GTPase and its target non-muscle myosin II (NMII), but the underlying molecular mechanism remains elusive. By combining the strength of *C. elegans* genetics with quantitative live-imaging, we show that the activity of the Formin CYK-1/mDia is a key determinant for chiral morphogenesis of *C. elegans* embryos. Using both loss and gain of function genetic perturbations we show that CYK-1/mDia is instrumental for the chirality of cortical flows and proper left-right symmetry breaking. Our results are consistent with a mechanism in which active tension and torque generation in the actomyosin layer are molecularly distinct and driven by NMII and CYK-1/mDia respectively.

SG42

**Linking Symmetry Breaking to Asymmetric Division in the Stomatal Lineage**

**A. Muroyama**[1], D. Bergmann[2]; [1]Stanford University, Stanford, CA, [2]Stanford University/HHMI, Stanford, CA.

Symmetry breaking is essential to coordinate cellular behaviors to pattern tissues in all multicellular organisms. However, our understanding of asymmetry-generating mechanisms in plants remains limited. Stomata, pores in the leaf epidermis that mediate gas exchange, are patterned by asymmetric divisions of precursor cells. In *Arabidopsis*, symmetry breaking in these cells is initiated by the polarization of BASL and BRX, plant-specific proteins that localize in a cortical crescent. This polarity domain is essential for proper division orientation and differential daughter cell fates. However, the mechanisms that link their polarization to asymmetric division remain unknown. By combining quantitative, long-term time-lapse imaging with pharmacological and genetic perturbations, we have uncovered two novel, cytoskeleton-driven pathways that link symmetry breaking to asymmetric division in plants. In one pathway, precursor asymmetry required mutual inhibition between the polarity crescent and membrane-associated microtubules. We found that anticlinal microtubules, while not required for initial polarization, restrict lateral spread of the polarity domain, conferring robustness. In turn, polarity proteins locally altered microtubule organization by suppressing microtubule stability at the membrane. Local microtubule depletion resulted in microtubule network anisotropy that is propagated through the cell cycle to position the division plane. In a parallel pathway, symmetry breaking at the membrane drives directional nuclear migration before and after division via a two-step “cytoskeletal hand-off” mechanism. We found that BASL and BRX repel the nucleus in a microtubule-dependent manner before division but attract it in an actin-dependent manner following division. This

coordinated nuclear movement potentially primes consecutive asymmetric divisions to ensure that daughter cells are properly distributed throughout the leaf epidermis. Taken together, we have uncovered an evolutionarily divergent polarity system that provides a framework to understand how symmetry breaking and asymmetric cell division are coordinated in plants.

SG43

**Stem Cell Mitotic Drive Ensures Asymmetric Epigenetic Inheritance and Distinct Cell Fates**

**R. Ranjan**, J. Snedeker, X. Chen; Johns Hopkins Univesrsity, BALTIMORE, MD.

Many stem cells utilize asymmetric cell division (ACD) to produce a self-renewed stem cell and a differentiating daughter cell. How non-genic information could be inherited differentially to establish distinct cell fates is not well understood. Here, we report that a stem cell-specific 'mitotic drive' ensures asymmetric epigenetic inheritance. We found that a series of spatiotemporally regulated asymmetric components, which ensure biased sister chromatid attachment and non-random segregation during ACD of *Drosophila* male germline stem cells (GSCs). First, sister centromeres are differentially enriched with proteins involved in centromere specification and kinetochore function. Second, temporally asymmetric microtubule activities and polarized nuclear envelope breakdown allow for the preferential recognition and attachment of microtubules to asymmetric sister kinetochores and sister centromeres. Abolishment of either the asymmetric sister-centromeres or the asymmetric microtubule activities results in randomized sister chromatid segregation. Together, these results provide the cellular basis for partitioning epigenetically distinct sister chromatids during stem cell ACDs, which opens new directions to study these mechanisms in other biological contexts.

SG44

**Generating Left-right Asymmetry through RNA Regulation in Kupffer's Vesicle**

J. Pelliccia, S. J. Y. Lee, **R. D. Burdine**; Princeton University, Princeton, NJ.

Axis specification is a critical step in establishing the body plan of developing organisms. Failure to correctly establish the axes can dramatically disrupt development and lead to disease; for example, failures in specifying the left-right axis lead to heterotaxia. In all vertebrates, left-sided identity is conferred by Nodal signaling in the left lateral plate mesoderm (LPM). *nodal* expression is restricted to the left by transient organs of asymmetry, such as Kupffer's Vesicle (KV) in zebrafish. These organs are thought to break left-right symmetry by both generating and sensing a cilia-driven asymmetric fluid flow. However, the mechanism by which asymmetric fluid flow restricts expression of *nodal* to the left side of the embryo is not yet fully understood. Here, we demonstrate that *nodal* is expressed dorsal to, but not in, the cells of KV, suggesting *nodal* is not the direct target of mechanisms downstream of cilia in this process. Instead, we show that the secreted Nodal inhibitor *dand5* is a more direct target of symmetry breaking. Asymmetric expression of *dand5* in the cells of KV is regulated downstream of cilia and the Polycystin channel proteins, and the 3' UTR of *dand5* is both necessary and sufficient to generate this asymmetric expression. We further demonstrate a role for both microRNA processing and the RNA-binding protein Bicaudal C homolog 2 (Bicc2) in generating this asymmetry. Taking these data together, we propose a role for Bicc2 in regulating post-transcriptional stability of *dand5* on the left side of the embryo. Destabilizing the mRNA would reduce the amount of Nodal inhibitor secreted on the left,

allowing Nodal to reach the threshold required for activation in the LPM, and establishment of the left-right axis.

SG45

**Active Locomotor Patterning in Algal Flagellates**

**K. Y. Wan**; University of Exeter, Exeter, UNITED KINGDOM.

Living creatures exhibit a remarkable diversity of locomotion mechanisms, evolving structures specialised for interacting with their environment. In the vast majority of cases, locomotor behaviours such as flying, crawling, and running, are orchestrated by nervous systems. Surprisingly, microorganisms can enact similarly complex movement gaits for swimming using multiple, fast-moving cellular protrusions called cilia and flagella. For example, different species of quadriflagellate algae can produce effective forward propulsion through a fluid, using species-dependent gaits such as the trot, the gallop, or the pronk. Our objective is to decipher how single-celled organisms achieve this sophistication of motion control.Recent theoretical and experimental evidence has already shown that interflagellar coordination in these new model species requires intracellular coupling through the basal apparatus. But is this coupling active or passive? Using a combination of high-speed live-cell imaging and micropipette manipulation, I resolve dynamic gaits at the level of individual flagella to reveal the active nature of locomotor patterning in diverse species. Novel, conserved behavioural features are characterised including beat intermittency, reversible rhythmogenesis, and coupling to ciliary mechanosensitivity. I show how the algal flagellar apparatus functions as a central pattern generator which encodes the beating of each flagellum in a network in a distinguishable manner - that is, no two flagella are the same. I will further demonstrate how activation/inactivation of a subset of flagella provides a novel symmetry-breaking mechanism for cell reorientation. Thus, the capacity to generate and coordinate complex locomotor patterns does not require neural circuitry but rather the minimal ingredients are already present in simple, unicellular organisms.

## Subgroup E: Kinesin Motors - What is Conventional?

SG46

**Direct Competition between Molecular Motors Defines Posterior Determination in *Drosophila* Oocytes.**

**W. Lu**[1], M. Lakonishok[1], A. Rich[2], M. Glotzer[2], V. I. Gelfand[1]; [1]Northwestern University Feinberg Sch Med, Chicago, IL, [2]University of Chicago, Chicago, IL.

The posterior pole of the *Drosophila* embryo is defined by an accumulation of *oskar (osk*) mRNA in the oocyte. Defects in *osk* mRNA localization prevent germ cell formation and abdomen specification. It is known that two major motor proteins, kinesin-1 and myosin-V, are essential for *osk* mRNA posterior localization. In this study, we used Staufen, an RNA-binding protein that colocalizes with *osk* mRNA, to visualize posterior determination in the oocyte. We find that the posterior localization of Staufen depends on three activities of kinesin-1: directed transport of Staufen particles toward the posterior pole, sliding microtubules to drive cytoplasmic streaming, and inhibition of Staufen particle accumulation at sites other than the posterior pole. Posterior localization of Staufen is defined by competition between kinesin-1 that removes Staufen from the cortex and myosin-V that anchors it. The outcome of this competition is determined by cortical microtubule density: it is won by myosin-V at the

posterior pole where the microtubule density is low but not at the other parts of the oocyte cortex. In agreement with this model, local depolymerization of cortical microtubules in the lateral cortex using optogenetics causes ectopic accumulation of Staufen. Thus, direct competition between two motors defines the initial localization of posterior determinants in the *Drosophila* oocyte.

SG47

**Mutations in the Chromokinesin Kif22 Disrupt Mitotic Chromosome Segregation and Cause Skeletal Dysplasia**

A. F. Thompson[1], P. R. Blackburn[2], M. Wagenbach[3], L. Wordeman[3], J. B. Lian[1], E. W. Klee[2], **J. Stumpff**[1]; [1]University of Vermont, Burlington, VT, [2]Mayo Clinic, Rochester, MN, [3]University of Washington, Seattle, WA.

The chromokinesin KIF22 (aka KID) generates polar ejection forces to promote mitotic chromosome congression and contributes to chromosome compaction during anaphase. However, the molecular mechanisms underlying these KIF22 functions are not completely understood. Interestingly, mutations in *KIF22* dominantly cause a rare skeletal development disorder called spondyloepimetaphyseal dysplasia with joint laxity- leptodactylic type (SEMDJL2). Specifically, mutations in proline 148 and arginine 149 of the KIF22 motor domain α2 helix cause SEMDJL2. Additionally, we report that two new skeletal dysplasia patients present with point mutations in the coiled-coil domain of KIF22. Our analyses of these mutations in cultured cells suggest they have minimal effects on the generation of polar ejection forces, but lead to anaphase chromosome segregation defects and disrupted nuclear morphology. We are currently investigating the mechanisms by which SEMDJL2 mutations specifically disrupt the anaphase functions of KIF22 using live cell imaging and single-molecule total internal reflection fluorescence (TIRF) microscopy.

SG48

**The Kinesin-6 Kif20B Regulates Abscission and Fate Outcomes of Neural Stem Cell Divisions in the Developing Brain**

K. McNeely, J. Little, **N. Dwyer**; University of Virginia, Charlottesville, VA.

The Kinesin-6 family of plus-end- directed microtubule motors has three mammalian members: Kif20A/MKLP2, Kif20B, and Kif23/MKLP1. Each has a distinct role in cytokinesis. The family is defined by homology in the motor domain, with a long insert in loop L6, but the stalks and tails are divergent. Kif20b evolved a much longer stalk than the other family members, with four coiled-coil domains. It is only found in vertebrate genomes. Cell-free assays showed that Kif20B is sufficient to slide, cross-link, and bundle microtubules, in an ATP-dependent manner. In neurons, we showed that Kif20B is required for normal microtubule packing in the axon, and localization of Shootin1 to the growth cone. We found that in dividing cells, it localizes to microtubules of the central spindle and midbody throughout cytokinesis, in a pattern distinct from Kif23 and Kif20A. Depletion of Kif20B in HeLa cells suggested that this motor facilitates late stages of midbody maturation and proper abscission timing. It may act in part by stabilizing microtubule bundles, but its molecular mechanisms of action are not well understood. Recently we have studied the roles of Kif20B in abscission of neural stem cells and growth of the cerebral cortex. We isolated a Kif20B loss-of-function mutant in a forward genetic mouse screen. The mutant embryos have small brains (microcephaly), craniofacial defects, and die postnatally. In the mutant brains, neuroepithelial stem cell (NESC) midbodies are wider and disorganized, suggesting

defects in abscission. However, binucleate cells were not detected. We found that most, but not all, of the microcephaly in the mutant is attributable to p53-dependent apoptosis of some NESCs. Here we report two new facets of the small brain phenotype of the Kif20B mutant. First is that by time-lapse imaging of abscission of NESCs in cortical explants, we found that Kif20B loss does not appear to block or delay abscission, but instead slightly accelerates it. Second, by assaying daughter cell fates of individual mutant NESC divisions, we found an increased propensity to exit the cell cycle and become post-mitotic neurons. This would prematurely deplete the stem cell pool. Surprisingly, this effect is p53-independent. Thus, Kif20B loss results in increased probability of two different fate changes for daughters of NESC divisions: p53-dependent apoptosis, and p53-independent cell cycle exit. These data suggest that the developing brain is vulnerable to subtle defects in abscission. Whether and how the changes in midbody structure or abscission timing we observed could influence signaling pathways is still mysterious. These studies provide insight into how a fundamental cell biological process such as cytokinesis is altered in different cell types during development and disease.

SG49

**Herpesviruses Carry Kinesin between Cells to Traffic Intracellularly to Nuclei**

C. Pegg, **G. Smith**; Northwestern University, Chicago, IL.

Neurotropic alpha-herpesviruses exhibit a trademark capacity to invade the nervous system where life-long persistence is established within the host. We report that this phenotype is supported by the incorporation of the cellular transport motor, conventional kinesin, as part of the virion substructure. Incoming capsids from a virus lacking kinesin-1 failed to reach the nucleus and instead accumulated at the centrosome. By producing virions carrying recombinant kinesin-1 fused to either a beta-lactamase reporter or containing a druggable peptide sequence, we demonstrate that kinesin-1 is captured into virions, carried cell to cell, delivered into the cytosol along with the capsid, and controlled by the capsid to promote its nuclear delivery.

SG50

**EMBO Young Investigator Lecture: Molecular Mechanism of Kinesin Motor Cooperation in Cell Division**

T. McHugh, **J. Welburn**; University of Edinburgh, Edinburgh, UNITED KINGDOM.

While most kinesins utilize ATP to walk on microtubules, the non-canonical Kinesin-13 family member MCAK is a potent microtubule depolymerase, that diffuses on microtubules and triggers catastrophe when at microtubule ends. How MCAK works mechanistically to interact with and depolymerize crowded microtubule ends in cell division is poorly understood. MCAK forms a compact structure in solution, held auto-inhibited by an interaction with its C terminus. We recently showed that the MCAK C terminus is displaced by the microtubule and MCAK adopts an extended conformation on the microtubule over 2 tubulin heterodimers, using biophysical methods and cross-linking/mass spectrometry. This allows the neck and N terminus to interact with the microtubule lattice but also with other proteins such as EB proteins and the Kinesin-8 Kif18b. We reported that, in mitotic cells MCAK requires both EB proteins and Kif18b to target to microtubule ends. We show that MCAK and EB require the motile properties of Kif18b to accumulate at plus ends of stabilized microtubules in vitro. Together, EB, Kif18b and MCAK form a low-affinity multivalent and interdependent network at microtubule plus ends. We demonstrate here the first cooperative behavior between kinesin motors. This cooperation is

essential for Kinesin-13 localization to microtubule plus ends in the context of molecular crowding to ultimately control mitotic microtubule length and spindle positioning.

SG51

**Analysis of the Immotile Kinesin-4 Motor Kif7 and Its Role in Hedgehog Signaling**

Y. Yue, M. Engelke, L. Blasius, **K. Verhey**; University of Michigan Medical School, Ann Arbor, MI.

Hedgehog signaling plays critical roles during development and tissue homeostasis. The kinesin-4 motor KIF7 is a conserved regulator of Hedgehog signaling and mutations in KIF7 are linked to human ciliopathies. In response to Hedgehog pathway activation, KIF7 localizes to the tip of the primary cilium in mammalian cells and facilitates the localization of Hedgehog effectors to the same location. However, unlike conventional kinesins, KIF7 is immotile and its ability to interact with microtubules is not regulated by ATP binding or hydrolysis. How the microtubule binding of KIF7 is regulated and relates to its functions in Hedgehog signaling are unclear. We demonstrate that KIF7 is regulated by autoinhibition as full-length KIF7 does not bind to microtubules whereas truncated motors bind statically to all microtubules in cells, including along the ciliary axoneme. Thus, unlike the behavior of purified motors in *in vitro* assays, truncated versions of KIF7 do not bind selectively to or track the plus ends of microtubules in cells. Using an inhibitable version of the heterotrimeric kinesin-2 motor KIF3A/KIF3B/KAP, we demonstrate that kinesin-2-driven intraflagellar transport (IFT) is required for localization of KIF7 to the cilium tip in response to Hedgehog stimulation. To understand how the unique microtubule-binding features of KIF7 are adapted for its role in Hedgehog signaling, we generated KIF7 variants with altered microtubule interaction phenotypes (no microtubule binding, increased microtubule binding, or microtubule-based motility) and examined their localization and functional output in KIF7-/- MEF cells. We demonstrate that KIF7 motors that lack microtubule binding activity behave similar to wild-type motors in their ability to a) localize to the cilium tip and b) facilitate accumulation of the Gli2 and Gli3 transcription factors at the cilium tip in response to Hedgehog pathway activation. In contrast, KIF7 motors capable of microtubule-based motility can a) localize to the cilium tip and b) drive the localization of Gli2 and Gli3 to the cilium tip even in the absence of Hedgehog pathway activation. Together this work suggests that autoinhibition and the immotile behavior of KIF7 are important for preventing the localization of Hedgehog effectors to the primary cilium in absence of Hedgehog signaling.

SG52

**Plant Kinesins: An Unconventional Bunch**

**G. Goshima**; Nagoya University, Nagoya, JAPAN.

We began to study plant kinesins several years ago, because they had been largely unexplored. Using the moss *Physcomitrella patens* as a model system, we have analysed plant kinesins' sub-cellular localisation and function, and determined their in vitro activities. The data obtained so far have been full of surprises: the knowledge gained on animal kinesins is scarcely applicable to moss orthologues. In this presentation, I will be introducing our recent 'unconventional' findings on kinesin-13 and kinesin-8, well-known microtubule depolymerases in non-plant species.

SG53

**Regulation of the Microtubule Organizing Kinesin HSET by the Cellular "Tubulin Economy"**

**R. Ohi**, E. G. Colicino; University of Michigan, Ann Arbor, MI.

Organization of microtubules (MTs) into higher order structures requires work provided by molecular motor proteins such as kinesins and cytoplasmic dynein. During cell division, the minus end-directed kinesin-14 HSET gathers MT minus ends, an activity that becomes essential in the presence of excess (>2) centrosomes. A non-motor MT-binding domain also enables HSET to cross-link and bundle MTs. How cells harness these activities to organize and remodel the mitotic spindle is not well understood. Since HSET overexpression increases spindle length and tapers spindle poles. Our current understanding of kinesin-14s has benefitted greatly from *in vitro*studies which have shown that fly Ncd and its vertebrate homologues (frog, XCTK2; human, HSET) are non-processive as single-molecules and are capable of organizing MTs into bundles or asters. We have shown that HSET toggles between these activities by associating with non-polymerized (soluble) tubulin, an interaction that causes motor clustering and a dramatic increase in motor processivity (Norris *et al.*(2018)). Based on these findings, we proposed that MT-associated proteins (MAPs) and kinesins that bind both soluble and polymerized tubulin have the potential to be regulated by the tubulin assembly cycle, a concept we term the "*Tubulin Economy*". Our current work is focused on testing this principle, with initial efforts directed at HSET as a model organizational factor. To understand if the ratio between HSET and tubulin concentrations is important for mitotic progression, we analyzed cell division in cells that inducibly overexpress HSET. As observed previously, we found that HSET overexpression causes MTs to taper at the poles. However, we also observed that cells delay in metaphase as a consequence of failed kinetochore-MT attachments. Bundled interpolar MTs also abound in these cells, suggesting that HSET inappropriately stabilizes non-K-MTs through bundling and that soluble tubulin levels are insufficient to drive the formation of proper K-MT attachments. Current efforts are focused on understanding how the non-motor MT-binding site of HSET interacts with tubulin and MTs, which may allow us to generate separation-of-function mutants in cells.

## Subgroup F: Machine Intelligence and Statistics in Cell Biology

SG54

**Revealing Architectural Order with Label-free Imaging and Deep Learning**

S. Guo[1], J. Folkesson[1], A. P. Krishnan[1], I. Ivanov[1], L. Yeh[1], B. Chhun[1], M. Keefe[2], D. Shin[2], N. Cho[1], M. Leonetti[1], T. Nowakowski[2], **S. B. Mehta**[1]; [1]Chan Zuckerberg Biohub, San Frnacisco, CA, [2]University of California, San Francisco, San Frnacisco, CA.

Emergence of architectural order among interacting components is a defining characteristic of living systems. Understanding how physiological functions emerge within cells and tissues and are dysregulated during disorders requires unbiased imaging of interaction among multiple components. Fluorescent labeling of more than 7 components in live cells remains difficult, especially in primary cells and tissues. Label-free imaging provides facile visualization of structures and reports intrinsic physical properties. Advances in deep learning now enable quantitative analysis of structures seen in label-free images.We describe joint optimization of polarization-resolved label-free imaging and deep learning to map architectural order[1] and employ it to study the human brain architecture. Human tissue is not only scarce but cannot be easily manipulated. Immunolabeling of primary tissue is time-consuming, can

introduce sample-to-sample variation, and is not compatible with live imaging. We visualize diverse structures in human brain tissue by mapping optical properties of density, birefringence, orientation, and scattering. We acquire training data by multiplexed imaging of label-free signatures and fluorescent reporters of specific structures. We report computationally efficient variants of U-Nets to predict tract distribution and cell types from intrinsic optical properties of the tissue. Our approach leads to predictive models that generalize to tissue sections not used in the training data. Our approach significantly increases the throughput at which information about brain cytoarchitecture can be acquired from scarce brain tissue samples. We expect that computational label-free imaging will be especially valuable when applied to archival tissue material.[1] Guo, S.-M., Krishnan, A.P., Folkesson, J., Ivanov, I., Chhun, B., Cho, N., Leonetti, M., and Mehta, S.B. (2019). Revealing architectural order with polarized light imaging and deep neural networks. BioRxiv 631101.

SG55

**Robust and Automated Detection of Subcellular Morphological Motifs in 3D Microscopy Images**

**M. K. Driscoll**, E. S. Welf, A. Jamieson, K. M. Dean, T. Isogai, R. Fiolka, G. Danuser; University of Texas Southwestern Medical Center, Dallas, TX.

Rapid developments in live-cell 3D microscopy enable imaging of cell morphology and signaling with unprecedented detail. However, tools to systematically measure and visualize the intricate relationships between intracellular signaling, cytoskeletal organization, and downstream cell morphological outputs do not exist in 3D. Here we introduce u-shape3D, a computer graphics and machine learning pipeline to probe molecular mechanisms underlying 3D cell morphogenesis and to test the intriguing possibility that morphogenesis itself affects intracellular signaling. u-shape3D includes a generic morphological motif detector that automatically finds 3D subcellular surface structures. It does so by decomposing the cell surface into convex patches, optionally merging these patches, and then classifying them by motif. Merging and classification is performed via support vector machines, a machine learning algorithm. Training data is generated in part by presenting users with the segmented cell surface and asking users to click on examples of motifs. We demonstrate the robustness of this motif detector by applying it to diverse systems, including blebs on melanoma and U2OS cells, filopodia on bronchial epithelial and melanoma cells, lamellipodia on dendritic and T cells, microvilli on breast cancer cells, extensions on microglial cells, and spines on neurons. These cells were imaged via various imaging modalities including high-resolution light-sheet microscopes, such as the lattice light-sheet, a standard commercial light-sheet microscope, and a laser scanning confocal microscope. In addition to motif detection, we also provide 3D tools to measure molecular localization near the cell surface, surface motion, and spatial statistics. All of these tools, as well as the motif detector, are accessible via a graphical user interface. As an application, we measure the differential association of $PIP_2$ and $Kras^{V12}$ with blebs on melanoma cells. Both signals polarize with blebs and associate with bleb edges, as expected for membrane-localized proteins, but only $PIP_2$ is enhanced on blebs. That $Kras^{V12}$ and $PIP_2$ spatially segregate with blebs, suggests that blebs may modulate intracellular signaling. Overall, our computational workflow enables the objective, automated analysis of the 3D coupling of morphodynamics with cytoskeletal dynamics and intracellular signaling.

SG56

**Dynamic Allocation of Computational Resources for Deep Learning-enabled Cellular Image Analysis**

D. Bannon[1], E. Moen[1], E. Borba[1], A. Ho[1], I. Camplisson[1], N. Koe[1], D. Kyme[1], B. Chang[1], T. Kudo[2], E. Osterman[3], W. Graf[1], **D. Van Valen**[1]; [1]California Institute of Technology, Pasadena, CA, [2]Stanford University, Stanford, CA, [3]Cloud Posse, Pasadena, CA.

Deep learning is transforming the ability of life scientists to extract information from images. These techniques have superior accuracy in comparison to conventional approaches and enable previously impossible analyses. As the capability of deep learning methods expands, they are increasingly being applied to large imaging datasets. However, the computational demands of deep learning present a significant barrier to large-scale image analysis. To meet this challenge, we have developed DeepCell 2.0, a platform for deploying deep learning models on large (>$10^5$-megapixel images) imaging datasets in the cloud. This software enables turn-key deployment of a Kubernetes cluster on all commonly used operating systems. By using a microservice architecture, our platform matches computational operations with their hardware requirements to reduce operating costs. Further, it scales computational resources to meet demand, drastically reducing the time necessary for analysis of large datasets. A thorough analysis of costs demonstrates that cloud computing for is economically competitive with on premise computing. By treating hardware infrastructure as code, this work foreshadows a new generation of software packages for biology in which computational resources are viewed as a dynamically allocated resource.

SG57

**Faster and Better: Taking Localization Microscopy Into Live Cells**

**S. Cox**; King's College London, London, UNITED KINGDOM.

Super-resolution microscopy is a powerful tool for imaging structures at a lengthscale of tens of nm, but its utility for live cell imaging is limited by the time it takes to acquire the data needed for an image. For localization microscopy the speed at which an image of a given structure can be acquired is directly linked to the structure being imaged, leading to a factor of more than 1000 difference in how fast a particular resolution can be achieved in different types of structure, even given identical performance of dyes and optics. Another result of this is that in almost all localization microscopy datasets there are images where fluorophores overlap. As an initial approach to this problem, we combined principle component analysis and a random forest classifier to allow data from overlapping fluorophores to be removed.However, this approach can only deal with a small degree of overlap. For localisation microscopy the acquisition time can be cut by more than two orders of magnitude by using advanced algorithms which can analyse dense data, trading off acquisition and processing time. Information can be traded for resolution: for example, the whole dataset can by modelled as arising from blinking and bleaching fluorophores (Bayesian analysis of Blinking and Bleaching), although at a high computational cost. However, all these approaches will come with a risk of artefacts, which can mean that the image does not resemble the underlying sample. We have recently developed Harr Wavelet Kernel (HAWK) analysis, a multi-timescale prefiltering technique which enables high density imaging without artefacts. The results of benchmarking with other techniques reveal that at high activation densities many analysis approaches may achieve high apparent precision, but poor accuracy. However, HAWK analysis produces images free from sharpening artefacts allowing accurate images to be rapidly taken. Furthermore, this

property of HAWK can be used to identify artificial sharpening artefacts and assess the quality of localisation microscopy images.

SG58

**Machine Learning Methods for Exploring the Spatial Dimensions of Gene Expression**

A. Imbert[1,2,3], F. Müller[4], E. Bertrand[5], **T. Walter**[1,2,3]; [1]Mines ParisTech, Paris, FRANCE, [2]Institut Curie, Paris, FRANCE, [3]INSERM, Paris, FRANCE, [4]Institut Pasteur, Paris, FRANCE, [5]Institut de Génétique Moléculaire de Montpellier (IGMM), Montpellier, FRANCE.

Messenger RNAs do not always distribute randomly inside cells: in some cases, mRNA molecules localize in specific regions of the cytoplasm, i.e. they distribute according to a specific localization pattern. Subcellular localization of mRNAs is thought of playing an important role for the spatio-temporal control of gene expression. However, the function and mechanism of RNA localization are not yet well understood. In addition, it is still unclear which localization patterns exist and which mRNAs distribute according to which localization pattern.These questions can be addressed by large-scale image-based assays, where individual mRNA molecules are visualized by single molecule FISH (smFISH). Here, we present an analysis framework to automatically analyze smFISH images for cell-based assays in order to infer the localization patterns according to which mRNA molecules distribute in cells. For this, we first segment cells, nuclei and individual RNA molecules using deep learning and traditional image analysis techniques. We then describe the spatial distribution of RNA molecules for each cell with features from spatial statistics, and apply supervised and unsupervised machine learning methods to infer the patterns of RNA localization. The results are validated with both manually annotated data and a simulation framework that we built for this purpose.Furthermore, we show how to use smFISH image simulation in order to train deep neural networks for the recognition of localization patterns. While deep neural networks are the best performing computer vision methods for image classification today, they typically require large amounts of annotated data. This requirement is often unrealistic for biological assays, where imaging protocols and sample preparation are less standardised than in medical imaging. Here, we demonstrate how to overcome the need for massive image annotation by image simulation.Finally, we apply these methods to a dual protein/RNA localization screen. Analysis of this screening data along with a series of follow-up experiments allowed us to identify mRNAs that are translated in specialized translation factories, potentially enabling new gene regulatory mechanisms.

SG59

**Computational Analysis of Cellular Processes Based on Quantitative Imaging Across Scales**

**J. Ellenberg**; EMBL, Heidelberg, GERMANY.

The recent resolution revolution in microscopy technologies allows unprecedented quantitative insights into the dynamic molecular machinery inside living cells. For the first time, imaging technologies have molecular resolving power and sensitivity and can be correlated to cover the whole range from structural detail of single macromolecular machines to imaging the architecture and fluxes of dynamic protein networks. This provides great opportunities for conceptually new insights into the underlying logic of living systems. However, delivering on this potential requires the development of powerful new computational image analysis tools, often based on machine learning, as well as suitable statistical models for four dimensional multimolecular systems in order to come to predictive mechanistic models. Openly sharing the underlying image data is key to fuel the development of such computational tools,

which allow to extract new knowledge and integrate data from many studies. This presentation will give examples at different scales where we have correlatively analysed quantitative live, single molecule, super-resolution and electron microscopy data to study the formation of large protein complexes and protein networks inside cells.

SG60

**Understanding Cell Morphodynamics with Machine Learning**

**B. Sun**; Oregon State University, Corvallis, OR.

Cell shape is an important biomarker that is directly linked to cell function. However, cell morphodynamics, namely the temporal fluctuation of cell shape is much less understood. We study the morphodynamics of MDA-MB-231 cells in type I collagen extracellular matrix (ECM). We employ machine learning to classify cell shape into five different morphological phenotypes corresponding to different migration modes. As a result, cell morphodynamics is mapped into temporal evolution of morphological phenotypes. We systematically characterize the phenotype evolutions including occurrence probability, dwell time, transition flux, and 3D migrational characteristics.We find that manipulating Rho-signaling enhances the morphodynamics and phenotype transitions. Using a tumor organoid model, we show that the distinct invasion potentials of each phenotypemodulate the phenotype homeostasis. Overall invasion of a tumor organoid is facilitated by individual cells searching for and commit-ting to phenotypes of higher invasive potential. In conclusion, we show that 3D migrating cancer cells exhibit rich morphodynamicsthat is regulated by ECM mechanics, Rho-signaling, and is closelyrelated with cell motility. Our results pave the way to the systematic characterization and functional understanding of cell morphodynamics as an new biomarker for normal and malignant cells.

SG61

**Snap47 and Trim67 Alter Mode of Vamp2-mediated Exocytic Fusion in Developing Cortical Neurons**

**F. Urbina**, S. Menon, S. Gupton; University of North Carolina: Chapel Hill, Chapel Hill, NC.

Exocytosis is required in developing neurons to supply material for plasma membrane expansion during morphogenesis. Classically, two modes of fusion have been reported. During full-vesicle-fusion (FVF), the fusion pore dilates and the vesicle collapses into the membrane. During kiss-and-run fusion (KNR) the fusion pore opens transiently for secretion, without adding plasma membrane material. We hypothesized that during neuronal morphogenesis, FVF-like exocytosis predominates to supply membrane material. To test this, we exploited VAMP2-pHluorin as a fluorescent readout of fusion pore opening. Images were analyzed using our automated analysis platform. Here we introduce a novel machine-learning method using multiple classifiers, including hierarchical agglomerative clustering, dynamic time warping, and feature selection with dimensionality reduction for unsupervised categorization of exocytosis. A majority-rule committee of 28 indices selected four discrete classes. This surprising result suggests classes diverging from FVF and KNR exist. All four classes were tetanus sensitive, indicating bona fide VAMP2-mediated fusion. HEPES addition increased the half-life of fluorescence decay in two classes, indicating decay resulting from re-acidification, consistent with KNR fusion. The HEPES insensitive classes displayed diffusion of fluorescence away from the fusion site, consistent with FVF-like behavior. One KNR and one FVF class exhibited immediate fluorescence decay after fusion pore opening, whereas the others demonstrated a delay in decay onset, consistent with the pore remaining open prior to diffusion or resealing. We named the four classes instantaneous FVF

(FVFi), delayed FVF (FVFd), instantaneous KNR (KNRi), and delayed KNR (KNRd). Modes were confirmed by following the fate of VAMP2 using VAMP2-TagRFP and VAMP2-pHluorin and by expressing a VAMP2 peptide that inhibits FVF. Mathematical modeling suggests FVF supplies sufficient plasma membrane material for morphogenesis in developing neurons. Deleting the E3 ubiquitin ligaseTrim67 significantly shifted the mode of exocytosis toward KNR fusion, without altering the frequency of exocytosis. We identified SNAP47 as a t-SNARE that interacts and colocalizes with TRIM67. SNAP47 levels increased upon deletion of Trim67. SNAP47 co-localized with a subset of VAMP2-mediated fusion events. Overexpression of SNAP47 in wildtype neurons partially phenocopied loss of Trim67, increasing KNRd exocytosis, whereas SNAP47 knockdown in Trim67-/- neurons rescued exocytic mode phenotypes. Together these results suggest that TRIM67 alters exocytic mode via SNAP47. This study provides novel quantitative and mechanistic insights into the modes of exocytosis and how they alter neuronal morphogenesis.

SG62

**Space-time-frequency Shape Mapping Reveals Harmonics in Contractile Oscillations During Cytokinesis**

M. E. Werner[1], D. D. Ray[1], C. E. Breen[1], A. Sattler[1], F. Jug[2], **A. S. Maddox**[1]; [1]UNC - Chapel Hill, Chapel Hill, NC, [2]Max Planck Institute of Molecular Cell Biology & Genetics, Dresden, GERMANY.

Cell and tissue shape changes in animals are driven by poorly understood rearrangements of the actomyosin cortical contractile cytoskeleton. In cytokinesis, a cortical actomyosin ring physically partitions one cell into two. To gain novel insights into the mechanisms of cell-autonomous cytokinetic contractility, we used the *C. elegans* zygote as a model cell type and imaged cytokinesis with unprecedented temporal resolution. We found that ring closure speed was not constant but instead underwent repeated cycles of acceleration and deceleration. Closure speed was also non-uniform around the ring and the focus of highest contraction speed traveled circumferentially. To quantify speed oscillations, we combined computational spatial oversampling, continuous wavelet transform, and mode decomposition. We found that the inward displacement dynamics of ring segments were the composite of several co-existing amplitude- and frequency-modulated wave modes with approximately 18, 36 and 72-second periodicity. Fitting to a wave-shape model revealed that the entire range of speed oscillations can be described by a single time-varying amplitude, a single time-varying frequency, and a shape factor, suggesting that speed oscillation are driven by a single oscillator. Last, to retain the spatial relationships among contracting segments of the cytokinetic ring, we performed mode decomposition in three dimensions on a space-time-frequency kymocube of our wavelet transform output. We found three major classes of frequency surfaces varying little over space and time. Principal component analysis of these two-dimensional modes confirmed that the frequencies of contractile oscillations are related as a harmonic and sub-harmonic around a fundamental frequency, which resembles the pacemaker kinetics of the cytokinetic master regulator RhoA. Periodicities of speed oscillations were only subtly changed during ring closure following partial depletion of a panel of conserved actomyosin regulators and structural components, while oscillation amplitudes were suppressed by reduction of force generation, and enhanced by reduction of network crosslinking. Taken together our results suggest that cytokinetic ring constriction is achieved by local oscillations between contraction and remodeling.

SG63

**Spatial Statistics in Bioimage Analysis**

**J. Olivo-Marin**, T. Lagache; Institut Pasteur, Paris, FRANCE.

The quantitative analysis of molecule interactions in bioimaging is key for understanding the molecular orchestration of cellular processes and is generally achieved through the study of the spatial colocalization between different populations of molecules. Most colocalization methods are based on pixel overlap between the previously denoised signal that is emitted from two (or more) different fluorescent labels, and use a global image correlation such as Pearson's or Manders' coefficients. These data, however, cannot be linked to physical parameters such as the real percentage of colocalizing molecules or the average colocalization distance. In addition, randomly distributed molecules can partially overlap, and it is hard to measure the statistical significance of the computed correlation indices. We will present a novel statistical method to analyze molecule colocalization that is based on the automatic detection of molecule fluorescent spots, followed by their representation as Point Processes and the statistical analysis of their spatial distribution. We will illustrate the method through examples in TIRF and 3D-STORM microscopy.

## Subgroup G: New Frontiers in Multifactor Regulation of Cytoskeleton

SG64

**Effects of Neuronal Drebrin a on Actin Dynamics**

**E. Grintsevich**; California State University Long Beach, Long Beach, CA.

Drebrin A is a major neuron-specific actin regulator which is highly enriched in dendritic spines (postsynaptic terminals). Drebrin is critical for synaptic plasticity and function, and its loss is a hallmark of many complex neurodegenerative disorders. It was originally categorized as an actin-stabilizing protein but recently more of its functions were identified. Here, we will focus on the unexpected effects of drebrin on actin dynamics and its newly discovered interaction with formin. Specifically, we found that drebrin can affect actin dynamics by inhibiting elongation of barbed ends of actin filaments. Moreover, drebrin can modulate actin assembly by inhibiting formin-assisted actin polymerization. Molecular details and biological implications will be discussed.

SG65

**Mechanosensation of Tight Junctions by Zo-1 Phase Separation and Flow**

**C. Schwayer**[1], K. Pranjic-Ferscha[1], A. Schauer[1], S. Shami Pour[1], M. Balda[2], M. Tada[3], K. Matter[2], C. Heisenberg[1]; [1]Institute of Science and Technology Austria (IST Austria), Klosterneuburg, AUSTRIA, [2]Institute of Ophthalmology, University College London, London, UNITED KINGDOM, [3]Department of Cell and Developmental Biology, University College London, London, UNITED KINGDOM.

Cell-cell junctions respond to mechanical forces by changing their organization and function. Tension-dependent conformational changes of junctional proteins are thought to underlie this junctional mechanosensitivity. Here we show that in the gastrulating zebrafish embryo, tight junctions (TJ) mechanosensitivity is mediated by actomyosin-driven flow of phase separated Zonula occludens-1 (ZO-1) clusters. We found that ZO-1 junctional accumulation at the contact between the Enveloping Layer (EVL) and the Yolk Syncytial Layer (YSL) closely scales with actomyosin tension. Actomyosin tension

triggers ZO-1 junctional accumulation by driving retrograde actomyosin flow within the YSL that transport non-junctional ZO-1 clusters towards the TJ. Non-junctional ZO-1 clusters form by phase separation, and their effective formation is dependent on the actin binding region (ABR) within the C-terminus of ZO-1. If the non-junctional ZO-1 pool is absent, TJ lose their mechanosensitivity, and, consequently, EVL-YSL movement is impaired. Thus, phase separation and flow of non-junctional ZO-1 confer mechanosensitivity to TJ.

SG66

**Protruding Actin Micropsikes Repair Failing Junctions to Maintain Cell-Cell Adhesion.**

**W. Brieher**, J. X. H. Li, V. Tang; University of Illinois, Urbana-Champaign, Urbana, IL.

Several research groups have reported fast actin turnover dynamics in cohesive sheets of epithelial cells. What is the purpose of all this actin assembly and disassembly in cells that are no longer moving or dividing due to contact inhibition? Using both light and electron microscopy, we have identified a population of protruding actin microspikes that operate continuously along lateral membranes in mature monolayers of kidney epithelial cells in culture (MDCK cells). We identified Arp2/3, EVL, and CRMP1 as three factors necessary for microspike formation. Knocking down the expression of any of these factors results in myosin II dependent tearing of cadherin-cadherin adhesive bonds. It thus appears that cadherin mediated cell-cell adhesion is a highly dynamic process and actin protrusive activity is used to constantly push lateral membranes together to keep the cells in contact and to rapidly repair cadherin adhesive bonds whenever they fail.

SG67

**Regulation of Actin and Microtubule Dynamics by Profilin Isoforms**

A. Henderson, M. Pimm, **J. L. Henty-Ridilla**; SUNY Upstate Medical University, Syracuse, NY.

Profilin proteins are abundant (>100 μM) actin-monomer binding proteins found in nearly all cells and are known to regulate critical cellular processes by modifying the dynamics of the actin and microtubule cytoskeletons including gene transcription, shape determination/morphogenesis, intracellular trafficking, motility, and division. Profilin-1 is the most ubiquitously expressed of the four isoforms and in many cases the most abundant isoform in mammalian tissues. Previous reports suggest that Profilin-1 and Profilin-2 perform redundant roles in cells, however, effects of the loss of these proteins cannot be fully rescued with other homologs. Profilin-1 plays a critical role in distributing actin monomers between different actin nucleation promoting factors and also influences microtubule dynamics through direct and indirect (e.g. formin-mediated) mechanisms. Whether Profilin-2 supports these roles have not been fully resolved. Here we compare the roles of Profilin-1 and Profilin-2 in regulating actin and microtubule structure and dynamics. Using STORM super-resolution microscopy, we visualized each Profilin isoform on actin and microtubule structures in Neuroblastoma-2A (N2A) cells. Specifically, we observed stronger Profilin-1 localization for the actin-rich cell regions compared with Profilin-2 which was more abundant on the sides of microtubules. Using TIRF microscopy we also compared the effects of each Profilin isoform on actin assembly alone and in the presence of actin nucleating formins or the Arp2/3 complex. Each Profilin isoform inhibits the spontaneous assembly of actin filaments, stimulates formin-mediated actin assembly, and inhibits Arp2/3 complex-mediated actin branching. Regardless of the nucleation system, Profilin-2 activities were ~5-fold less efficient and strongly correlated with reduced affinity for actin monomers. These results support the idea that Profilin isoforms influence actin and microtubule

dynamics in neuronal systems with Profilin-1 a stronger regulator of actin assembly compared to Profilin-2, and Profilin-2 is a stronger regulator of microtubule dynamics than Profilin-1.

SG68

**The Drosophila Melanogaster Rab Gap RN-tre Plays a Role in Regulating Non-muscle Myosin Ii Localization and Function**

A. Platenkamp[1], E. Detmar[2], L. Sepulveda[1], A. Ritz[1], S. L. Rogers[2], **D. A. Applewhite**[1]; [1]Reed College, Portland, OR, [2]University of North Carolina, Chapel Hill, NC.

To identify novel regulators of non-muscle myosin II (NM II) we performed a targeted RNAi screen using a stable Drosophila melanogaster S2 cell line expressing EGFP-tagged regulatory light chain (RLC) of NM II and mCherry-Actin. This screen yielded a single hit, the Rab-specific GTPase-activating protein (GAP) RN-tre. Using total internal reflection fluorescence (TIRF) microscopy, we found that RNAi depletion of RN-tre led to a loss of NM II RLC localization in a manner that phenocopied depletion of proteins in the Rho pathway. This loss of localization corresponded to a loss of function as well—depletion of RN-tre led to an increase in actin retrograde flow rates and a decrease in S2R+ cells' ability to constrict in a cellular contractility assay. We next wanted to determine if RN-tre's regulation of NM II is dependent on its GAP activity. Interestingly, over-expression of constitutively active Rabs 5, 6, and 19, all of which are thought to be inactivated by RN-tre, failed to alter NM II RLC localization. Instead, we found that expression of constitutively active Rho, Rho-kinase (Rok), and the phosphomimetic NM II RLC could each rescue the loss of localization following RN-tre depletion. Furthermore, we assayed the cells for phosphorylated RLC by immunostaining and found a substantial decrease in phosphomyosin-positive cells following RN-tre depletion. In addition, depletion of RN-tre led to a decrease in the amount of active Rho as compared to controls. Collectively, our results suggest that RN-tre plays an important regulatory role in NM II RLC distribution, phosphorylation, and function, likely through conserved Rho signaling and is an example of cross-talk between the secretion machinery and actomyosin contractility.

SG69

**Vrp1/WIP Activates Wsp1/WASp Nucleation Promoting Factor Activity At Sites of Endocytosis in Fission Yeast**

**C. D. MacQuarrie**, M. James, V. Sirotkin; SUNY Upstate Medical University, Syracuse, NY.

Arp2/3 complex-mediated branched-actin nucleation is important across species for cell motility and endocytosis. Branched actin assembly is stimulated by Nucleation Promoting Factors (NPFs), such as Wiskott-Aldrich Syndrome protein (WASp). WASp works in complex with WASp Interacting Protein (WIP), which protects WASp from degradation in mammals. Human patients with mutations in either WASp or WIP develop Wiskott-Aldrich Syndrome, a serious immune disorder. An important unresolved question is how branched actin assembly machinery is regulated and positioned to efficiently drive membrane deformation.We investigate mechanisms of branched actin assembly in a favorable model system, endocytic actin patches in fission yeast *S. pombe*. Here WASp homologue Wsp1 remains stable in the absence of WIP homologue verprolin Vrp1, providing an ideal environment to study WIP/Vrp1 function. Wsp1 and Vrp1 exist in a transient complex with myosin-1 Myo1 where Wsp1 and Myo1 activate the Arp2/3 complex to form the branched actin network necessary to internalize endocytic structures. *S. pombe* Vrp1 enhances Myo1 NPF activity *in vitro*, but its physiological role remains unknown. To uncover the role of Vrp1 on actin patch assembly *in vivo*, we generated internal domain

deletions in the *vrp1*$^{+}$ gene, expressed these mutations under the endogenous promoter in cells expressing endocytic proteins tagged with fluorescent mGFP, and examined the effects of these mutations on endocytic patch dynamics using quantitative spinning disk confocal microscopy.We found Vrp1 localization to sites of endocytosis is entirely dependent on its C-terminal WASp Binding Domain (WBD). Additionally, using an *in vivo* protein binding assay, we discovered the Vrp1 Proline Rich Domain (PRD) directly binds the Myo1 tail, independent of Wsp1. When this Wsp1-Vrp1 interaction is disrupted by removing the Vrp1 WBD, the number of actin molecules localized to sites of endocytosis is significantly reduced. Surprisingly, removal of the Myo1-interacting PRD or the actin binding WH2 domains did not impact actin patch accumulation, suggesting Vrp1 activates Wsp1 NPF activity independent of the PRD and WH2 domains. This reduced accumulation of actin correlates with a reduction in the distance endocytic vesicles internalize, suggesting Vrp1 activation of Wsp1 provides force needed for membrane deformation. Further, using a novel *in vivo* actin polymerization assay, we observed the Vrp1 WBD, but not the PRD or WH2 domains, was necessary to enhance Wsp1 mediated actin assembly. This data provides novel evidence that Vrp1 regulates endocytosis by activating Wsp1 NPF activity.

SG70

**Regulation of Inf2-mediated Actin Polymerization through Site-specific Lysine Acetylation of Actin Itself**

**M. A, 03755**; Dartmouth college, hanover, NH.

INF2 is a formin protein that accelerates actin polymerization and also severs actin filaments. A common regulatory mechanism for formins is autoinhibition, through interaction between the N-terminal diaphanous inhibitory domain (DID) and C-terminal diaphanous autoregulatory domain (DAD). We recently showed that INF2 utilizes a variant of this mechanism which we termed ‘facilitated autoinhibition’, whereby a complex consisting of cyclase-associated protein (CAP) bound to lysine-acetylated actin (KAc-actin) is required for INF2 inhibition, in a manner still requiring DID. We also showed that de-acetylation of actin in the CAP/KAc-actin complex activates INF2. Here, we use lysine-to-glutamine mutations as acetyl-mimetics to map the relevant lysines on actin for INF2 regulation, focusing on three positions: K50, K61 and K328. Biochemically, purified K50Q-, K61Q and K328Q β-actin polymerize with similar equilibrium and kinetic properties to WT β-actin or rabbit skeletal muscle (RSK) actin, both in the absence and presence of INF2. When complexed with CAP2, however, K50Q- and K61Q- inhibit RSK-actin polymerization by full-length INF2, but not by INF2 lacking DID. When expressed at low levels in U2OS cells, K50Q- and K61Q-actin inhibit INF2-mediated actin polymerization. Direct binding studies show that the CAP WH2 domain binds INF2-DID with sub-micromolar affinity but has weak affinity for actin, while INF2-DAD binds CAP/K50Q-actin 5-fold better than CAP/WT-actin, suggesting an inhibition model whereby CAP/KAc-actin serves as a bridge between INF2 DID and DAD. In U2OS cells, INF2 is 70 and 8-fold less abundant than CAP1 and CAP2, respectively, suggesting that there is sufficient CAP for full INF2 inhibition.

SG71

**Role of Coronin 7 in Cellular Homeostasis**

**S. Jansen**; Washington University St. Louis, St. Louis, MO.

Coronin 7 (Coro7) is part of a multi-protein family that regulates actin cytoskeleton dynamics, and hence many essential biological processes ranging from cell migration, and intracellular trafficking to T-cell immune response, muscle contraction and wound healing. While the mechanisms and functions of the other Coronins have been studied extensively, the physiological roles and actin-regulatory activities of Coro7 remain largely unknown. This is quite surprising, as Coro7 is structurally unique amongst its family members, suggesting that it might employ different mechanisms than observed for the other Coronins to shape and dynamically remodel actin networks. In addition, Coro7 has been involved in Golgi organization and Golgi to plasma membrane transport, two processes that critically depend on actin cytoskeleton dynamics, although the precise roles and the underlying molecular mechanisms of Coro7 remain to be elucidated. To get a better insight into the function of Coro7 in intracellular trafficking, as well as into its actin-regulatory activities, we studied the real-time dynamics of a fluorescently tagged Coro7. As reported, we found that Coro7 is involved in the transport of many different cargoes from the Golgi, and our preliminary data strongly suggests that this function of Coro7 occurs through the actin cytoskeleton. Interestingly, we also observed that Coro7 became much more dynamic upon starvation of cells, and would accumulate on vesicles reminiscent of autophagosomes. In line with this, these Coro7 coated vesicles were shown to recruit the canonical autophagy markers, p62 and LC3, as well as form on actin scaffolds. We further demonstrated that the formation of autophagosomes as well as autophagic flux are severely affected in cells depleted of Coro7. Altogether, this points to Coro7 as an overlooked actin-regulatory factor that has a central role in maintaining and regulating cellular metabolism, and more work is needed to identify its actin-regulatory partners, and molecular activities.

SG72

**A Clip-170-induced +Tip Network Superstructure Has Characteristics in Cells Consistent with a Liquid Condensate**

**Y. O. Wu**[1,2], G. Fernandes[1], A. T. Bryant[1,2], H. V. Goodson[1,2]; [1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, [2]Integrated Biomedical Sciences Graduate Program, University of Notre Dame, Notre Dame, IN.

Proper regulation of microtubule (MT) dynamics is critical for many cellular processes such as cell division and intracellular transport. Microtubule plus-end tracking proteins (+TIPs) dynamically track growing MT tips and play a key role in this regulation. +TIPs interact with each other in a complex web of intra- and inter- molecular interactions known as the +TIP network. Explanations for the purpose of the +TIP:+TIP interactions include localizing MT regulators to growing MT tip and relieving their autoinhibition. Our group is interested in the possibility that the +TIP network also has a physical function: we suggest that it creates a dynamic assembly that surrounds the fragile MT tip, constraining its structural fluctuations and therefore promoting MT assembly (Gupta, Bioessays. 2014). The observation that many +TIP network proteins are multivalent and contain intrinsically disordered regions suggests that the +TIP network as assembled on MT tips might constitute a liquid condensate (also known as liquid droplet or membraneless organelle). Such a condensate could potentially form a sleeve-like structure at the tip, providing an attractive model for how the +TIP network might physically promote MT polymerization. It is difficult to test this hypothesis directly. However, previous studies

have shown that overexpression of the +TIP CLIP-170 induces large structures that do not co-localize with membrane markers but do contain CLIP-170 and other members of the +TIP network. We hypothesize that these overexpression-induced structures ("patches") might reflect the biological properties of the endogenous +TIP network, and so we are investigating whether they have the properties predicted for liquid condensates.Video microscopy experiments show that the GFP-CLIP-170 induced condensates can elastically deform and undergo fission and fusion. Fluorescence Recovery After Photobleaching (FRAP) experiments demonstrate that CLIP-170 dynamically exchanges both within a patch and between a patch and the cytoplasm. These results indicate that the patches have properties consistent with liquid condensates and are not simply protein aggregates. Immunofluescence experiments show that the patches contain a range of +TIP network proteins but exclude molecules found in other liquid condensates. Taken together, these results indicate that the CLIP-170 induced patches in cells are phase-separated liquid condensates consisting of +TIPnetwork proteins, and they suggest that the endogenous +TIP network might also form liquid droplets at MT tips.

SG73
**Actin Cytoskeleton Self-organization**
**D. Kovar**; University of Chicago, Chicago, IL.

Cells assemble functionally diverse actin cytoskeleton networks with distinct architectures and dynamics to drive fundamental processes such as polarization, endocytosis, motility and division. The specific characteristics of different actin filament networks (actin filament density, organization and dynamics) are determined through the coordination action of specific sets of actin binding proteins (ABPs) with complementary binding properties. Cells typically assemble and use multiple F-actin networks simultaneously within the same cytoplasm. Consequently, F-actin networks must self-organize from a common pool of shared actin monomers and overlapping sets of ABPs. We are investigating the direct and indirect interactions between self-organized F-actin networks, which are critical for establishing their unique identities and functions within a common cytoplasm, and to determine the underlying molecular mechanistic principles that govern these interactions.

## Subgroup H: Nucleoporin Roles in Tissue Architecture, Development, and Genetic Disease

SG74
**Disassembly and Reassembly of the Nuclear Pore Complex in C9orf72 Als/FTD, An RNA Mediated Event**
**J. D. Rothstein**, A. Coyne, B. Zaepfel, L. Hayes; Johns Hopkins University, Baltimore, MD.

Nucleocytoplasmic transport, controlled by the nuclear pore complex, has recently emerged as a pathomechanism underlying neurodegenerative diseases including C9orf72 ALS/FTD. However, little is known about the underlying molecular events. Using super resolution structured illumination microscopy of twenty three nucleoporins in nuclei from C9orf72 iPSC derived neurons and postmortem human motor cortex we identify a unique subset of eight nucleoporins lost from the neuronal nuclear pore complex. POM121, an integral scaffolding nucleoporin, appears to coordinate the disassembly and reassembly of the nuclear pore complex in human neurons impacting nucleocytoplasmic transport, and subsequent cellular toxicity in C9orf72 iPSNs. These data suggest that POM121 is an integral nucleoporin

in the maintenance of the nuclear pore in human neurons and loss of POM121 from the nuclear pore complex, as a result of expanded C9orf72 ALS/FTD repeat RNA, initiates a pathological cascade affecting nuclear pore complex integrity, function, and overall neuronal survival.

SG75

**Nuclear Pore Complexes in the Regulation of T Cell Survival and Function**

J. Borlido, S. Sakuma, M. Raices, **M. D'Angelo**; Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA.

Nuclear pore complexes (NPCs) are multiprotein channels that connectthe nucleus with the cytoplasm.These structures are built by the repetition of 32 different proteins known as nucleoporins or nups. In recent years, it has become evident that the expression of many NPC components varies among different cell types and tissues and that mutations in several nups result in tissue-specific phenotypes. These findings indicate that NPCs can be specialized to play specific roles. What the physiological functions of these cell type-specific structures are remain largely unknown. We recently discovered that the loss of the tissue-specific NPC component Nup210 causes a severe deficit of naïve $CD4^{+}$T cells. Nup210-deficient $CD4^{+}$T lymphocytes develop normally but fail to survive in the periphery due to their inability to transmit tonic T cell receptor (TCR) signals, and to increased sensitivity to Fas-mediated cell. We have now identified that depletion of another NPC component from a different pore domain also significantly reduces the number of naïve $CD4^{+}$T cells. Notably, our evidence suggests that these nucleoporins play distinct roles in maintaining T cell homeostasis. Our results establish NPCs as important cell-intrinsic regulators of T cell physiology and expose these structures as novel players in the adaptive immune system.

SG76

**Nucleoporin Nup88 Is Required for Proper Muscle Differentiation and Neuromuscular Junction Formation**

R. Jühlen[1], V. Martinelli[1], **B. Fahrenkrog**[2]; [1]Universite Libre de Bruxelles, Charleroi, BELGIUM, [2]Universite Libre De Bruxelles, Charleroi, BELGIUM.

Fetal akinesia deformation sequence (FADS) is characterized by reduced fetal movement in utero (fetal akinesia) and considered as neuromuscular disorder. Known genetic causes for FADS are mutations in genes linked to signalling at the neuromuscular junction (NMJ), such as *RAPSN* or *MUSK,* and we have previously shown that bi-allelic mutations in the nucleoporin NUP88 lead to lead to a lethal form of FADS. Here we show that depletion of Nup88 impairs muscle cell differentiation as well as clustering of the acetylcholine receptor (AChR) at the plasma membrane in mice myotubes and in skeletal muscle of *nup88*$^{-/-}$zebrafish. Proper AChR clustering involves podosomes, conical actin-enriched structures at the plasma membrane. Podosome structure in Nup88-depleted myotubes is altered and their number is increased. Similarly, we observed aberrant podosome structure and number in fibroblasts derived from individuals affected by FADS. FADS cells further suffer from abnormal contractile actin-myosin modules and enhanced maturation of focal adhesions due to increased activation of RhoGTPases. Defective actin-myosin organization perturbs also the microtubule and intermediate filament network in FADS fibroblasts. Our results thus establish a consequential link between dysregulation of the actin cytoskeleton, muscle cell maturation and AChR patterning at NMJs. Intact NMJs are critical for proper skeletal muscle contraction and especially for fetal movement. Thus, FADS disorders are likely the result

of pathologic actin microfilament regulation involving proteins responsible for signalling at NMJs and most interestingly, NUP88.

SG77

**Nup133: a Structural Nucleoporin Involved in Kidney and Brain Disorders: Functional Insights From Studies in Embryonic Stem Cells**

A. Berto[1], C. Cianciolo Cosentino[2], S. Pelletier[1], B. Souquet[1], E. Freed[3], E. Lacy[3], J. Loffing[2], S. Neuhauss[2], **V. Doye**[1]; [1]Institut Jacques Monod - CNRS / Université de Paris, Paris, FRANCE, [2]University of Zurich, Zurich, SWITZERLAND, [3]Developmental Biology Program, Memorial Sloan Kettering, New York, NY.

Recent genetic studies of patients with steroid-resistant nephrotic syndrome (SRNS) identified causative mutations in genes encoding structural nuclear pore proteins (nucleoporins) including components of the Y-complex [1-4]. Among these, hypomorphic mutations that reduce levels of Nup107 and Nup133 result in microcephaly in addition to SRNS, a pathology referred to as Galloway-Mowat syndrome [5,6]. However, why mutations that affect ubiquitously expressed nucleoporins produce organ specific disorders remains unknown.Using splice morpholino-mediated gene knockdown in zebrafish, we showed that moderate *nup133* deficiency leads to glomerular damage despite the lack of gross defect in NPC assembly [7]. In contrast, a translation-blocking morpholino that more effectively depletes Nup133 protein caused both glomerular defects and microcephaly [6]. These data indicate that the severity of phenotype and range of tissue involvement depend on the extent of Nup133 depletion or functional alteration; with the kidney being the organ with the highest sensitivity..Previous studies on a null mutation in mice revealed that *Nup133* is essential for embryonic development but not for the proliferation of pluripotent mouse embryonic stem cells (mESCs) [8]. Unexpectedly we found that Nup133 is dispensable for nuclear pore scaffold assembly in mESCs. We also discovered that loss of either full length Nup133 or only its central domain, specifically perturbs the formation of the nuclear basket [9]. Experiments-in-progress using monolayer and embryoid body cultures, found that differentiating *Nup133*$^{-/-}$ ESCs undergo high levels of cell death. However, *Nup133*$^{-/-}$ ESCs escaping cell death properly repress markers of pluripotency and acquire a morphology and marker expression profile suggestive of a differentiated fate. Yet, we have identified a restricted subset of genes differentially expressed between differentiating wild type and *Nup133*$^{-/-}$ ESCs. Findings will be presented from our recent studies investigating the extent to which the specific transcriptome alterations reflect perturbation of nuclear basket assembly.[1] Alazami et al. *Cell Rep* 10, 148-161 (2015)[2] Miyake et al. *Am J Hum Genet* 97, 555-566 (2015)[3] Park *et al Nephrol Dial Transplant* 32, 1013-1017 (2017)[4] Braun et al. *J Clin Invest* 128, 4313-4328 (2018)[5] Rosti et al. *J Med Genet* 54, 399-403 (2017)[6] Fujita et al. *Ann Neurol* 84, 814-828 (2018)[7] Cianciolo Cosentino, Berto et al., *Sci. Reports*, *9:4750* (2019)[8] Lupu et al., *Dev Cell* 14, 831-842 (2008)[9] Souquet et al., *Cell Rep* 23, 2443-2454 (2018)

SG78

**RanGAP Targeting to the Nuclear Envelope Is Essential for Development in *Drosophila*.**

**S. Chen**, M. Lyanguzova, K. Plevock Haase, H. Lee, A. Arnaoutov, M. Dasso; National Institutes of Health, Bethesda, MD.

The Ran GTPase is critical for nuclear-cytoplasmic transport, nuclear envelope (NE) assembly, and mitotic spindle formation. These processes are driven by gradients of GTP-bound Ran (Ran-GTP) and GDP-bound Ran (Ran-GDP). During interphase, Ran’s chromatin-bound nucleotide exchange factor,

RCC1, maintains high levels of Ran-GTP within nuclei. Conversely, Ran's GTPase activating protein, RanGAP, resides in the cytoplasm and promotes Ran-GTP hydrolysis, so that Ran-GDP is predominant in the cytosol. In fungi, RanGAP is diffusely distributed throughout the cytosol. However, multicellular organisms localize RanGAP to the NE. In vertebrates, RanGAP becomes SUMOylated, facilitating its association to the nucleoporin RanBP2 on the cytoplasmic face of the nuclear pore complex (NPC).We used cultured mammalian cells and *Drosophila* to investigate the functional consequences of RanGAP localization in metazoans. We used CRISPR/Cas9 gene editing to mutate human RanGAP (hRanGAP) in DLD-1 cells, preventing its SUMOylation and NPC targeting. Surprisingly, this change had no impact on cell viability and caused no obvious perturbations of nuclear transport or mitosis. The mechanism of *Drosophila* RanGAP (dRanGAP) targeting at the nuclear envelope had not been reported. We found that while dRanGAP associates to the NPC through binding to dRanBP2, the targeting mechanism is distinct from mammals to the extent that it occured via a direct association between dRanGAP and dRanBP2 without involvement of SUMOylation. We identified the domains in both proteins that mediate their binding and used CRISPR/Cas9 gene editing to generate dRanBP2 mutants (dRanBP2$^{short}$) that abolish dRanGAP anchorage to NPCs through a 23-amino-acid deletion. Homozygous RanBP2$^{short}$ mutants exhibited no apparent growth defects as larvae. However, they died as pupae without gas bubble expulsion or eversion of the cephalic complex. This developmental arrest was rescued by a direct fusion of dRanGAP to dRanBP2$^{short}$, indicating that recruitment of dRanGAP to dRanBP2 per se was necessary for the pupal ecdysis sequence during development.Collectively, our results indicate that while the localization of RanGAP to the NE is widely conserved in multicellular organisms, the targeting mechanisms are not. Further, we find a previously unreported requirement for this localization in critical tissue developmental processes, and we are currently working to understand the precise molecular role of dRanBP2-dRanGAP interaction during metamorphosis.

SG79

**Nucleoporin Megator Controls Male X Chromosome Transcriptional Output through Interactions with the MSL Complex**

**J. Aleman**, Y. Lan, J. Gospocic, M. Capelson; University of Pennsylvania, Philadelphia, PA.

The nuclear pore complex (NPC) is well known for its role in nuclear-cytoplasmic transport. A role for the NPC in gene expression has been an emergent topic in recent years as certain nucleoporins have been found in the nuclear interior, binding to chromatin. Megator, (Mtor) makes up the nuclear basket of the NPC and appears to form an intranuclear matrix-like structure that binds along chromatin in Drosophila polytenized salivary gland nuclei. Since Mtor has been implicated in both RNA biogenesis and dosage compensation, I examined the effect of Mtor on localization of a non-coding RNA that is part of the dosage compensation (MSL) complex, roX1. Using RNA FISH in Drosophila salivary gland nuclei, we detected an increase in nuclear soluble roX1 in Mtor-depleted conditions. The increased presence of roX1 was due to increased levels of transcription of roX1 in male nuclei. In addition to roX1, a number of other X-linked genes exhibited a male-specific increase in expression via qPCR assays upon depletion of Mtor. To confirm the male-specific upregulation of X-linked genes, we performed RNA-Seq in male and female salivary glands in both control and Mtor knockdown conditions. Our results confirmed our initial finding - the most notable change in gene expression observed upon Mtor depletion was an upregulation of X-linked genes in males that are also MSL complex targets. Further genetic interaction studies combining Mtor depletion lines with MSL complex component mutations resulted in rescue of the upregulation of X gene expression phenotype as well as enhancement of male viability. These results

suggest that Mtor normal function is to attenuate dosage compensated gene expression. Overall this work uncovers a novel gene regulatory role for a nuclear-scaffold forming nucleoporin in the context of the epigenetic phenomenon of dosage compensation.

SG80

**Elys and Nup153 Anchor the Nuclear Pore Complex to Nuclear Lamins**

**M. Kittisopikul**[1,2], T. Shimi[1,3], M. Tatli[4], Y. Zheng[5], O. Medalia[4,6], K. Jaqaman[2], R. D. Goldman[1]; [1]Northwestern University, Chicago, IL, [2]UT Southwestern Medical Center, Dallas, TX, [3]Tokyo Institute of Technology, Yokohama, JAPAN, [4]University of Zurich, Zurich, SWITZERLAND, [5]Carnegie Institution for Science, Balitmore, MD, [6]Ben Gurion University of the Negev, Beer-Sheva, ISRAEL.

The nucleoporins Elys and Nup153 are localized on the nucleoplasm side of the nuclear pore complex (NPC) where they contribute to the structure of the nucleoplasmic ring and basket of the NPC and may thus interact with the nuclear lamina (NL). The NL contains type-V intermediate filament proteins that assemble into isoform specific fibrous meshworks. We probe how the lamin meshworks influence the distribution of NPCs using 3D structured illumination microscopy (3D-SIM) with sub-pixel computational image analysis. We quantitatively describe how the distribution of NPCs is defined by the lamin meshwork fibers in wild-type mouse embryonic fibroblasts (MEFs), lamin isoform knockouts, and nucleoporin knockdowns. For each case, we determine the distance between NPCs and the lamin fibers by using adaptive resolution multi-orientation image analysis to localize the center of each structure on a subpixel basis with 10 nm precision. The results demonstrate that the centers of a majority of NPCs lie between 60 and 100 nm from lamin A, B1, and B2 fibers, a distance slightly greater than the NPC radius. This analysis reveals that NPCs are laterally associated with lamins A, B1, and B2. In contrast, this spatial relationship is not detected for LC fibers. Relative to LC fibers, NPCs appear to be randomly positioned according to a uniform distribution. Taking advantage of the enlarged meshworks of lamin fibers in lamin A/C (LA/C) and B1 (LB1) knockouts, we show that NPCs follow the lamin fibers forming the perimeter of lamin depleted holes in LB1 and LA meshworks, respectively. When Nup153 and Elys are knocked down, NPCs are shifted away from lamin fibers comprising the sparse meshworks in lamin knockouts and fill gaps created in dense wild-type lamin meshworks. These results suggest that LA, LB1, and B2 act through specific nucleoporins to regulate the normal distribution of NPCs. Cryo-EM tomography combined with immunogold labeling reveals that there are direct physical interactions between lamin fibers and the nucleoplasmic ring of the NPC. Our quantitative approach combining 3D-SIM, cryo-electron tomography and computational image analysis sheds light on how lamins and nucleoporins interact and how the NL is organized. These interactions are pre-requisite to understanding a wide range of diseases, including the laminopathies caused by mutations in lamin A.
Supported by NIH grants RO1GM106023,T32CA080621, and R35GM119619.

SG81

**Differential Turnover of Nup188 Controls Its Levels At Centrosomes and Role in Centriole Duplication**

N. Vishnoi, K. Dhanasekeran, M. Chalfant, I. Surovstev, M. Khokha, **P. Lusk**; Yale University Sch Med, New Haven, CT.

We previously identified a copy number variant in the *NUP188* gene in a patient with Heterotaxy and congenital heart disease, which we linked to a role for Nup188 at the bases of cilia. Nup188 is best understood as a scaffold component of the nuclear pore complex (NPC), but its function at cilia bases

remains ill defined. We have now more fully explored the mechanisms that physically and functionally segregate Nup188 between the pericentriolar material (PCM) and NPCs throughout the cell cycle. Pulse-chase fluorescent labeling approaches indicate that Nup188 populates centrosomes with newly synthesized protein that does not exchange with NPCs even after mitotic NPC breakdown. In addition, the steady-state level of Nup188 at centrosomes is controlled by the sensitivity of the PCM pool, but not the NPC pool, to proteasomal degradation. Proximity-labeling, co-immunoprecipitation and super-resolution microscopy supports that Nup188 interacts with components of PCM including Cep192 and the centriolar satellite component, PCM1. Consistent with this, Nup188 plays a role in centriole duplication at or upstream of Sas6 loading. Together, our data establish Nup188 as a functional component of PCM and potentially provides insight into the pathogenesis of congenital heart disease.

SG82

**FRAGILE X-related Proteins and Dynein Facilitate Interphase Nuclear Pore Assembly Preventing Ectopic Phase Separation of Nucleoporins.**

A. Agote-Arán[1], S. Schmucker[1], K. Jerabkova[1], A. Berto[2], C. Kleiss[1], L. Pacini[3], S. Awal[1], L. Guerard[4], H. Moine[1], J. Mandel[1], S. Jacquemont[5], C. Bagni[6], **I. Sumara**[1]; [1]IGBMC, Illkirch, FRANCE, [2]Institut Jacques Monod, Paris, FRANCE, [3]University of Rome Tor Vergata, Rome, ITALY, [4]Biozentrum, University of Basel, Basel, SWITZERLAND, [5]University of Montreal, Montreal, QC, CANADA, [6]University of Lausanne, Lausanne, SWITZERLAND.

Protein phase separation emerges as a fundamental mechanism controlling cellular homeostasis. Nucleoporins (NUPs) build a highly organized Nuclear Pore Complexes (NPCs) at the nuclear envelope (NE) but several NUPs phase separate into a sieve-like hydrogel within the central channel of the NPCs to regulate the nucleocytoplasmic exchange. In the cytoplasm, a large excess of soluble NUPs has been reported but it is currently unknown how can the cell prevent aberrant phase separation and demixing of the cytoplasmic NUPs. Here we show that Fragile X-related protein 1 (FXR1) can interact with several NUPs and facilitate their assembly at the NE during interphase through a microtubule and dynein-dependent mechanism. Downregulation of FXR1 or closely related orthologs FXR2 and Fragile X mental retardation protein (FMRP) leads to the accumulation of the aggregated NUPs, the Cytoplasmic Nucleoporin Granules (CNGs). Likewise, several models of the Fragile X syndrome (FXS) characterized by a loss of FMRP, display NUP localization defects and accumulation of CNGs. Moreover, the CNGs-containing cells display changes in the nuclear morphology. Our results reveal an unexpected role of FXR protein family and dynein in the spatial regulation of nucleoporin phase separation.

SG83

**TorsinA and Neuronal Nuclear Pore Complex Biogenesis**

**S. Kim**[1], S. S. Pappas[2], S. J. Barmada[1], W. T. Dauer[2]; [1]University of Michigan, Ann Arbor, MI, [2]UT Southwestern, Dallas, TX.

TorsinA is a AAA+ protein localized to the endoplasmic reticular (ER)/nuclear envelope (NE) endomembrane space. A loss-of-function mutation in the gene encoding torsinA causes DYT1 dystonia, a neurodevelopmental movement disorder. Yet, the biological function of torsinA remains largely unknown and the molecular defects underlying DYT1 dystonia remain poorly understood. In mouse primary neurons, we find a dramatic upregulation in nuclear pore complex (NPC) biogenesis during neuronal maturation. In contrast to wild-type neurons, torsinA-null neurons develop increasingly

mislocalized clusters of NPCs. These clusters appear to represent halted intermediate states of NPC assembly, as they contain early- but not late-recruited NPC components. Consistent with this notion, torsinA-null neurons exhibit defects in NPC-mediated nucleocytoplasmic transport. Interestingly, NPC density is unaffected in torsinA-null neurons, suggesting that torsinA helps maintain NPC localization and assembly, but not number. Similar to prior findings *in vivo*, primary cultures of torsinA-null neurons develop evaginations of the inner nuclear membrane (NE buds). The emergence of NE buds coincides with the formation of mislocalized NPC clusters, implicating an association between these events. Considered together, our findings suggest that torsinA plays previously unidentified roles in the localization and assembly of new NPCs during a key period of neuronal development, implicating aberrant NPC biogenesis in the pathogenesis of DYT1 dystonia.

SG84

**Chromatin-bound Nucleoporins Promote Heterochromatin Repair Dynamics**

T. Ryu, C. See, C. Merigliano, C. P. Caridi, D. Arya, **I. Chiolo**; University of Southern California, Los Angeles, CA.

Heterochromatin mostly comprises repeated DNA sequences prone to ectopic recombination. In *Drosophila* cells, 'safe' homologous recombination (HR) repair of heterochromatic double-strand breaks (DSBs) relies on the relocalization of repair sites to the nuclear periphery before Rad51 recruitment and strand invasion. Nuclear actin filaments and myosins actively drive relocalization to nuclear pores and inner nuclear membrane proteins (INMPs), and anchoring to the pores requires the Nup107 complex. We recently discovered an additional function of three nucleoporins in relocalization. Nup88, Nup98 and Sec13 promote relocalization downstream from Smc5/6. Strikingly, the nucleoplasmic function of these components, and not their association with the nuclear periphery, is required for relocalization. Nup88, Nup98 and Sec13 are recruited to heterochromatic DSBs before relocalization, and they are needed to mobilize repair sites (i.e., they mediate movement, rather than anchoring at the nuclear periphery), perhaps through their function in phase separation. Defects in this pathway result in heterochromatin repair defects and instability, revealing its importance in genome integrity. These findings reveal a novel off-pore role of nucleoporins in chromosome dynamics and genome stability in a multi-cellular eukaryote.

## CSCB/ASCB Subgroup: Organelle Membrane Contact Sites and Cell Plasticity Control

SG85

**Architecture of Interfaces between Lipid Droplets Revealed by Electron Cryo-tomography**

**I. Ganeva**[1], K. Lim[2], J. Boulanger[1], P. Hoffmann[1], D. Savage[2], W. Kukulski[1]; [1]MRC LMB, Cambridge, UNITED KINGDOM, [2]Institute of Metabolic Sciences, Addenbrooke's Hospital, Cambridge, UNITED KINGDOM.

Lipid droplets (LDs) are responsible for regulated storage and release of neutral lipids. LDs feature a neutral lipid core enclosed by a phospholipid monolayer and surface proteins involved in lipid metabolism. CIDE proteins govern the number and size of LDs in cells. CIDEC is crucial for fat storage in white adipocytes where it mediates fusion between LDs. This process is fundamentally different from fusion of bilayer-surrounded compartments. CIDEC accumulates at interfaces between LDs and facilitates directional neutral lipid transfer from the smaller to the larger LD. Deciphering the structure

of the LD-LD interface is key in understanding the molecular mechanism of lipid transfer.Here, we use live cell imaging and electron cryo-tomography of vitrified human cells, thinned by focussed-ion beam milling. By live cell imaging we reveal that LD interface formation is a highly dynamic process. LDs contact each other repeatedly before eventually merging. Lipid transfer is completed within minutes. Our electron cryo-tomography shows that at the interface between LDs, the two monolayers are intact and in close apposition. LDs exhibit large-scale shape deformations and adjacent monolayers get as close as 5 nm. Depending on the size difference between the LDs, the interface morphology varies: i) minimal deformation, ii) flattening of LDs similar in size, or iii) smaller LD locally imposing curvature to larger LD. These differences possibly reflect distinct stages of interface formation. The interfaces contain a dense protein layer and apposing monolayers display waviness, indicating local packing disturbances. Currently, we use *in vitro* reconstitution to test if CIDEC is required and sufficient to establish the LD-LD interface architecture we observe *in situ*.Our results suggest that CIDEC-mediated neutral lipid transfer does not occur through a fusion pore formed between two LDs, but through close apposition of two intact monolayers. How the dynamics of contact formation exactly relate to the interface architecture remains to be seen. While the interface organization is conceptionally similar to known organelle contact sites involved in lipid transfer, the distance between the two monolayers is significantly shorter. Local disturbances in phospholipid packing possibly lower the energy barrier and allow neutral lipids to cross from one LD to the other.

SG86

**Organelle Membrane Contacts Revealed by Enhanced FIB-SEM Systems**

**C. Xu**[1], S. Pang[1], G. Shtengel[1], G. Parlakgul[2], A. P. Arruda[2], G. S. Hotamisligil[2], H. F. Hess[1]; [1]Janelia Research Campus, HHMI, Ashburn, VA, [2]Sabri Ülker Center, Department of Genetics & Complex Diseases, Harvard T.H. Chan School of Public Health, Boston, MA.

3D imaging of large volume with fine enough resolution to distinguish a variety of membrane structures is essential to study organelle membrane contact sites. Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) offers superior isotropic resolution better than 10 nm, which is necessary to provide 3D tomography of the cellular ultrastructure and spatial relationship of various intracellular organelles. However, deficiencies of a conventional system in its imaging speed and long-term system stability cap the maximum volume to ~ $10^3$ $\mu m^3$, which is less than ~ 10% of a typical mammalian cell. We have developed the enhanced FIB-SEM platform that accelerates image acquisition and significantly improves reliability of conventional FIB-SEM, expanding the imageable volume by several orders of magnitude to greater than $10^7$ $\mu m^3$ while maintaining an isotropic resolution of 8 x 8 x 8 $nm^3$ voxels. We imaged mouse liver tissues (~$10^6$ $\mu m^3$ each) from four different biological conditions to better understand the specific role of ER-mitochondria contact sites in the control of glucose homeostasis. Dramatic structural changes of ER were observed during the feeding and fasting cycles. Moreover, detailed analysis of segmented FIB-SEM datasets allowed us to quantify the impact of fasting, feeding, and obesity on hepatic ER morphology. Higher resolution further improves the interpretation of otherwise ambiguous details. Nearly all organelles can be resolved and classified with whole cell imaging at 4 nm voxel resolution. We have routinely imaged entire cultured cells at this resolution to study the close contacts among various organelles. At the forefront of 3D imaging innovations, the enhanced FIB-SEM technology pushes the envelope of image acquisition and system reliability, offering a novel package suited for studying organelle membrane contact sites from single cells to large tissues.

SG87

**Phase Separation on Synapse Formation, Transmission and Plasticity**

**M. Zhang**; Hong Kong University of Science and Technology, Hong Kong, HONG KONG.

Neurons are most compartmentalized among all cell types in animals. Emerging evidences in recent years indicate that phase separation is a mean for cells to organize highly condensed biological assemblies with very broad functions and regulatory properties in different subcellular regions. Such condensed assemblies formed via phase separation are termed as biological condensates or memberaneless compartments. Molecular machineries dictating synaptic transmissions in both presynaptic boutons and postsynaptic densities of neuronal synapses are such biological condensates. In this talk, I will discuss how phase separation can build dense synaptic molecular assemblies, highlight unique features of such condensed assemblies in the context of synaptic development and signaling. I will also talk about how Stargazin in complex PSD-95-asembled postsynaptic complexes form highly concentrated and dynamic condensates via phase separation to regulate AMPAR receptor synaptic targeting, clustering and synaptic transmission.

SG88

**Cryo-Electron Microscopy Characterization of Purified Vap-A Engaged in *In Vitro* Membrane Contact Sites**

**M. Dezi**[1], A. Di Cicco[2], E. De la Mora Lugo[2], J. Bigay[3], D. Castano-Diez[4], A. Bertin[2], B. Antonny[3], B. Antonny[3], B. Mesmin[3], D. Levy[1]; [1]Laboratoire Physico Chimie Curie, Institut Curie, PSL Research University, CNRS UMR 168, Sorbonne Université, Paris, FRANCE, [2]Laboratoire Physico Chimie Curie, Institut Curie, PSL Research University, CNRS UMR 168, Sorbonne Universit, Paris, FRANCE, [3]CNRS, Institut de Pharmacologie Moléculaire et Cellulaire, Université de Nice Sophia-Antipolis, Valbonne, FRANCE, [4]BioEM Lab, C-CINA, Biozentrum, University of Basel, Basel, SWITZERLAND.

VAMP-associated protein (VAP) is a small, conserved transmembrane protein present in the ER. VAP-A and VAP-B, two isoforms, interact through a FFAT motif with more than 100 cytoplasmic proteins. Many of them, when complexed with VAP-A or VAP-B, form membrane contact sites (MCS) that connect ER to various organelles [1,2]. These narrow cytosolic gaps (<30 nm) are of key importance for intracellular lipid transport, intracellular signaling, organelle inheritance and lipid metabolism. They have received considerable attention in recent years due to their involvement in certain metabolic diseases. We reconstructed the first functional MCS between ER-like proteoliposomes containing a cytosolic domain of VAP-A and PI4P containing Golgi-like liposomes in presence of the oxysterol binding protein (OSBP). We have shown that in this MCS OSBP is able to transfers cholesterol from ER using the energy associated with counter-transport of PI4P from trans-Golgi apparatus [3,4]. Here, we expressed, purified and reconstituted full length VAP-A in vesicles with variable protein density. Then, we designed an *in vitro* MCS model system to control the formation of contact sites. We combined Cryo-Electron Microscopy and Tomography to describe the architecture of the VAP-A alone or engaged in MCS. Data analysis provided evidence of unexpected features of OSBP and VapA playing an important role in contact formation. First, we shown that the OSBP N-terminus regions act as an entropic barrier to control protein orientation and increase protein dynamic at membrane contact sites [5]. Secondly, Cryo-EM results show a strong flexibility of Vap-A that can span from 8 towards 17 nm depending on local protein density. As a consequence, this behavior enables Vap-A structural adaptation, necessary to form MCS of varying lengths. Moreover, we were able to show that the presence of ORD domain increase

disorder distribution of tethers in the MCS regions that might be necessary to allow ORD movements between two facing membranes.

SG89

**The Interaction between Non-fusogenic Sec22b-syntaxin Complexes and Extended-synaptotagmins Promotes Neurite Growth and Ramification**

A. Gallo[1], F. Giordano[2], L. Danglot[1], T. Binz[3], C. Vannier[1], **T. Galli**[1]; [1]IPNP, INSERM U1266, Paris, FRANCE, [2]I2BC, CNRS, INSERM, Gif-sur-Yvette, FRANCE, [3]Institut für Zellbiochemie, OE4310, Hannover, GERMANY.

Axons and dendrites are long and often ramified neurites that need particularly intense plasma membrane expansion during the development of the nervous system. Neurite growth depends on non-fusogenic Sec22b-Syntaxin1 SNARE complexes at endoplasmic reticulum (ER)-plasma membrane (PM) contacts. Here we show that Sec22b interacts with the endoplasmic reticulum lipid transfer proteins Extended-Synaptotagmins (E-Syts) and this interaction depends on the Longin domain of Sec22b. Overexpression of E-Syts stabilizes Sec22b-Stx association, whereas silencing of E-Syts has the opposite effect. Overexpression of wild-type E-Syt2, but not mutants unable to transfer lipids or attach to the ER, increase the formation of axonal filopodia and ramification of neurites in developing neurons. This effect is inhibited by a clostridial neurotoxin cleaving Stx1, expression of Sec22b Longin domain and a Sec22b mutant with extended linker between SNARE and transmembrane domains. We conclude that Sec22b-Stx1 ER-PM contact sites contribute to PM expansion by interacting with E-Syts.

SG90

**New Insights Into Cholesterol Metabolism: Covalently Linkage to Proteins and Tissue Communication**

**B. Song**; Wuhan University, WUHAN, CHINA.

Hedgehog (Hh) has been known as the only cholesterol-modified morphogen playing pivotal roles indevelopment and tumorigenesis. A major unsolved question is how Hh signaling regulates the activity of Smoothened (SMO). We performed an unbiased biochemical screen and identified that SMO was covalently modified by cholesterol on the Asp95 (D95) residue through an ester bond. This modification was inhibited by Patched-1 (Ptch1) but enhanced by Hh. The SMO(D95N) mutation, which could not be cholesterol modified, was refractory to Hh-stimulated ciliary localization and failed to activate downstream signaling. Furthermore, homozygous SmoD99N/D99N (the equivalent residue in mouse) knockin mice were embryonic lethal with severe cardiac defects, phenocopying the Smo-/- mice.In addition, we identify glycoprotein nonmetastatic melanoma protein B (Gpnmb) as one liver-WAT cross-talk factor in lipogenesis. Hepatic SREBP pathway inhibition leads to increased transcription of the Gpnmb and promotes the processing of the membrane protein to a secreted form. Gpnmb stimulates lipogenesis in WAT and exacerbates diet-induced obesity and insulin resistance. In humans, Gpnmb is tightly associated with body mass index and is a strong risk factor for obesity. Gpnmb inhibition by a neutralizing antibody or liver specific knockdown improves metabolic parameters including weight gain reduction and increased insulin sensitivity, likely by promoting the beiging of WAT. These results suggest that Gpnmb is a liver-secreted factor regulating lipogenesis in WAT, and that Gpnmb inhibition may provide a therapeutic strategy for obesity and diabetes. We will also present our latest progresses on cholesterol metabolism.

SG91

**Lipid Scramblase Tmem16k Is An Interorganelle Regulator of Endosomal Sorting**

**M. Petkovic**, J. Oses-Prieto, A. Burlingame, L. Jan, Y. Jan; University of California, San Francisco, San Francisco, CA.

It is emerging that essential cellular processes can be coordinated via interorganelle communication at membrane contact sites (MCS) between distinct organelles. However, the identity and function of such membrane contact sites in maintaining cellular physiology remain an open scientific question. Here, we report that TMEM16K, an endoplasmic reticulum lipid scramblase linked to spinocerebellar ataxia (SCAR10), is a critical interorganelle regulator of the endolysosomal pathway. We identify endosomal transport as a major functional cluster of TMEM16K in proximity biotinylation proteomics analyses. TMEM16K forms contact sites with endosomes, interacting with the small GTPase RAB7 and phosphatidylinositol 3-phosphate (PtdIns(3)P). Our study further implicates TMEM16K lipid scrambling activity in endosomal sorting at these sites. Absence of TMEM16K leads to dysfunction of the endolysosomal pathway, which human disease point mutants cannot rescue, and progressive neuromuscular impairment in mouse models, consistent with observed human pathology. Thus, TMEM16K-containing ER-endosome contact sites represent clinically relevant platforms for regulating endosomal sorting.

SG92

**Dissecting the Crosstalk between Lysosomes and Mitochondria**

**C. E. Hughes**, T. K. Coody, M. Jeong, J. A. Berg, D. R. Winge, A. L. Hughes; University of Utah, Salt Lake CIty, UT.

Mitochondria perform a wide variety of important cellular functions, ranging from energy production, to metabolism, to innate immune signaling. Mitochondria do not exist in isolation, but in a wider cellular context in which their structure and function depends on the presence of other cellular organelles. Lysosomes, in particular, heavily influence mitochondrial function, and the lysosome-mitochondrial relationship is important for maintaining mitochondrial health. Disruption of this organelle interdependence is thought to be at the heart of cellular aging and cause numerous age-related diseases, but what drives the functional connection between lysosomes and mitochondria remains unknown. We carried out genetic screens in yeast to uncouple lysosomes (vacuoles in yeast) from mitochondria and better understand the mechanics of the vacuole-mitochondria relationship. From these screens, we found that an increase in cellular iron uptake restored mitochondrial respiration in the absence of vacuolar function. Likewise, iron suppressed defects in mitochondrial structure and function brought on by vacuolar dysfunction. These data suggest that the main role of vacuoles in mitochondrial function is maintenance of a pool of bioavailable iron. Consistent with this idea, vacuole-deficient cells are starved for iron, despite being iron replete. The lack of iron bioavailability caused by vacuole impairment was exacerbated by amino acids, in particular, cysteine. Given that an important role of the vacuole is the compartmentation of amino acids, and failure of vacuoles would result in a buildup of cytoplasmic amino acids, we propose that excess cytoplasmic amino acids cause a lack of iron bioavailability which negatively impacts mitochondrial function. How cytoplasmic cysteine limits iron bioavailability is an open question, but our data suggest a role for reactive oxygen species, known disruptors of iron homeostasis, in the process. Together, this work sheds light on the vacuole-

mitochondria connection, and establishes compartmentation of amino acids as an important strategy cells use to limit the toxicity of excess cytoplasmic amino acids.

SG93

**Highspeed GI-TIRF-SIM Microscopy Reveals Extensive Co-assembly of Vimentin Intermediate Filaments with Peripheral ER-matrices**

**A. S. Moore**[1], M. Kittisopikul[2,3], A. Vahabikashi[2], R. D. Goldman[2], J. Lippincott-Schwartz[1]; [1]Howard Hughes Medical Institute, Ashburn, VA, [2]Northwestern University, Chicago, IL, [3]UT Southwestern Medical Center, Dallas, TX.

Vimentin intermediate filaments (VIFs) form an extensive intracellular network that coordinates a diverse list of cellular functions including the regulation of cell shape, force transmission, cytoplasmic mechanics and organelle positioning. Previous work has shown that VIFs surround and tether multiple organelles, including mitochondria, Golgi, lipid droplets, pigment granules and the nuclear envelope. However, if and how vimentin associates with the endoplasmic reticulum (ER), one of the largest and most morphologically complex organelles in the cell, is unknown. Here, using STED, 3D SIM, and grazing incidence TIRF-SIM (GI-TIRF-SIM) microscopy, we find that VIFs form extensive contacts with the ER. In COS-7, U2-OS, and mouse embryonic fibroblasts (MEFs), we observe VIF densities, or “knots,” that co-assemble with and stabilize highly concentrated matrices of ER tubules. Using an adaptive resolution multi-orientation segmentation algorithm, we find that VIF knots remain stably associated with ER matrices as both structures undergo persistent retrograde flow toward the perinuclear region. In peripheral regions devoid of assembled VIFs, we note a clear absence of ER matrices. Thus, we find that VIF knots represent a stable molecular marker for dynamic ER matrices, structures that were previously characterized only by their morphological complexity. Though vimentin frequently co-localizes with other cytoskeletal filaments, we do not observe enhanced F-actin or microtubule density at ER matrices. These results suggest one of three models: that VIFs pattern the morphology and distribution of ER, that ER matrices template VIFs, or both.

SG94

**Lipid Droplet Assembly Factor-1 and Seipin Form a Lipid Droplet Assembly Complex**

**J. Chung**, X. Wu, T. J. Lambert, Z. Lai, T. C. Walther, R. V. Farese Jr.; Harvard University, Boston, MA.

Lipid droplets (LDs) originate from the endoplasmic reticulum (ER) to store triacylglycerol (TG) and cholesterol esters. The ER protein seipin was shown to localize to ER-LD contacts soon after LDs form, but what determines the sites of initial LD biogenesis in the ER is unknown. Here we identify TMEM159, now re-named lipid droplet-assembly factor 1 (LDAF1), as an interaction partner of seipin. Together, LDAF1 and seipin form a ~600kDa oligomeric complex that copurifies with TG. LDs form at LDAF1-seipin complexes, and re-localization of LDAF1 to the plasma membrane co-recruits seipin and redirects LD formation to these sites. Once LDs form, LDAF1 dissociates from seipin and moves to the LD surface. In the absence of LDAF1, LDs form only at significantly higher cellular TG concentrations. Our data suggest that the LDAF1-seipin complex is the core protein machinery that facilitates LD biogenesis and determines the sites of their formation in the ER.

SG95

**Regulation and Compartmentalization of Fatty Acid Metabolism At Membrane Contact Sites**

**H. Hariri**, M. Henne; UT Southwestern Medical Center, DALLAS, TX.

Fatty acids (FA) are toxic to cells and thus sequestered in the form of triacylglycerides in cytoplasmic organelles called lipid droplets (LD) that bud from the endoplasmic reticulum (ER). We recently showed that, in response to nutrient stress, yeast LDs cluster adjacent to the vacuole/lysosome, but how this LD accumulation is spatially coordinated remains unknown. We report that ER-vacuole tether Mdm1 spatially regulates LD biogenesis at ER-vacuole contacts to maintain ER lipid homeostasis and protect the cell from lipotoxicity. Mdm1 binds to LDs and free FAs via its uncharacterized PX-Associated (PXA) domain, and co-enriches with fatty acyl-CoA ligase Faa1 at LD bud sites. Consistent with this, loss of *MDM1* perturbs free FA activation and subsequent TAG generation, elevating cellular FAs that perturbs ER morphology and sensitizes yeast to FA-induced lipotoxicity. Our data support a model where Mdm1 organizes free FA activation adjacent to the vacuole to promote LD production in response to stress. This maintains ER lipid homeostasis by efficiently segregating away toxic FAs into LDs. These findings establish a functional significance for organelle contacts in organizing FA metabolism and lipid flux. We are currently investigating the mechanism by which Mdm1 coordinates the metabolic fates of free FAs at organelle contact sites through a combination of *in vitro* reconstitution, global lipidomics, and state-of-the-art cryogenic focused ion beam (Cryo-FIB) milling and electron microscopy.

SG96

**Emr1 Is Required for the Assembly of the Endoplasmic Reticulum-mitochondria Encounter Structure Complex**

F. Rasul, **C. Fu**; University of Science and Technology of China, Hefei, Anhui, CHINA.

Membrane contact sites allow inter-organelle communication. The endoplasmic reticulum-mitochondria encounter structure (ERMES) complex creates contact sites between the ER and mitochondria, playing crucial roles in mitochondrial fission, mtDNA inheritance, lipid transfer, and autophagy. The mechanism regulating the formation of the ERMES complex is unclear. Here, we show that the uncharacterized mitochondrial membrane protein Emr1 is required for the formation of the ERMES complex. The absence of Emr1 significantly decreases the number of ERMES foci. Moreover, Emr1 interacts with the ERMES core components Mdm12 and Mmm1 and colocalizes with them on mitochondria. Similar to ERMES mutant cells, cells lacking Emr1 display defective mitochondrial morphology and impaired mitochondrial segregation, which can be rescued by an artificial tether capable of linking the ER and mitochondria. We further demonstrates that the cytoplasmic region of Emr1 is responsible for promoting ERMES formation. Thus, this work reveals a crucial regulatory protein necessary for ERMES formation and provides mechanistic insights into understanding the dynamic regulation of ER-mitochondria communication.

SG97

**Architecture and Dynamics of Membrane Contact Sites Involving Osbp**

**B. Mesmin**[1], D. Jamecna[1], J. Bigay[1], D. Kovacs[1], T. Péresse[2], M. Dezi[3,4], J. Polidori[1], M. Subra[1], M. Magdeleine[1], J. Bignon[2], F. Roussi[2], D. Lévy[3,5], B. Antonny[1]; [1]Institut de Pharmacologie Moléculaire et Celluaire, CNRS, Université Côte d'Azur, Valbonne, FRANCE, [2]Institut de Chimie des Substances Naturelles, CNRS, Université de Paris-Saclay, Gif-sur-Yvette, FRANCE, [3]Laboratoire Physico Chimie Curie, Institut Curie, CNRS, Paris, FRANCE, [4]CNRS, Institut de Minéralogie, de Physique des Matériaux et de Cosmochimie, UPMC, Institut de Recherche pour le Développement, MNHN, Paris, FRANCE, [5]Sorbonne Universités, UPMC, Paris, FRANCE.

Oxysterol-binding protein (OSBP) transports cholesterol from the endoplasmic reticulum (ER) to the trans-Golgi network (TGN) and bridges the membranes of these two compartments by binding to phosphatidylinositol 4-phosphate (PI4P) and Arf1 at the surface of the TGN and to the receptor VAP-A at the ER. We have shown that cholesterol transfer by OSBP is spatio-temporally coupled to the turnover of PI4P, which is synthesized by kinases at the TGN, transferred to the ER by OSBP and then hydrolyzed by Sac1 phosphatase. We study the architecture and dynamic properties of OSBP and its partners at ER-TGN membrane contact sites in various cellular models and using reconstituted membrane systems. We have shown that the intrinsically disordered N-terminal region of OSBP controls the distribution and lateral mobility of the OSBP/VAP-A complex within contact sites, thus limiting its density through a crowding effect. We are currently dissecting the OSBP cycle and its consequences on the spatial and temporal organization of membranes at the nanoscale by taking advantage of the intrinsic fluorescence of an OSBP inhibitor and by using various microscopy approaches.

SG98

**Molecular Mechanisms of MTORC1 Signal Regulation At Inter-organelle Contacts**

C. Lim, O. Davis, H. Shin, **R. Zoncu**; University of California, Berkeley, Berkeley, CA.

The molecular mechanisms through which cells sense nutrients remain largely unknown, but their elucidation is key to our understanding of metabolic regulation both in normal and disease states. At the center of nutrient sensing and growth regulation is an ancient protein kinase known as the mechanistic Target of Rapamycin Complex 1 (mTORC1). A central aspect of mTORC1 function that has so far remained poorly understood is its ability to sense lipids. We recently discovered that mTORC1 senses an important lipid, cholesterol, at the lysosome. In particular, addition of cholesterol to cholesterol-depleted cells triggers the recruitment of mTORC1 from the cytoplasm to the surface of lysosomes, concomitant with restoration of the kinase activity of mTORC1. This effect of cholesterol requires the Rag GTPases, which serve as a bridge between mTORC1 and the lysosomal surface. We also identified dedicated lysosomal proteins that convey cholesterol information to the Rag GTPases to trigger mTORC1 activation. Using targeted manipulations of the lipid content of selected organelle populations combined with reconstitution-based assays of mTORC1 activation, we are elucidating key aspects of this newly identified signaling pathway. In particular, I will present ongoing work concerning i) the cellular location of the cholesterol pools that regulate mTORC1 ii) the role of ER-lysosome contacts in relaying cholesterol for mTORC1 activation and iii) the molecular mechanisms through which cholesterol induces mTORC1 recruitment to the lysosomal surface. Moreover, our work is illuminating how aberrant cholesterol sensing by mTORC1 could disrupt cellular quality control and drive the metabolic and neurodegenerative disease, Niemann-Pick type C.

## Subgroup J: Visualizing Immune Cell Activation

SG99

**Mechanism of Chimeric Antigen Receptor (CAR) Signaling**

R. Dong[1], K. Libby[2], R. Vale[1], **X. Su**[2]; [1]UCSF, San Francisco, CA, [2]Yale University, New Haven, CT.

The chimeric antigen receptor (CAR) enables T cells to specifically target and kill cancer cells. Despite of its success in clinical trials, the cellular mechanism of how CARs signal to downstream pathways remains unclear. Here we investigated the molecular mechanism underlying CAR-induced T cell activation. We report that CARs form microclusters as a signaling platform right after antigen engagement. In about half of the cell populations CAR microclusters undergo inward movement to form a disc-like structure in the cell center. This is very similar to the cSMAC that has been reported for the endogenous T cell receptor (TCR). However, in the other half populations, CAR microclusters remain separated, sometimes even undergoing outward movements. These data suggest a more diverse pattern of self-assembly of CAR on the membrane as compared to TCR. Furthermore, we revealed that LAT, a scaffold protein essential for TCR microclusters, is dispensable for forming microclusters and immunological synapses following CAR activation. Consistent with that, the LAT binding partner Gads and SLP76 could be directly recruited to and activated at the CAR microclusters. Together, these data suggest the cytoplasmic domain of CAR, which contains multivalent binding elements for downstream effectors, could serve as a new signaling scaffold, that partially replaces the function of LAT in recruiting and organizing signaling. We will discuss how the rewiring of signaling pathways downstream CAR would affect signaling kinetics and amplification medicating T cell activation.

SG100

**Actomyosin Networks in T Cell and B Cell Function**

**J. Hammer**, J. Wang, D. Schrock; National Heart, Lung, and Blood Institute, NIH, Bethesda, MD.

Upon antigen recognition, T cells exhibit rapid, large-scale reorganizations of their actin and microtubule cytoskeletons that are required for effector functions like the secretion of granules in the direction of the antigen presenting cell (APC). Such events require specialization of the T cell's actin cortex at its site of contact with the APC, commonly referred to as the immunological synapse (IS). Work from many labs including our own has shown that that antigen engagement results in the formation of two distinct actin networks at the T cell IS. The first network, which comprises the outer dSMAC portion of the IS, is composed of a branched actin network generated by the Arp2/3 complex functioning downstream of Rac-GTP/PIP3/WRC/WAVE2 acting at the outer edge of the radially symmetric IS. Once the T cell is fully spread, continued assembly of this network drives its inward/retrograde flow until it disassembles abruptly at the dSMAC/pSMAC boundary. The second network, which comprises the medial pSMAC portion of the IS, is composed of actin arcs generated by the formin Dia1 acting at the IS outer edge. These arcs are organized into concentric, contractile structures by bipolar filaments of myosin 2A and move inward until they disassemble abruptly at the pSMAC/cSMAC boundary. These two networks drive TCR microcluster centralization by distinct mechanisms: direct binding to actin in the branched network comprising the dSMAC and a sweeping/frictional coupling mechanism driven by the actomyosin arcs comprising the pSMAC. Notably, this later contractile structure may also be important for sustained integrin activation required for robust T cell/APC adhesion, and it may be the source of the forces

exerted by the T cell on the APC that enhance target cell killing. Finally, we now find that B cells create very similar actin networks at their IS, and that integrin engagement enhances the formation of their actomyosin arcs, making them the major actin structure at synapses made by primary B cells. We discuss these results in light of the known role for myosin 2-dependent pulling forces in testing BCR: antigen affinity and driving the extraction of antigen from APCs.

SG101

**Biomechanical Profiling of the Immune Synapse in Space and Time**

**M. De Jesus**[1,2], D. Vorselen[3], P. Shah[4], J. Theriot[5], M. Huse[1]; [1]Immunology Program, Memorial Sloan Kettering Cancer Center, New York, NY, [2]Louis V. Gerstner, Jr., Graduate School of Biomedical Sciences, New York, NY, [3]Department of Biochemistry and Howard Hughes Medical Institute, Stanford University, Stanford, Palo Alto, CA, [4]Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, [5]Department of Biology, University of Washington, Seattle, Seattle, WA.

The immune synapse formed by cytotoxic CD8+ T lymphocytes (CTLs) against their target cells is not only biochemically dynamic, but also biomechanically so. Our laboratory has recently uncovered a proportionality between the magnitudes of exerted forces and killing efficiency of CTLs, suggesting that mechanical forces at the synapse are functional elements of the cytolytic program (i.e. their major cellular function). **Thus, we are greatly interested in describing the force patterns of these "killer" immune synapses in space and time, and contrasting them with other classes of lytic and non-lytic T-cell synapses.** To this end, we observe the interactions of T cells with elastic, immunostimulatory hydrogel spheres with very high spatiotemporal resolution. We have thus far discovered that CTLs exert two major classes of forces on these substrates: (1) they burrow into targets to form concave interfaces ("craters"), through which (2) dynamic, actin-based protrusive forces ("fingers") are projected. This may reflect the default mechanical configuration of an immune synapse, and suggests that synapses on real target cells appear flat due to the viscoelastic nature of the target cell cytoplasm. We are currently studying cell-intrinsic and cell-extrinsic regulators of these synaptic forces, and attempting to measure the extent to which these forces are coordinated with biochemical outputs (e.g. granzyme B release). We are also currently profiling the forces exerted by cytotoxic CD8+ and CD4+ T cells, naïve T cells, non-lytic helper T cells, regulatory T cells, natural killer cells, and T cells bearing chimeric antigen receptors (CARs). We are hopeful that this kind of "biomechanical taxonomy" across the various classes and states of T-cells may illuminate some of the ways in which physical and chemical activities are meaningfully integrated by cells of the immune system to perform their many functions.

SG102

**Supramolecular Adhesion Domains and Secretome of the Immunological Synapse**

**M. L. Dustin**; University Oxford/Kennedy Inst Rheumatol, Oxford, UNITED KINGDOM.

T cells communicate through direct cell-cell contact across gaps of only 13 nm (immunological synapse) or through secreted cytokines. I will present data from my lab on the role of CD2 expression in immunological synapse structure and on an intermediate mode of communication based on T cell export of vesicles and non-vesicular particles to antigen presenting cells across immunological synapses. I will present data that CD2-CD58 interactions amplify TCR signals and the low CD2 expression has a similar impact to engaging PD1 on signaling parameters (https://www.biorxiv.org/content/10.1101/589440v1). Vesicles that are generated in the immunological

synapse by budding from the plasma membrane into the extracellular space are referred to as synaptic ectosomes (SYNECT) (https://www.biorxiv.org/content/10.1101/600551v2). SYNECTs combine both antigen recognition and effector function and thus may maintain specificity even if they escape the initial synaptic interaction. In contrast, cytotoxic CD8+ T cells release non-lipid bilayer enveloped cytotoxic microparticles into the synaptic cleft (Balint, Fisher, Kessler. Harkiolaki and Dustin, in preparation). SMAPs are held together by multiple non-covalent protein-protein and protein-carbohydrate interactions and are referred to as supramolecular attack particles (SMAPs). SMAPs lack TCR, but appear to incorporate innate recognition molecules. I will describe the composition, structure and function of SYNECTs and SMAPs and discuss potential applications.

SG103

**Microcluster Formation At Sites of T Cell Activation**

**L. Samelson**; NCI/NIH, Bethesda, MD.

Engagement of the T cell antigen receptor (TCR) results in the formation of microclusters containing many signaling molecules. Subsequent signaling events lead to structural rearrangements that produce an immune synapse between the T cell and antigen presenting cell. Microclusters form within seconds of TCR engagement and are the basic signaling units required for T cell activation. To understand the key events that lead to microcluster formation, we have imaged the transmembrane signaling molecule LAT using high speed, minimally phototoxic lattice light sheet microscopy (LLSM). The increase in temporal resolution afforded by LLSM enabled simultaneous identification of LAT microclusters and intracellular vesicular pools at rates fast enough to image microcluster formation. We also observed the activated T cell surface with higher spatial and temporal resolution using Total Internal Reflection Fluorescence Simulated Interference Microscopy. Using this latter technique, we have demonstrated the formation of sub-domains within microclusters and have followed the kinetics of microcluster formation. Regulation of microcluster formation is currently under investigation.

SG104

**T-cell Priming Is Enhanced by Maturation-dependent Stiffening of the Dendritic Cell Cortex**

**D. Blumenthal**[1,2], J. K. Burkhardt[1,2]; [1]Children's Hospital of Philadelphia, Philadelphia, PA, [2]Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA.

Priming of T-cell responses by dendritic cells (DCs) is essential for protective immunity against pathogen invasion and cancer. T cell activation involves multiple receptor-ligand interactions occurring in concert at a specialized cell-cell contact site termed the immunological synapse (IS). Recently, it has become clear that T cell activation at the IS depends on the application of mechanical forces exerted by the T-cell actin cytoskeleton. Since the DC acts as the substrate for T cell activation, we reasoned that the biophysical properties of the DC cortex are likely to provide important mechanical cues. While conventional imaging approaches have greatly advanced our understanding of T-DC interactions, they do not provide information on the biophysical properties of the cells and their role in relaying mechanical cues at the IS. We therefore turned to atomic force microscopy to measure the cortical stiffness of DCs. We show that as part of the maturation process induced by inflammatory stimuli, DC cortical stiffness is increased 2-3 fold, in an actin cytoskeleton dependent manner. Using pharmacological inhibitors and DCs from KO mice, we identify several actin regulatory pathways downstream of Rho GTPases that govern the maturation-associated modulation of DC stiffness. By

manipulating the stiffness of T-cell substrates using stimulatory hydrogels or DCs expressing mutant cytoskeletal proteins, we show that increasing stiffness over the range observed for DC maturation lowers the agonist dose needed to initiate T-cell activation. Thus, DC cortical stiffness is a novel, biophysical costimulatory mechanism that functions in concert with canonical receptor-ligand signaling cues. Taken together, our data reveals that maturation-associated changes in the DC cytoskeleton alter its biophysical properties to create a platform for enhanced mechanotransduction, facilitating T cell priming.

SG105

**Cd47 Positions Sirpa to Prevent Integrin Activation and Engulfment**

**M. A. Morrissey**, R. D. Vale; UCSF, San Francisco, CA.

Macrophages must engulf dead cells, debris, and pathogens, while selecting against healthy cells to prevent autoimmunity. Healthy cells express CD47 on their surface, which activates the SIRPA receptor on macrophages to suppress engulfment. Cancer cells overexpress CD47 to evade clearance by the innate immune system, making the CD47-SIRPA signaling axis an appealing therapeutic target. However, the mechanism by which CD47-SIRPA inhibits engulfment remains poorly understood. Here, we dissect SIRPA signaling using a reconstituted target with varying concentrations of activating and inhibitor ligands. We find that SIRPA is excluded from the phagocytic synapse between the macrophage and its target unless CD47 is present. Artificially directing SIRPA to the kinase-rich synapse in the absence of CD47 activates SIRPA and suppresses engulfment, indicating that the localization of the receptor is critical for inhibitory signaling. CD47-SIRPA inhibits integrin activation in the macrophage, reducing macrophage-target contact and suppressing phagocytosis. Chemical activation of integrins can override this effect and drive engulfment of CD47-positive targets, including cancer cells. These results suggest new strategies for overcoming CD47-SIRPA inhibition of phagocytosis with potential applications in cancer immunotherapy.

SG106

**The Epithelial Innate Immune System Quantifies Microbe Associated Molecular Patterns through An Epigenetic Digital Signaling Mechanism**

**H. Clark**, C. McKenney, S. Regot; Johns Hopkins, Baltimore, MD.

Epithelial cells perform a delicate role in the innate immune system: they must protect against biological threats without damaging the host or its commensal microbiota. Accordingly, Microbe Associated Molecular Patterns (MAMPs) must be accurately identified and quantified to execute appropriate responses. Here we use live cell imaging of innate immune signaling in mammary epithelial monolayers to study the mechanisms by which tissues and individual cells enforce appropriate quantitative responses to MAMPs and inflammatory cytokines. We conducted a comprehensive screen measuring single-cell nuclear translocation and transcriptional activation of NF-κB upon stimulation of Toll Like Receptors (TLRs) 1/2, 3, and 5, as well as IL-1R and TNFR. Our results show that epithelial cells respond to bacterial MAMPs as a population by differentially activating fractions of cells in an all-or-nothing, digital manner. This digital signaling is maintained through epigenetic mechanisms that regulate receptor expression levels in fractions of cells. This regulation allows epithelial cells to sub-specialize within the context of a monolayer to sense identity and strength of bacterial inputs.

SG107

**Stim1 Associates with Vap B and Regulates Calcium Dynamics.**

**D. Holowka**[1], B. Baird[1], C. Stefan[2]; [1]Cornell University, Ithaca, NY, [2]University Corllege London, London, UNITED KINGDOM.

The store-operated $Ca^{2+}$-entry (SOCE) pathway is integral to the physiology and function of numerous cell types including neurons and immune cells. In many immune cells, the $Ca^{2+}$sensor protein STIM1 is localized throughout the endoplasmic reticulum (ER) upon basal resting conditions. Depletion of $Ca^{2+}$ER stores by FcεRI signaling or the SERCA inhibitor thapsigargin induces STIM1 translocation to the cortical ER where it couples to and activates the $Ca^{2+}$channel protein Orai1 at the plasma membrane (PM). We find that the ER-localized VAP-B protein associates with the STIM1 protein during SOCE in RBL mast cells, as monitored by fluorescence resonance energy transfer (FRET). A dominant negative form of VAP-B (P56S) implicated in amyotrophic lateral sclerosis (ALS) is impaired in this interaction. Moreover, association of STIM1 with Orai1 is reduced by expression of the P56S mutant protein, in parallel with the partial inhibition of SOCE. Likewise, antigen-induced $Ca^{2+}$uptake into mitochondria is impaired in cells expressing the mutant VAP-B (P56S) protein. Consistent with these results, siRNA knockdown of the VAP-A and VAP-B isoforms results in impaired SOCE as well as mitochondrial $Ca^{2+}$uptake stimulated by antigen or thapsigargin. Altogether, our results indicate that the ER-localized VAP-B protein is involved in SOCE and inter-organelle $Ca^{2+}$handling.

SG108

**Netosis Proceeds by Cytoskeleton and Endomembrane Disassembly and Pad4-mediated Chromatin De-condensation and Nuclear Envelope Rupture**

**H. R. Thiam**[1], S. L. Wong[2,3,4], R. Qiu[5], M. Kittisopikul[5], A. Vahabikashi[5], A. E. Goldman[5], R. Goldman[5], D. D. Wagner[2,3,6], C. M. Waterman[1]; [1]Cell and Developmental Biology Center, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD, [2]Program in Cellular and Molecular Medicine, Boston Children's Hospital Boston, Boston, MA, [3]Department of Pediatrics, Harvard Medical School, Boston, MA, [4]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, SINGAPORE, [5]Department of Cell and Molecular Biology, Northwestern University Feinberg School of Medicine, Chicago, IL, [6]Division of Hematology/Oncology, Boston Children's Hospital Boston, Boston, MA.

Neutrophil extracellular traps (NETs) are web-like DNA structures decorated with histones, antimicrobial and cytotoxic proteins released by activated neutrophils. Initially described as a way for neutrophils to trap and neutralize pathogens during innate immunity, NETs are now widely implicated in the detrimental effects of several autoimmune diseases. Peptidylarginine deiminase 4 (PAD4) citrullinates histones and is required for NETs formation (NETosis) in mouse neutrophils. While various molecular mechanisms mediating NETosis are being revealed, the cellular events driving NETs release are still unclear. Here, we determined the sequence of cellular events in NETosis, and examined the role of PAD4 in these events. We performed high resolution time-lapse microscopy of mouse and human neutrophils (PMN) and differentiated HL60 neutrophil-like cells (dHL-60) labelled with fluorescent markers of the cytoskeleton and organelles and stimulated with ionomycin or lipopolysaccharides to induce NETosis. We found that stimulated neutrophils eject NETs after decondensation of the nuclear DNA in the nucleus, rupture of the lamin meshwork and nuclear membrane allowing the release of decondensed DNA to the cytosol, disassembly of the actin, microtubule and vimentin intermediate filaments networks, vesiculation of the endoplasmic reticulum and plasma membrane and finally rupture of the

plasma membrane. More importantly, we found that these cellular pathways occur in a specific and well conserved temporal order suggesting a requirement for a precise sequence for progression through NETosis. Indeed, inhibition of actin disassembly, one of the first cellular events, blocked NETs release. To examine the role of PAD4 in NETosis we isolated neutrophils from PAD4-deficient mice and generated a PAD4-knock down (KD) HL60 CRISPR line. We found that chromatin de-condensation, lamin meshwork and NE rupture and extracellular DNA release required the enzymatic and nuclear localization activities of PAD4. Thus, NETosis proceeds by a well conserved, step-wise sequence of cellular events culminating in the PAD4-mediated expulsion of DNA. Our data further suggest that targeting these cellular pathways might be a better approach for controlling NETosis progression than targeting the various divergent signaling pathways. Indeed, plasma membrane microvesicle shedding, actin and lamin meshwork disassembly, DNA de-condensation, nuclear envelope and plasma membrane rupture are now potential therapeutic targets for NETosis inhibition.

SG109

**Repair of Plasma Membrane Wounds Caused by Bcr-antigen Interaction Is Mediated by Lysosomal Exocytosis and Promotes Antigen Uptake.**

**W. Song**, F. Y. Maeda, J. van Haaren, N. Andrews; University of Maryland, College Park, MD.

B lymphocytes survey antigen through the B cell receptor (BCR), which initiates the activation of B cells to mount antibody responses against infectious agents. Polarization of the BCR and lysosomes towards the antigen interaction site are important steps that lead to antigen acquisition and presentation to T cells. Lysosomal enzymes were proposed to participate in extraction of surface-immobilized antigen by B cells (1), but the trigger for lysosomal enzyme secretion at the B cell immunological synapse remained unknown. Plasma membrane (PM) wounding triggers lysosomal exocytosis, a process required for PM repair in several cell types (2). We previously showed the importance of lysosomal exocytosis in the repair of B cell PM wounds caused by pore-forming toxins (3). This study addresses whether PM wounding occurs when B cells engage antigen, and whether PM wounding and repair affect antigen acquisition. We found that pseudo and bona fide antigen but not transferrin, when immobilized to beads or planar lipid bilayers, wound the B cell PM in an antigen dose-dependent manner. The actin motor non-muscle myosin II (NMII) and the BCR polarize towards antigen interaction sites before PM wounding, and NMII inhibition reduces the number of wounded cells. The lysosomal protein Limp2 is exposed at sites of antigen engagement, and inhibition of lysosomal exocytosis or the lysosomal enzyme acid sphingomyelinase increase the number of wounded cells. Thus, antigen-induced PM wounding is followed by lysosome-mediated PM repair. Antigen internalization occurs after polarization of lysosomes towards antigen-binding and PM wounding sites, and is decreased when antigen-induced PM wounding is reduced by the NMII inhibitor. These results suggest that forces generated by NMII within B cells following engagement of immobilized antigen by the BCR can wound the PM, and that lysosome-mediated wound repair can facilitate internalization of antigen from presenting surfaces. This work is supported by the NIH grant GM064625 to NWA and WS.1. Yuseff, M-I, Reversat, A, Lankar, D, Diaz, J, Fanget, I, Pierobon, P, Randrian, V, Larochette, N, Vascotto, F, Desdouets C, Jauffred B, Bellaiche, Y, Gasman, S, Darchen, F, Desnos, C and Lennon-Duménil, A-M. *Immunity* 35: 361-374, 2011. 2. Reddy, A, Caler, E V and Andrews, N W. *Cell* 106: 157-169, 2001. 3. Miller, H, Castro-Gomes, T, Corrotte, M, Tam, C, Maugel, TK, Andrews, NW and Song, W. *J. Cell Biol.* 211: 1193-1205, 2015.

## Subgroup K: Bacterial Cell Organization

SG110

**Biogenesis and Subcellular Organization of Lipid-bounded Organelles in Bacteria**

**A. Komeili**, J. Wan, C. Grant; University of California, Berkeley, Berkeley, CA.

Lipid-bounded organelles are often cited as the defining feature that separates eukaryotes from the architecturally primitive cells of bacteria. However, numerous bacteria use lipid-bounded organelles to execute essential, and at times toxic, biochemical reactions in a compartmentalized fashion. What are the evolutionary relationships between bacterial and eukaryotic organelles? How do bacterial organelles contribute to the health and survival of organisms in nature? And, can bacterial organelles to be exploited to develop groundbreaking biomedical applications? To answer these questions, my group has pioneered the development of non-standard model systems for the mechanistic study of organelles in diverse microorganisms. In our signature work, we have defined the genetic and biochemical basis for the formation and function of the magnetosomes of magnetotactic bacteria. Magnetosomes are lipid-bilayer invaginations of the cell membrane that direct the formation of nanometer-sized magnetic crystals are produced. Individual magnetosomes are assembled into chains allowing magnetotactic bacteria to use geomagnetic fields as a guide for low oxygen environments. A major thrust of our work is to understand the cell biological features of magnetosomes. Here, I will present our recent work on the molecular mechanisms that regulate the size of magnetosomes and their arrangement within the cell. I will also describe the discovery of a novel iron-accumulating lipid-bounded organelle named the ferrosome that is found in diverse bacteria including resident members of the gut microbiome and opportunistic pathogens.

SG111

**A Bacterial Biomolecular Condensate Sequesters a Signaling Pathway that Drives Spatial Regulation of Asymmetric Cell Division**

**K. Lasker**, L. von Diezmann, W. Moerner, L. Shapiro; Stanford University, Stanford, CA.

Selective recruitment and concentration of signaling proteins within membraneless compartments is a ubiquitous mechanism for subcellular organization. We combined single-molecule tracking and super-resolution microscopy, light-induced spatial mutations, reaction-diffusion modeling, and spatially-resolved transcriptional profiling to study signal exchange in and out of a <200 nm cytoplasmic microdomain at the cell poles of the asymmetrically dividing bacterium *Caulobacter crescentus*. We show that the signaling proteins in a core developmental pathway that culminates in the activation of the cell fate transcription factor CtrA are transiently confined to the polar microdomain. Restricted rates of entry into and escape from the microdomain enhance phospho-signaling, leading to a steep submicron gradient of activated CtrA. Thus, nanoscale protein assemblies can modulate signal propagation with fine spatial resolution, and in *Caulobacter*, this modulation serves to prime asymmetric genome readout.The polar microdomain is defined by self-assembly of the negatively charged, disordered protein PopZ that directly binds signaling proteins *in vivo*. We demonstrate that entry into the PopZ microdomain is selective by showing that cytosolic proteins lacking a microdomain binding partner cannot penetrate the microdomain despite being similar in size and charge to proteins that do enter. These microdomain-excluded-proteins can be recruited to the microdomain through light-induced dimerization between the excluded protein and PopZ, suggesting that microdomain binding

controls entry. Finally, the selective properties of PopZ are independent of geometry: in *Caulobacter* cells with a deformed spherical shape, PopZ becomes a droplet that maintains its selectivity properties.

SG112

**Spatial Regulation of a Biomolecular Condensate in Bacteria**

**A. Vecchiarelli**; University of Michigan, Ann Arbor, MI.

Liquid-liquid phase separation (LLPS) is becoming a well-studied phenomenon in the formation of biomolecular condensates, or "membraneless organelles" in eukaryotic cells. In bacteria, phase-separating proteins are only now emerging. *For all domains of life, little is known about how non-lipid-bound organelles are spatially regulated in the cell*. Almost half of the global $CO_2$ fixation occurs inside two non-lipid-bound organelles: the pyrenoid, found in eukaryotic algae, and the carboxysome, found in cyanobacteria. At the foundation of $CO_2$ fixation in these compartments is the most abundant enzyme on Earth - Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RuBisCO). Microbes evolved a $CO_2$ concentrating mechanism that encapsulates and concentrates RuBisCO with $CO_2$. It has long been thought that the Rubisco matrix in the pyrenoid and carboxysome are crystalline. However, several groups have recently shown that these Rubisco matrices exhibit liquid-like behavior - a paradigm shift and a great leap forward in our understanding of these $CO_2$-fixing organelles. Our lab has identified the two-factor system responsible for the maintenance of carboxysome distribution (McdAB) in cyanobacteria. McdA is an ATPase that forms dynamic protein gradients on the condensed bacterial chromosome (or nucleoid). The McdA gradients emerge in response to a partner protein, McdB, which associates with the carboxysome cargo. The emergent oscillations of McdA on the nucleoid equally spaces carboxysomes down the length of the cell by an unknown mechanism. Without the McdAB system, carboxysomes form asymmetrically-inherited aggregates that are rapidly lost from a cell population. We present data showing that, like its carboxysome cargo, McdB undergoes liquid-liquid phase separation *in vitro*. *We describe the carboxysome and its McdAB system as a tractable model for understanding how ATP-driven protein gradients can control the size, structure, and spatial organization of a biomolecular condensate or "membraneless organelle" in bacteria.*

SG113

***E. Coli*Selectively Restricts Access to Its DNA During Times of Stress**

**E. A. Abbondanzieri**, A. Meyer; University of Rochester, Rochester, NY.

In stationary-phase *Escherichia coli*, Dps (DNA-binding protein from starved cells) is the most abundant protein component of the nucleoid. Dps compacts DNA into a dense crystalline complex and protects it from damage. Dps has also been proposed to act as a global regulator of transcription. We directly examined the impact of Dps-induced compaction of DNA on the activity of RNA polymerase (RNAP). Strikingly, deleting the *dps* gene decompacted the nucleoid but did not significantly impact global patterns of *in vivo* transcription during stationary phase. Complementary *in vitro* analyses demonstrated that Dps does not block RNAP from binding promoters. In contrast, the activity of restriction enzymes decreased sharply with Dps-DNA complex formation. Single-molecule assays further demonstrated that Dps can dynamically condense around elongating RNAP without impeding its progress. Based on these findings, we conclude that Dps forms a dynamic structure that excludes some DNA binding proteins yet allows RNAP free access to the buried genes, a behavior consistent with phase-separated organelles. Dps provides the first identified example of a DNA-binding protein that can completely decouple DNA

condensation from transcriptional regulation, providing bacteria greater freedom to tailor transcriptional responses to various sources of stress while protecting the genome from damage. We propose that Dps achieves this decoupling by creating a phase-separated organelle in bacteria that neither excludes nor strongly concentrates proteins associated with transcription.

SG114

**Diversity, Structure, Function, Assembly & Engineering of Primitive Protein-based Organelles: Bacterial Microcompartments**

**C. Kerfeld**; MSU and LBNL, Berkeley, CA.

Bacterial microcompartments (BMCs), are widespread among Bacteria; they are multienzyme-containing proteinaceous organelles bounded by a selectively permeable protein shell. For example, the carboxysome is a self-assembling metabolic module for $CO_2$ fixation found in all cyanobacteria. These large (~100-500 nm) polyhedral bodies sequester Carbonic Anhydrase and RuBisCO within a protein shell, thereby concentrating substrates and protecting RuBisCO from oxygen generated by the light reactions. In general, BMCs sequester segments of metabolic pathways, sequester toxic and/or volatile intermediates and, essentially function as bacterial organelles. Bioinformatically, we have shown that these organelles are widespread among the Bacterial Kingdom. Because carboxysomes and other BMCs function to organize reactions that require special conditions for optimization, including the sequestration of substrates, cofactors, or toxic intermediates and the protection of oxygen sensitive enzymes, they have received considerable attention as templates for synthetic nanoreactors in bioengineering and as metabolic modules for programming synthetic microbial consortia.

SG115

**Intricate Subcellular Organization and Trafficking During Bacteriophage Replication**

**J. Pogliano**; University of California, San Diego, La Jolla, CA.

Jumbo phages PhiKZ, PhiPA3, and 201phi2-1 assemble a nucleus-like structure upon infection of *Pseudomonas*cells. A proteinaceous shell produced by the phage surrounds the replicating phage DNA and compartmentalizes proteins according to function. Phage and host proteins involved in DNA replication, DNA repair and RNA transcription occur inside the phage nucleus while ribosomes and metabolic enzymes are located in the cytoplasm and excluded from the nucleus. A tubulin based spindle composed of PhuZ monomers assembles early during lytic growth and plays multiple roles during the life cycle of the phage. At early stages of infection, the PhuZ spindle uses dynamic instability to position the phage nucleus at midcell. Later during infection the spindle uses treadmilling to traffic newly assembled capsids to the surface of the nucleus to initiate DNA packaging. Treadmilling PhuZ filaments also rotate the phage nucleus resulting in capsids being distributed around the nucleus surface. We are currently exploring the structure and function of the phage nucleus using a variety of approaches. This highly regulated phage replication system bears resemblance to the eukaryotic nucleus and may bring insight into the evolution of intracellular organization and trafficking.

SG116

**Spatial Organization of Bacterial Cells by the Bactofilin Cytoskeleton**

**M. Thanbichler**; Philipps University, Marburg, GERMANY.

In recent years, it has become evident that bacteria contain a variety of cytoskeletal proteins that are critical for their fitness and survival. These include homologs of actin, tubulin and intermediate filament proteins as well as bacteria-specific factors, such as the bactofilins. Bactofilins constitute a new group of cytoskeletal proteins with a conserved β-helical fold that assemble into stable polymeric structures without the need for nucleotide cofactors. They are almost ubiquitous among bacteria and often present in multiple paralogous copies per species, suggesting that they have critical cellular functions. However, their biological roles are still poorly understood. This talk will highlight recent structural and functional studies on the roles of bactofilin polymers in selected bacterial model organisms. The results obtained indicate that bactofilins serve as multi-purpose cytoskeletal scaffolds that recruit interacting proteins to the (sub)polar regions of the cell, thereby regulating a range of different processes. This functional diversity underscores the complexity of mechanisms that have evolved to control the spatiotemporal organization of bacterial cells.

SG117

**Bidirectional Ftsz Filament Treadmilling Promotes Membrane Constriction Via Torsional Stress**

**D. Ramirez**, A. Merino-Salomon, M. Heymann, P. Schwille; Max Planck Institute of Biochemistry, Munich, GERMANY.

FtsZ is a key component in bacterial cell division, being the primary protein of the presumably contractile Z ring. Reconstituted in vitro, it shows two distinctive features that could so far however not be mechanistically linked: self-organization into directionally treadmilling vortices on solid supported membranes, and shape deformation of flexible liposomes. In cells, circumferential treadmilling of FtsZ was shown to recruit septum-building enzymes, but an active force production remains elusive. To determine direct contributions of FtsZ to membrane constriction, we designed a novel in vitro assay based on soft lipid tubes pulled from FtsZ decorated giant lipid vesicles (GUVs) by an optical tweezers. FtsZ actively transformed these tubes into spring-like structures, where GTPase activity promoted spring compression. Operating the optical tweezers in lateral vibration mode and assigning spring constants to FtsZ coated tubes, we found that that FtsZ indeed exerts pN forces upon GTP hydrolysis, through torsional stress induced by bidirectional treadmilling.

SG118

**Z Ring Assembly Is Regulated by FtsZ Filament Binding Proteins**

**G. R. Squyres**[1], S. R. Barger[2], B. R. Pennycook[3], J. Ryan[4], V. Yan[5], E. C. Garner[1]; [1]Harvard University, Cambridge, MA, [2]SUNY Upstate Medical University, Syracuse, NY, [3]Imperial College London, London, UNITED KINGDOM, [4]Ludwig Maximilian University of Munich, Munich, GERMANY, [5]Technische Universität Dresden, Dresden, GERMANY.

Cell division in bacteria is orchestrated by a group of proteins that work together to carry out cytokinesis and synthesize new cell wall at the division site. Filaments of FtsZ, a bacterial homolog of tubulin, form a “Z ring” at the middle of the cell that recruits other cell division proteins and constricts as the cell divides. While many of these proteins have been identified, the mechanisms of cell division remain poorly understood. Recent work has emphasized the importance of the properties of FtsZ filaments in the cytokinesis process. Using TIRF microscopy, we have shown that FtsZ filaments treadmill around the division site in the gram-positive bacterium *Bacillus subtilis*, and that these dynamics are of key significance in bacterial cell division.Now, we investigate whether and how FtsZ filament assembly and

treadmilling dynamics are regulated. We specifically investigate the FtsZ binding proteins, a group of proteins known to bind directly to FtsZ at the division site. These proteins have been proposed to regulate both FtsZ dynamics and bundling *in vitro*. To investigate this, we use live-cell single molecule lifetime measurements as a quantitative reporter of FtsZ's treadmilling dynamics independent of both spatial resolution and local filament density. We ask whether and how FtsZ binding proteins control filament structure and/or dynamics during the bacterial cell cycle. Surprisingly, our results suggest that these FtsZ binding proteins do not regulate FtsZ filament kinetics directly, but rather mediate Z ring assembly through filament bundling. Functional regulation of filaments by bundling, mediated by accessory filament-binding proteins, is a common feature of eukaryotic cytoskeletal systems, but has rarely been seen in bacteria. We propose that this regulated FtsZ filament bundling is a prerequisite for normal Z ring formation and cytokinesis.

SG119

**Regulating Cell Wall Synthesis for Bacterial Cell Division**

**E. D. Goley**; Johns Hopkins University School of Medicine, Baltimore, MD.

Bacterial cell division is orchestrated by a multi-protein machine (the divisome) centered on the tubulin-like GTPase FtsZ. Cell wall synthases within the divisome synthesize new peptidoglycan (PG) to drive cell envelope constriction. Evidence suggests intimate coupling between the structure and dynamics of FtsZ and the dynamics and activity of PG synthases, but the mechanisms of this coupling are unclear. Using the model alphaproteobacterium *Caulobacter crescentus,* we recently identified an activation pathway from FtsZ through a binding partner, FzlA, to the PG synthases FtsW and FtsI (FtsWI). FzlA is required to activate FtsWI, but also to direct the geometry of PG insertion so that cells maintain their characteristic crescent shape throughout the process of division. FzlA requires both its ability to bind FtsZ and its conserved C-terminus for its role in cell division. Using co-immunoprecipitation, we implicated the conserved DNA translocase FtsK as a second binding partner for FzlA that we hypothesize tranduces a signal from FzlA downstream towards activation of FtsWI. Collectively our results suggest a molecular pathway from FtsZ-FzlA through FtsK to activation of FtsWI for cell wall synthesis during cell division in alphaproteobacteria.

SG120

**A Bifunctional ATPase Drives Tad Pilus Extension and Retraction**

**C. K. Ellison**[1,2], J. Kan[3,4], J. L. Chlebek[1], K. R. Hummels[1], G. Panis[5], P. H. Viollier[5], N. Biais[3,4], A. B. Dalia[1], Y. Brun[6,1]; [1]Department of Biology, Indiana University, Bloomington, IN, [2]Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, [3]Biology Department, CUNY Brooklyn College, Brooklyn, NY, [4]Graduate Center of CUNY, Brooklyn, NY, [5]Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, Geneva, SWITZERLAND, [6]Département de microbiologie, infectiologie et immunologie, Université de Montréal, Montreal, QC, CANADA.

A wide-spread class of prokaryotic motors powered by secretion motor ATPases drive the dynamic extension and retraction of extracellular fibers, such as type IV pili (T4P). Among these, the tight adherence (tad) pili are critical for surface sensing and biofilm formation. As for most other motors belonging to this class, how tad pili retract despite lacking a dedicated retraction motor ATPase has remained a mystery. Here we find that a bifunctional pilus motor ATPase, CpaF, drives both activities through ATP hydrolysis. We show that mutations within CpaF result in a correlated reduction in the

rates of extension and retraction that directly scales with decreased ATP hydrolysis and retraction force. Thus, a single motor ATPase drives the bidirectional processes of pilus fiber extension and retraction.

## Subgroup L: Bottom-Up Cell Biology

SG121

**Recruitment of Mrnas to P Granules by Gelation with Intrinsically-disordered Proteins**

C. Lee, A. Putnam, **G. Seydoux**; HHMI, Johns Hopkins University Sch Med, Baltimore, MD.

Animals with germ plasm assemble cytoplasmic RNA granules (germ granules) that segregate with the embryonic germ lineage. How germ granules assemble and recruit RNA is not well understood. Here we characterize the assembly and RNA composition of the germ (P) granules of *C. elegans*. ~500 maternal mRNAs are recruited into P granules by a sequence independent mechanism that favors mRNAs with low ribosome coverage. Translational activation correlates temporally with P granule exit for two mRNAs that code for germ cell fate regulators. mRNAs are recruited into the granules by MEG-3, an intrinsically disordered protein that condenses with RNA to form nanoscale gels. Our observations reveal parallels between germ granules and stress granules and suggest that cytoplasmic RNA granules are reversible super-assemblies of nanoscale RNA-protein gel condensates.

SG122

**Cell-free Expression of Sun Proteins to Construct Artificial Nuclear Membranes**

**A. Liu**; University of Michigan, Ann Arbor, MI.

The linker of nucleoskeleton and cytoskeleton (LINC) is a conserved nuclear envelope-spanning molecular bridge that is responsible for the mechanical integration of the nucleus (through binding with lamin) with the actin cytoskeleton. LINC complexes are composed of the inner and outer nuclear membrane KASH and SUN proteins, respectively. The direct interaction between KASH and SUN proteins within the perinuclear space of the nuclear envelope is critical for LINC complex-mediated force transmission into the nucleoplasm. Despite recent structural insights into the molecular architecture of the LINC complex, our mechanistic understanding of LINC complex assembly remains limited by the lack of an experimental system for the *in vitro* reconstitution and manipulation of LINC complexes composed of full-length SUN proteins within the context of a lipid bilayer. Here, we describe artificial nuclear membranes (ANMs) as a simple reconstitution platform based on mammalian cell-free expression. By incorporating full-length SUN proteins into lipid bilayer membranes, we demonstrate that SUN1 and SUN2 are oriented in ANMs such that their C-terminal KASH-binding SUN domains are solvent-exposed. In addition, we use ANMs to determine that SUN2 possesses a single transmembrane domain, while SUN1 possesses three. Finally, we show that ANMs containing SUN1 or SUN2 bind synthetic KASH peptides, thereby reconstituting the core of an assembled LINC complex. To our knowledge, this work represents the first *in vitro* reconstitution of LINC complexes in artificial lipid bilayers using cell-free expression, which will be invaluable for testing proposed models of LINC complex assembly and may facilitate *in vitro*reconstitution of mechanotransduction.

SG123

**Life Without Ancestors?**

**P. Schwille**; Max Planck Inst Biochem, Martinsried, GERMANY.

One of the pillars of cell theory is the recognition that any life form, consisting of one or more living cells, results from another living cell. Although this insight was a huge step towards making biology a scientific discipline, and freeing the concept of life from metaphysics and speculation, it left us with a fundamental riddle: how did the first cell, how did life originate? Although we will probably never be able to answer this question historically, it is worth searching for the necessary conditions of a potential transition from a chemical system to a biological system - life without a living ancestor. Using bottom-up synthetic biology of simplistic reconstituted systems, we attempt to identify the fundamental principles of how reaction systems acquire, one by one, the particular features and functions of living systems. Our particular emphasis is on the origin of cell division, and how we could build a minimal system that is able to divide autonomously.

SG124

**Challenges of in Vitro Reconstitution of Kinetochore-Microtubule Attachment: Bi-orientation, Multivalency, Force Sensing**

**A. Musacchio**; Max-Planck-Inst. of Molecular Physiology, Dortmund, GERMANY.

Chromosome bi-orientation is prerequisite to successful cell division. Capture of spindle microtubules takes place at complex protein structures named kinetochores. Kinetochore activity is subject to a feedback control mechanism, referred to as error correction, which promotes the selective stabilization of bi-oriented attachments while preventing the stabilization of incorrect ones. This mechanism is believed to respond to a sensor monitoring the consequences of forces acting on kinetochores when they are bi-oriented (correct attachments) or not (incorrect or incomplete attachments). The molecular details of this force sensor and of its downstream responses remain largely unclear. Focusing on the human kinetochore as model system, we have taken a biochemical reconstitution approach to dissect this very complex question. Our medium- to long-term goal is a comprehensive reconstitution of kinetochore function that may collectively involve ~100 constitutive and regulatory proteins. On the shorter term, we are focusing on various unresolved questions, including the rather obscure implications of kinetochore multivalency for microtubule binding. Each microtubule-binding site contains approximately 8 copies of the 4-subunit Ndc80 complex, the primary microtubule binder. Using a controllable multimerization approach, we demonstrated that Ndc80 multivalency promotes dramatic increases of the microtubule-binding affinity, as well as an improved ability to track depolymerizing microtubules and to capture force generated during depolymerization (Volkov *et al*. eLife 2018; Huis in ‘t Veld *et al*. 2019, BioRxiv, https://doi.org/10.1101/675363). During bi-orientation, kinetochore composition matures: certain proteins are shed and others acquired. Among the latter is the 3-subunit Ska complex, also a microtubule binder. Previous work had shown that Ska recruitment requires Ndc80, but the interaction had not been reconstituted in vitro, leading to hypothesize that force is required to expose an otherwise cryptic binding site. Contrary to this, we identified conditions for robust binding of Ndc80 and Ska in absence of force (Huis in ‘t Veld et al. 2019, *op. cit*.), suggesting that Ndc80 is not a force-controlled direct binding platform. In addition, while control of Ska recruitment in vivo depends on a short “loop” region in the Hec1 subunit of the Ndc80 complex, the loop is dispensable for the interaction with Ska *in vitro*, pointing to its fundamental role in signaling bi-orientation. In our current

pipeline, we perform experiments in vitro with defined protein compositions, and re-introduce recombinant proteins in cells via electroporation to assess the match of observations in vitro and in vivo. The considerable potential of this approach will be discussed.

SG125

**Realtime Chromosome Assembly on Naked DNA in *Xenopus* Eggextract**

**M. Sun**[1], C. Bustamante[1], T. Hirano[2], R. Heald[1]; [1]UC Berkeley, Berkeley, CA, [2]RIKEN, Wako, JAPAN.

In eukaryotic cells, a variety of cellular processes, such as transcription, DNA replication, take place in the content of chromatin. Due to the processivity of those events, chromatin structure is required to be highly dynamic. Also, high-order chromatin structure changes dramatically throughout the cell cycle. Precise packaging of DNA molecules and faithful structural rearrangement of high-order chromatin structures are absolutely essential for cell division and its nuclear functions. However, due to its complexity, the dynamic process of DNA folding and chromatin assembly has not been directly observed. In our study, we developed an *in vitro*cell-free system in *Xenopus*egg extract, in which real-time chromatin assembly and force-induced disassembly is directly monitored using optical traps. We report real-time stepwise DNA compaction dynamics upon introduction of high-speed egg extract at low tension (1-2 pN), as well as the complete stepwise disassembly under high tension (10-15 pN). Moreover, we compared chromatin assembly/disassembly dynamics in the presence and absence of ATP. The DNA compaction in the absence of ATP indicates the direct binding of key chromosomal proteins (such as histones) to DNA; whereas the DNA compaction in the presence of ATP yields to more dynamic behaviors. We observe a much higher degree of DNA compaction in metaphase extract compared to that of interphase extract. Moreover, we performed immunodepletion of key chromosomal proteins, such as core histones, and linker histone H1 in *Xenopus*egg extract. We observed that with nucleosome depletion, chromatin condensation process slows down dramatically. Surprisingly, the final degree of condensation is similar in nucleosome depleted extract, compared to that in the mock depletion control. However, chromatin assembled in nucleosome-free extract is structurally much less stable, and opens up spontaneously without additional external force. Our results shed light on chromatin dynamics in different orders of chromatin assembly.

SG126

**Actin Bundle Assembly by Formins and Mechanics**

**A. Jegou**, E. Suzuki, J. Chikireddy, G. Romet-Lemonne; Institut Jacques Monod - CNRS, PARIS, FRANCE.

Formins are central players in the assembly of most actin networks in cells, but how their activity is affected by the geometrical constraints imposed by the network architectures, such as filament crosslinking and formin spatial confinement, is still unclear. We have combined microfluidics and micropatterning techniques to investigate in vitro the dynamics of actin filament bundles induced by fascin and elongated by mDia1 formins. We have measured the impact of geometrical constraints on formin elongation rates and processivity. We show that filament bundling by fascin reduces formin activity. Strikingly, when formins are surface-anchored and elongate cross-linked filaments, formin elongation rate immediately decreases and processivity is greatly reduced, depending on the cumulative impact of formin rotational and translational freedoms, revealing an unexpected crosstalk between the constraints at the filament and the formin levels. Those results highlights the importance of molecular details of the formin anchoring to the plasma membrane in cells to efficiently modulate formin activity.

SG127

**Excitable Extract Makes Waves**

**J. Landino**[1], M. Leda[2], A. Michaud[3], W. Bement[3], A. Vecchiarelli[1], A. Goryachev[2], A. L. Miller[1]; [1]University of Michigan, Ann Arbor, MI, [2]University of Ediburgh, Edinburgh, UNITED KINGDOM, [3]University of Wisconsin, Madison, WI.

Reconstitution of biological systems is an essential tool for uncovering the molecular mechanisms that underlie cellular function. Here we describe the development of an *ex vivo* system that reconstitutes cortical excitability on a supported lipid bilayer. In cells, cortical excitability is a phenomenon characterized by waves of active Rho (Rho-GTP) and actin polymerization that are thought to “prime” cells to initiate actomyosin-dependent events such as cytokinesis and cell migration. Our group has previously characterized cortical excitability in developing *Xenopus laevis* embryos and starfish oocytes[1]. We found that cortical waves are amplified by overexpression of the RhoGEF Ect2 and inhibited by actin filaments (F-actin). These results suggest that cortical excitability exists as an activator-inhibitor system, where both positive (Ect2-dependent) and negative (F-actin-dependent) feedback loops exist simultaneously to induce wave formation and propagation. We have generated an excitable *ex vivo* system using actin-intact *Xenopus* egg extract on a supported lipid bilayer[2]. These components sufficiently reconstitute a robust wave of Rho-GTP followed by a wave of actin polymerization. The phase shift between the waves of Rho-GTP and F-actin is comparable to *in vivo* cortical waves. This single wave, however, is quickly extinguished by the formation of a dense network of bundled actin filaments, which inhibits continued wave propagation. We find that adding a drug that inhibits actin polymerization creates an environment where multiple waves of Rho-GTP can travel, unobstructed, on the bilayer. These propagating waves of active Rho have a regular periodicity and exhibit patterning that closely resembles F-actin waves in *Xenopus* embryos. This work raises new questions about F-actin-mediated negative feedback in cortical excitability; for example, how is active Rho “turned off” in the absence of actin polymerization? Additionally, the excitable extract system provides a powerful environment for investigating the role of RhoGAPs, select lipids (e.g. $PI(4,5)P_2$, $PI(3,4)P_2$), as well as other downstream effectors (Anillin, Myosin II), in mediating negative feedback that regulates cortical wave patterning.1. Bement *et al*. (2015) Activator-inhibitor coupling between Rho signaling and actin assembly make the cell cortex an excitable medium. *Nat Cell Biol*. 17(11): 1471-1483. 2. Nguyen *et al*. (2014) Spatial organization of cytokinesis signaling reconstituted in a cell-free system. *Science*. 10; 346 (6206):244-7.

SG128

**Studying Self-organized Pattern Formation with Human Embryonic Stem Cells**

**A. Warmflash**; Rice University, Houston, TX.

The ability of stem cells to self-organize in vitro provides a promising avenue for understanding embryonic patterning that is complementary to in vivo studies. Moreover, such in vitro systems may be the only way to understand human development due to ethical and practical considerations. We have shown that a combination of geometric confinement and application of signaling molecules induces human embryonic stem cells (hESCs) to undergo self-organized developmental patterning. Treatment of hESCs confined to circular colonies with BMP4 leads to patterns consisting of extraembryonic cells and the three embryonic germ layers organized along the radial axis of the colony. Patterning is controlled by a combination of the colony boundary and waves of endogenous Wnt and Nodal signaling. I will

discuss the contributions of the boundary, these two signaling waves, and cell movements to the resulting cell fate patterns in these colonies. These analyses suggest that the dynamics of the waves, rather than static signaling gradients, contain the information for pattern formation.

SG129
**Self-organization in Intestinal Organoid Development**
**P. Liberali**; Friedrich Miescher Institute, Basel, SWITZERLAND.

Intestinal organoids are complex three-dimensional structures that mimic the cell-type composition and tissue organization of the intestine by recapitulating the self-organizing ability of cell populations derived from a single intestinal stem cell. Crucial in this process is a first symmetry-breaking event, in which only a fraction of identical cells in a symmetrical sphere differentiate into Paneth cells, which generate the stem-cell niche and lead to asymmetric structures such as the crypts and villi. Here we combine single-cell quantitative genomic and imaging approaches to characterize the development of intestinal organoids from single cells. We show that their development follows a regeneration process that is driven by transient activation of the transcriptional regulator YAP1. Cell-to-cell variability in YAP1, emerging in symmetrical spheres, initiates Notch and DLL1 activation, and drives the symmetry-breaking event and formation of the first Paneth cell. Our findings reveal how single cells exposed to a uniform growth-promoting environment have the intrinsic ability to generate emergent, self-organized behaviour that results in the formation of complex multicellular asymmetric structures.

## Subgroup M: Building Complexity to Understand the Microtubule Cytoskeleton: From Regulation of Microtubule Dynamics to Coordination of Motor Ensembles

SG130
**Harnessing the Dynamic Microtubule End: Lessons From Reconstruction Studies in Vitro**
W. Luo[1], Q. Shen[2], C. Lin[2], **E. L. Grishchuk**[1]; [1]University of Pennsylvania, Philadelphia, PA, [2]Yale University, New Haven, CT.

Dynamic microtubule end is a highly complex assembly of tubulin dimers with different conformations and biochemical composition. Various microtubule-associated proteins can bind to and modify these variable structures, regulating dynamics of the microtubule ends and attaching them to specialized cellular sites, such as the kinetochores of mitotic chromosomes. To learn about how microtubule ends behave when bound to kinetochores, we reconstruct these interactions using purified tubulin and microtubule-binding proteins with the help of various motility assays. A promising novel approach to reconstruct the microtubule end-attachment sites with defined molecular compositions is to employ DNA origami scaffolds, which can be programed to have precisely controlled stoichiometry and spatial positioning of conjugated proteins. We synthesized a ring-shaped DNA origami with the outer diameter ~60 nm, which roughly corresponds to the area for a microtubule end binding at the kinetochore. These scaffolds are visualized via fluorescent labels and they can be immobilized on coverslip surface via biotin linkages. Using SNAP proteins fused to GFP-binding nanobodies, we conjugated 10-20 purified GFP-tagged proteins to each scaffold, forming the coverslip-immobilized multimolecular clusters of microtubule-binding proteins. Clusters of Ndc80 complexes, the major microtubule-binding kinetochore component, support lateral diffusion of stabilized microtubules and show weak binding to the microtubule ends, as expected for this microtubule wall-binding protein. However, clusters of the TOG-

domain protein CLASP2, which is required for tubulin incorporation at the kinetochore-bound microtubule ends, exhibit strong binding specifically to the plus-ends of stabilized microtubules. We show that CLASP2 recognizes the nucleotide-dependent conformation of the terminal tubulin dimers, providing a novel molecular mechanism to suppress microtubule catastrophe and promote tubulin flux at mitotic kinetochores.

SG131

**The Mitotic Crosslinking Protein PRC1 Acts as a Viscous Dashpot Against Relative Microtubule Sliding**

**S. Forth**; Rensselaer Polytechnic Institute, Troy, NY.

Cells use cytoskeletal networks to perform complex physical tasks throughout diverse processes such as the assembly of the mitotic spindle during cell division. These dynamic networks consist of microtubules that are organized, bundled, and transported by motor and non-motor proteins that produce 'active' pushing and 'passive' frictional forces to achieve proper assembly and perform mechanical work. How these mesoscale forces are regulated at the micron-scale by ensembles of nanometer-sized proteins has been unclear. We are addressing this knowledge gap by directly measuring force production across microtubule bundles using optical tweezers and simultaneously observing by single molecule fluorescence microscopy the localization of key proteins that regulate network stability. Here, we demonstrate that ensembles of PRC1, an essential non-motor crosslinking protein needed both to assemble bridging fibers during metaphase and build the central spindle in anaphase, are viscous frictional elements whose resistance to motor-driven microtubule sliding scales linearly with velocity and local protein concentration. Our direct experimental measurements and computational simulations describe how PRC1 molecules can both diffuse within the overlapping microtubule network and track a partially reflective barrier at microtubule plus-ends in order to act as a viscous dashpot against microtubule sliding motions. This mechanical property can then help to establish stable rates of both chromosome and pole separation. These results set the groundwork for understanding higher-order microtubule networks as "machines" that use simple rules to modulate their force production and control the spatiotemporal organization of the dynamic cytoskeleton.

SG132

**Kinesin-5 Mechanisms in Bipolar Mitotic Spindle Assembly**

**M. Betterton**; University of Colorado-Boulder, Boulder, CO.

Kinesin-5 motors are essential to bipolar mitotic spindle assembly in many organisms. Kinesin-5 homotetramers crosslink antiparallel microtubules and step toward microtubule plus ends to slide apart the microtubules and separate mitotic spindle poles. Therefore, kinesin-5 plus-end-directed motility has been considered its key mitotic role. This model of kinesin-5 function is at odds with observations that kinesin-5s traffic in both directions along microtubules and localize at spindle poles, near microtubule minus ends. How kinesin-5 bidirectional trafficking and spindle-pole localization contribute to bipolar spindle assembly are not fully understood. We are using an interdisciplinary approach of cell biology, genetics, imaging, and computational modeling to study kinesin-5 function. In our computational model of fission-yeast spindle assembly, simulated spindles do not become bipolar if kinesin-5 is purely plus-end directed. This occurs because minus-end-directed motility enhances kinesin-5 spindle-pole localization important for proper force generation to separate spindle poles. In fission-yeast cells, the kinesin-5 motor Cut7 moves bidirectionally on spindle microtubules, consistent with previous work in

vitro. In addition, spindle-pole separation to form a short bipolar spindle can occur when most motors remain localized at one spindle-pole body. Our results suggest that kinesin-5 bidirectional motility and spindle-pole localization contribute to force generation to assemble a bipolar spindle.

SG133

**Building a Functional Kinetochore: From Microtubule to Centromere**

**G. E. Hamilton**[1], L. A. Helgeson[1], E. I. Henry[1], A. Zelter[1], M. J. MacCoss[1], C. A. Asbury[1], Y. N. Dimitrova[2], T. N. Davis[1]; [1]University of Washington, Seattle, WA, [2]Genentech Inc., South San Francisco, CA.

Perhaps the largest and most intricate of all microtubule-associated protein machines, the kinetochore consists of over fifty different proteins organized into subcomplexes. Their architecture is branched, with more copies of the outer microtubule-binding subcomplexes than centromeric nucleosomes at their base. The kinetochore performs vital mechanical functions during mitosis: coupling chromosome movement to microtubule dynamics and sensing the tension that signals chromosome biorientation. Much is known about the microtubule-coupling interface of the kinetochore, and previous work has demonstrated that the Ndc80 subcomplex binds to microtubules and transmits force to the Mis12 subcomplex (Mis12c$^{MIND}$). However, it has been unclear how the distinct, underlying branches within the centromere-proximal inner kinetochore contribute to the kinetochore's load-bearing ability. Here, using a set of six recombinantly purified budding yeast kinetochore subcomplexes, we show that they can spontaneous self-assemble into functional, load-bearing tethers between dynamic microtubule tips and nucleosomes containing the centromere-specific histone variant. We quantify the load-bearing and microtubule tip-coupling abilities of these reconstituted kinetochores using an optical trap and demonstrate that there are two distinct and essential paths of force transmission through the inner kinetochore. These two paths, one through CENP-QU$^{OA}$ and the other through CENP-C$^{Mif2}$, are stronger in combination than alone. Moreover, our work corroborates the finding that the Mis12c$^{MIND}$-inner kinetochore interface is regulated by the major mitotic kinase, Aurora B, which alleviates autoinhibition within Mis12c$^{MIND}$. Intriguingly, these two paths of force transmission through the inner kinetochore appear differentially sensitive to Aurora B phosphorylation. Taken together, this work represents a major advance towards the goal of reconstituting a complete, functional kinetochore from purely recombinant proteins.

SG134

**Biochemical Reconstitution of Branching Microtubule Nucleation**

**R. Alfaro-Aco**, A. Thawani, S. Petry; Princeton University, Princeton, NJ.

The microtubule cytoskeleton supports cell function by giving cells their shape, organizing their interior and segregating chromosomes. Microtubules are nucleated from specific locations at precise times in the cell cycle, and several of these microtubule nucleation pathways converge to form a particular architecture in the cell. Although many microtubule nucleation pathways have been identified, it remains poorly understood which factors comprise them and how they shape the microtubule cytoskeleton. Here, using purified proteins we biochemically reconstitute branching microtubule nucleation, a nucleation pathway where microtubules originate from pre-existing microtubules, which is essential for spindle assembly and chromosome segregation in many cell types and species. We found that besides the microtubule nucleator gamma-tubulin ring complex (γ-TuRC), the two branching effectors augmin and TPX2 are required to efficiently nucleate new microtubules from pre-existing ones.

TPX2 has a key role in this process, as its binding sites along the microtubule generate regularly-spaced patches that serve as the sites of microtubule nucleation by recruiting augmin and γ-TuRC. We found that the preferred branching angle was less than 90 degrees, and the absence of TPX2 resulted in shallower branch angles. Our results demonstrate how the microtubule nucleator γ-TuRC is brought to its site of nucleation to cause branching microtubule nucleation, a process critical for spindle assembly and axon architecture. We anticipate our results to help explain how other microtubule nucleation pathways give rise to a cell's microtubule cytoskeleton. Furthermore, our work serves as a stepping stone to reconstitute complete microtubule structures such as the mitotic spindle and to engineer specific microtubule architectures in health and disease.

SG135

**TinA Enables Kinesin-14/KlpA for Spindle Pole Localization**

G. Feng[1], A. Popchock[1], X. Xiang[2], **W. Qiu**[1]; [1]Oregon State University, Corvallis, OR, [2]Uniformed Services University of the Health Sciences, Bethesda, MD.

Kinesin-14s are C-terminal kinesin motor proteins that function to anchor the microtubule minus ends at the microtubule organizing center (MTOC; the spindle pole body in fungi and the centrosome in animal cells) and thus play important roles in mitotic spindle assembly. However, how kinesin-14 motors localize to the MTOC remain poorly understood. Our previous work revealed that unlike all other kinesin-14s, kinesin-14/KlpA from *Aspergillus nidulans* uniquely exhibits plus-end-directed processive motility on single microtubules as individual homodimers. Here, we found that KlpA localizes preferentially to the MTOC in wildtype A. nidulans but decorates the entire length of spindle microtubules with no preferential localization in mutant *A. nidulans* lacking a KlpA cofactor protein called TinA. Using total internal reflection fluorescence microscopy, we further found that purified TinA directly interacts with KlpA in vitro and causes KlpA to switch direction and exhibit processive minus-end-directed motility on single microtubules. Collectively, our results show that TinA is a key cofactor protein for KlpA localization to the spindle pole body. Ongoing work in the lab is focused on dissecting the molecular and structural basis of the KlpA-TinA interaction.

SG136

**Slow Microtubule Binding Kinetics of Membrane-bound Kinesin Predicts High Motor Copy Numbers on Intracellular Cargo**

**R. Jiang**[1], S. Vandal[2], S. Park[1], S. Majd[3], E. Tüzel[2], W. O. Hancock[1]; [1]Pennsylvania State University, University Park, PA, [2]Worcester Polytechnic Institute, Worcester, MA, [3]University of Houston, Houston, TX.

Bidirectional vesicle transport along microtubules is necessary for cell viability and function, particularly in neurons. When multiple motors are attached to a vesicle, the distance a vesicle travels before dissociating is determined by the race between detachment of the bound motors and attachment of the unbound motors. Motor detachment rates ($k_{off}$) can be measured via single-molecule experiments, but motor reattachment rates ($k_{on}$) are generally unknown, as they involve diffusion through the bilayer, geometrical considerations of the motor tether length, and the intrinsic microtubule binding rate of the motor. To understand motor attachment dynamics during vesicle transport, we quantified the microtubule accumulation rate of fluorescently-labeled kinesin-1 motors in a 2D system where motors were linked to a supported lipid bilayer. From the first-order accumulation rate at varying motor

densities, we extrapolated a $k_{off}$ that matched single-molecule measurements, and measured a two-dimensional $k_{on}$ for membrane-bound motors binding to the microtubule. Using a theoretical model to interpret our data and a computational model to simulate our experiments, this $k_{on}$ is consistent with kinesin-1 being able to reach roughly 20 tubulin subunits when attaching to a microtubule. By incorporating cholesterol to reduce membrane diffusivity, we demonstrate that this slow $k_{on}$ is not limited by the motor diffusion rate, but instead is determined by the intrinsic motor binding rate. For intracellular vesicle trafficking, this two-dimensional $k_{on}$ predicts that long-range transport of membrane-bound vesicles requires at least 5 motors for vesicles in the 30 nm range and 800 motors for vesicles in the 500 nm range.

SG137

**Motor-specific Regulation by Maps - Tau and Map7 Differentially Regulate Kinesin and Dynein Motors to Direct Transport of Intracellular Cargoes**

**A. R. Chaudhary**[1], L. Balabanian[1], H. Lu[2], K. M. Trybus[2], A. G. Hendricks[1]; [1]McGill University, Montreal, QC, CANADA, [2]University of Vermont, Burlington, VT.

Cargoes are shuttled to different locations in the cell along microtubules by plus-end directed kinesin and minus-end directed dynein motors. While many cargoes are transported by similar sets of kinesin and dynein motors, they exhibit different transport characteristics and are localized differently in the cell. Emerging evidence suggests that microtubule associated proteins (MAPs) regulate kinesin and dynein to target intracellular transport. Two neuronal MAPs, tau and MAP7, control the motility of organelles by regulating the forces exerted by kinesin and dynein teams. We isolated endogenous cargoes driven by teams of kinesin-1, kinesin-2, and dynein motors and reconstituted their motility in vitro. On tau-decorated microtubules, we observe more minus-end directed motility, while cargoes moving along MAP7 decorated microtubules demonstrate more plus-ended motility. Furthermore, the specific set of motor proteins associated with a cargo determine how its transport will be affected by MAPs. Early endosomes are enriched for kinesin-1, while kinesin-2 is the dominant plus-end directed motor on late endosomes. Previous studies have shown that tau inhibits kinesin-1 more strongly than kinesin-2 or dynein. Accordingly, we observe that early endosomes are more sensitive to tau expression in COS-7 cells. Collectively, our results indicate that MAPs can differentially regulate the load-dependent binding and unbinding rates of specific motor proteins to enable cargo trafficking to precise locations in the cell.

## Subgroup N: Epithelia and Their Stem Cells

SG138

**Regulation of Cell Migration, Cell Adhesion, and Cytoskeletal Dynamics During Epithelial Morphogenesis**

**A. Ewald**; Johns Hopkins University, Baltimore, MD.

Epithelial tissues are a fundamental building block of metazoan organs and also the most frequent site of cancer in humans. They are initially specified in the embryo but are capable of dramatic changes in structure and function during adult development, injury, and malignant progression. We seek to parse epithelial morphogenesis to a discrete series of generative cell behaviors and to resolve the regulation of these cell behaviors to spatiotemporal dynamics in molecular activity and force generation. We use

the murine mammary gland as a model system as it provides abundant tissue and powerful genetics, is capable of reinitiating morphogenesis in response to hormone and growth factor signals at any age, and is amenable to systematic analysis both in vivo and in real-time in 3D culture. We recently used a combination of molecular biosensors, force inference methods, and finite element modeling to produce a basic model of how cells elongate epithelial tubes through a combination of asymmetric cell division, directed cell migration, and radial intercalation. We are presently focused on understanding the role of: TGF-beta mediated regulation of migration speed in bifurcation of epithelial tubes, E-cadherin based cell adhesion in mediating cell survival, and of both the actin and microtubule cytoskeletons in regulating epithelial architecture and migration dynamics. In each topic, we iterate between normal and neoplastic development in order to more precisely understand both the normal mechanisms of organogenesis and how these mechanisms are coopted in disease.

SG139

**Patterning Principles of the Mammalian Small Intestine**

**K. McKinley**[1,2], X. Qiu[1], D. Yang[1], F. de Sauvage[3], J. Bush[1], O. Klein[1], R. Vale[1,2]; [1]UCSF, San Francisco, CA, [2]Howard Hughes Medical Institute, San Francisco, CA, [3]Genentech, South San Francisco, CA.

The mammalian small intestine exhibits a remarkable capacity for renewal during homeostasis and regeneration after injury. This capacity is powered by stem cells, which are housed at the base of invaginations of the epithelium, called crypts. Remarkably, following stem cell loss due experimental and pathological assaults, cells that have already made fate commitments can revert back into stem cells to restore the stem cell pool. However, the mechanisms that direct certain cells to revert to stemness have been elusive. Existing models suggest that specific cell types are responsible for restoring the stem cell pool, either as designated “reserve” or “revival” stem cells, or due to intrinsic features such as chromatin organization. I will describe our recent work using live imaging of organoids to determine the mechanisms that re-establish stem cells at the base of crypts during regeneration.

SG140

**Patterning Collective Cell Motion in Epithelial Morphogenesis**

**D. Devenport**; Princeton University, Princeton, NJ.

How cells assemble into precise spatial patterns from undifferentiated progenitors is a fundamental but still poorly understood question in developmental biology and tissue engineering. Using the mouse embryonic skin as a model system, which is decorated with regularly spaced, globally polarized hair follicles (HFs) that arise through self-organized epidermal-dermal signaling and planar polarized morphogenesis, we have established methods to perform long-term live imaging of epidermal development to capture the individual and collective cell behaviors that drive polarized morphogenesis of mammalian hair follicles. Using cell tracking methods to monitor the behaviors of every cell within developing hair placodes over the course of polarization, our live imaging approach revealed an unanticipated and novel pattern of collective cell movements that generates both morphological and cell fate asymmetry of developing follicles. Here we show how spatial patterning of hair follicle progenitors through Wnt and Shh pathways establish a morphogenetic program of collective cell motion. We show unanticipated robustness in this morphogenetic program, though feedback between cell fate specification and cell motility.

SG141

**Visualizing Stem Cell Dynamics During Tissue Maintenance in Living Epithelia**

C. K. Brock, S. T. Wallin, O. E. Ruiz, K. M. Samms, A. Mandal, E. A. Sumner, **G. T. Eisenhoffer**; 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. Dying cells provide instructive cues that can influence surrounding cells to proliferate, but how these signals are transmitted to their healthy neighbors to control individual cellular behaviors and population dynamics during tissue homeostasis and regeneration remains poorly understood. To visualize the removal and replacement of stem cells within a living epithelium, we induced death specifically in a subset of fluorescently labeled stem cells in the developing zebrafish epidermis and used high-resolution time-lapse imaging to observe the dynamics of the remaining healthy stem cells to proliferate and sustain tissue homeostasis. Combining this approach with pharmacological and genetic perturbation facilitated the identification of novel mechanisms required to induce stem cell divisions that restore cell numbers in the tissue. Our data 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 induced 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.

SG142

**Mechanisms of Stable Force Transmission in Contractile Epithelia**

**A. C. Martin**, C. Ko; Massachusetts Inst Technol, Cambridge, MA.

During development, tissues are sculpted into forms that are important for their ultimate function. These changes in tissue shape are powered by force propagated between cells, which generate collective cell behavior. We have investigated mechanisms that enable force to be stably transmitted between presumptive *Drosophila* mesoderm cells, cells that invaginate during early development. Using live cell imaging at high time resolution and pharmacological and genetic perturbations, we have determined that the attachment of the apical actomyosin network to adherens junctions is dynamic during apical constriction. We showed that apical actomyosin networks release adherens junctions and that this connection is rapidly reestablished through actin turnover. Interestingly, we found that these dynamics are regulated by an apical microtubule cytoskeleton. This apical microtubule cytoskeleton is organized by a non-centrosomal microtubule-organizing center that forms from actomyosin contraction. We are further investigating the dynamics of this attachment and mechanisms that regulate detachment or reattachment.

SG143

**Collective Mapk Signaling Dynamics Coordinates Epithelial Homeostasis**

T. Aikin, A. Peterson, M. Pokrass, H. Clark, **S. Regot**; Johns Hopkins University, Baltimore, MD.

Epithelial tissues are constantly challenged by individual cell fates such as apoptosis or extrusion while maintaining the barrier function. During oncogenesis, cell behaviors and signaling states also differ between mutant and normal cells but little is known about their communication. Previous studies have shown that oncogene expressing cells in epithelial monolayers are often extruded. However, the role of neighboring cells remains controversial. Here we show that the temporal patterns of MAPK activity are decoded by the ADAM17-EGFR paracrine signaling axis to coordinate directed migration of neighboring cells towards shedding cells promoting extrusion of aberrantly signaling cells. Interestingly, both cell populations trigger ERK signaling but with qualitatively different temporal dynamics. Concurrently, neighboring cells increase proliferation to maintain cell density while the oncogene expressing cells undergo cell cycle arrest. Furthermore, we found that either sustained or pulsatile activity of the stress MAPK p38 elicits the same paracrine signaling response suggesting that this pathway constitutes a quality control mechanism to eliminate and replace unfit cells from the epithelial monolayer. Our results demonstrate that MAPK signaling dynamics simultaneously regulates individual cell fates and the paracrine signaling necessary to coordinate collective cell behavior at the tissue level.

SG144

**Mechanical Regulation of Epithelial Branching Morphogenesis**

**C. M. Nelson**; Princeton University, Princeton, NJ.

The morphogenetic patterning that generates three-dimensional epithelial tissues requires dynamic concerted rearrangements of individual cells with respect to each other. We have developed microfluidic approaches to investigate the mechanical forces and downstream signaling responsible for generating the airways of the lung. I will discuss how we combine these experimental techniques with computational models to uncover the physical forces that drive development of complex epithelial geometries. I will also describe efforts to uncover and actuate the different physical mechanisms used to build the airways in lungs from birds, mammals, and reptiles.

SG145

**Morphogenetic Control of Epithelial Topology**

**K. Ishihara**[1,2], A. Mukherjee[2], E. Gromberg[3], T. Krammer[3], M. Shahbazi[4], M. Zernicka-Goetz[4], J. Brugués[1,2], F. Jülicher[2], E. Tanaka[3]; [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]Research Institute of Molecular Pathology, Vienna, AUSTRIA, [4]University of Cambridge, Cambridge, UNITED KINGDOM.

The epithelium is a fundamental tissue architecture that defines the boundary of many organs. While past studies have proposed how changes in the cell's mechanical properties induce local shape changes, the statistical properties of epithelial tissues have not been addressed. What are the cell biological and physical conditions that determine whether an epithelium remains connected, or divides into multiple, topologically distinct epithelia? To address this issue, we study epithelial morphogenesis by culturing mouse embryonic stem cells as free-floating 3D aggregates and differentiating them to neuroepithelia.

Within 4 days, a continuous apical membrane domain forms in the interior of the tissue as a result of collective cell polarization and epithelialization. Treatment with retinoic acid induces the apical membrane to split up into multiple spherical structures, or fluid-filled cysts. We hypothesize that apical surface area and its topology are controlled by retinoic acid-mediated down regulation of PODXL, an apical membrane protein with a negatively charged extracellular domain. Indeed, PODXL heterozygote cells show fragmented apical surfaces in the absence of retinoic acid, and PODXL overexpression show continuous epithelium overcoming the effect of retinoic acid. We develop a biophysical framework that connects the mechanics of epithelial cells to the statistical mechanics of fluid membranes. Our theory allows us to predict epithelial topology from the balance of actomyosin contractility and repulsion of PODXL molecules at the apical membrane. Thus, we elucidate the cell biological basis for retinoic acid-mediated morphogenesis, and propose that epithelial self-organization can be conceptually understood in analogy to how surfactant molecules self-assemble.

## Subgroup O: Lipids and Proteins in the Secretory Pathway- Homeostasis and Stress

SG146

**The Role of Calnexin in Regulating ER Proteostasis of RESET Substrates**

N. Sharma, N. M. Lott, D. Mandal, **P. Satpute-Krishnan, 20817**; Uniformed Services University, Bethesda, MD.

RESET is a protein quality control pathway that clears diverse misfolded GPI-anchored proteins, including human disease mutants of prion protein (PrP) [1], and select transmembrane proteins out of the endoplasmic reticulum (ER) to the Golgi. RESET contrasts with ER associated degradation and autophagy pathways that retain misfolded proteins for degradation at the ER. During RESET, misfolded proteins are released by the ER-resident chaperone, calnexin (CNX), and bound by p24-family members for vesicular transport to the Golgi. The misfolded proteins subsequently transit the cell surface en route to lysosomes where they are destroyed. Thus, steady-state turnover of RESET substrates is concomitant with ER-export. However, physiological and chemical ER stressors dramatically enhance ER-export and consequent lysosomal degradation.Here we address the questions (i) what are the mechanisms regulating constitutive ER-export of RESET substrates during steady-state conditions and (ii) how do ER-stress conditions enhance ER-export of RESET substrates for subsequent degradation? For these studies, we combined imaging with biochemical analysis, including pulse-chase and pull-downs, using a previously characterized misfolding variant of PrP (PrP*[1]) as a model substrate. Our results suggest that the flux of RESET is regulated by competition of other unfolded/misfolded proteins for CNX-binding under steady-state conditions. Chemically blocking new expression of CNX substrates dramatically inhibits steady-state ER-export and degradation of CNX substrates. Conversely, an increase of competitor binding interactions with CNX during physiologically induced ER-stress conditions, such as during the upregulation of specific secretory pathway proteins, increases the rate of ER-export and degradation of RESET substrates. We present a new model that displacement from CNX by newly synthesized CNX substrates regulates the constitutive and stress-enhanced turnover of RESET substrates.[1] Satpute-Krishnan, *et al*. ER stress-induced clearance of misfolded GPI-anchored proteins via the secretory pathway. Cell. 2014; **158**:522-533

SG147

**Ufmylation of Rpl26 Links Translocation-associated Quality Control to Endoplasmic Reticulum Protein Homeostasis**

L. Wang, Y. Xu, **Y. Ye**; National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

**Protein biogenesis at the endoplasmic reticulum (ER) in eukaryotic cells is monitored by a protein quality control system named ER-associated protein degradation (ERAD). While there has been substantial progress in understanding how ERAD eliminates defective polypeptides generated from erroneous folding, how cells remove nascent chains stalled in the translocon during co-translational protein insertion into the ER is unclear. Here show that ribosome stalling during protein translocation at the ER induces the attachment of UFM1, a ubiquitin-like modifier, to two conserved lysine residues near the COOH-terminus of the 60S ribosomal subunit RPL26 (uL24). Strikingly, RPL26 UFMylation enables the degradation of stalled nascent chains, but unlike ERAD or previously established cytosolic ribosome-associated quality control (RQC), which uses proteasome to degrade their client proteins, ribosome UFMylation promotes the targeting of a translocation-arrested ER protein to lysosomes for degradation. RPL26 UFMylation is upregulated during erythroid differentiation, which helps cells to cope with increased secretory flow, and compromising UFMylation impairs protein secretion, and ultimately hemoglobin production. We propose that in metazoan, co-translational protein translocation into the ER is safeguarded by a UFMylation-dependent protein quality control mechanism, which when impaired causes anemia in mice and abnormal neuronal development in humans.**

SG148

**Role of Lipids in Protein Sorting During Export From the Endoplasmic Reticulum**

**M. MUÑIZ**; UNIVERSITY OF SEVILLE, SEVILLA, SPAIN.

Protein sorting in the secretory pathway is crucial to maintain cellular compartmentalization and homeostasis. To address the role of lipids as sorting determinants in membrane trafficking, we have investigated in yeast how GPI-anchored proteins having a very long chain sphingolipid moiety are differentially exported from the endoplasmic reticulum (ER). By using 3D high resolution live imaging microscopy, we provide direct in vivo evidence that sphingolipid acyl chain length is critical for lateral clustering and sorting of GPI-anchored proteins into selective ER exit sites. We also uncover a quality control system that regulates the ER export of GPI-anchored proteins by monitoring the incorporation of very long chain sphingolipid moiety into the GPI anchor.

SG149

**The ERSU Pathway Coordinates Sphingolipids and ER Functional Homeostasis**

**M. Niwa**, F. Pina, J. T. Chao, A. Tam, Y. Lai, L. Wang; University of California, San Diego, La Jolla, CA.

The endoplasmic reticulum (ER) - a gateway to the secretory pathway - performs a variety of critical functions, including cellular lipid synthesis, detoxification, production of secretory and cell surface proteins, and intracellular calcium regulation. In response to the increased demands of ER function during the lifetime of a cell, ER function must change according to immediate needs. Overall ER functional homeostasis is regulated by the unfolded protein response (UPR) pathway. We demonstrated

that ER stress blocks ER inheritance, leading to a halt in the cell cycle. This process is mediated, in part, by transfer of the septin ring from the bud neck, halting the cell cycle in cytokinesis. Proper inheritance of functional ER during the normal cell cycle is ensured by the ER stress surveillance (ERSU) pathway in *S. cerevisiae,* and is independent of the UPR. Indeed, while the UPR is activated by IRE1, an ER stress sensor/transmembrane kinase and endoribonuclease, the ERSU pathway is mediated by Rtn1, an ER transmembrane component, Wsc1, and the PKC1-SLT2 MAP kinase cascade. Our discovery that IRE1 is not involved in ERSU pathway activation suggested that activating signals for the ERSU pathway are unlikely to be those that activate IRE1, such as proteotoxic stress. Indeed, we found that ER stress resulted in the increase of sphingolipids, in part mediated by their increased biosynthesis. Further investigation revealed that among the ER stress-induced sphingolipids, a temporal increase in phytosphingosine (PHS), an early sphingolipid biosynthetic product, leads to the activation of ERSU hallmark events including the block of cortical ER inheritance, septin ring transfer to the bud neck, Slt2 phosphorylation, and cytokinesis cell cycle arrest. Furthermore, addition of myriocin, which inhibits the first step of the sphingolipid biosynthetic pathway, to cells treated with ER stress inducers diminished activation of ERSU pathway hallmark events. We also found that aureobasidin A, which, in effect, blocks the conversion of PHS to downstream complex sphingolipids, induces the ERSU pathway. Conversely, knockout of genes involved in ultimate down-regulation of PHS synthesis resulted in ERSU pathway activation. Our finding that PHS activates the ERSU pathway parallels our findings that similar sphingolipids activate ATF6, one of the three UPR-initiating components in mammalian cells. Our further studies will focus on the molecular mechanisms by which ER stress orchestrates sphingolipid homeostasis and ER functional status during the cell cycle.

SG150

**How Form Defines Function: Insight Into Lipase Regulation**

**S. Neher**[1], K. Gunn[1], E. Egelman[2]; [1]UNC Chapel Hill, Chapel Hill, NC, [2]University of Virginia, Charlottesville, VA.

In order to ensure energy homeostasis, living organisms must partition nutrients to cells for immediate use or to storage for future use. Triglycerides are the most energy-dense form of nutrients, and lipoprotein lipase (LPL) plays a key role in the spaciotemporal regulation of triglyceride utilization. LPL is a secreted lipase that hydrolyzes triglycerides from circulating lipoproteins, making free fatty acids available to the underlying tissues. Improper partitioning of fatty acids to the peripheral tissues upsets lipid homeostasis and can have major metabolic consequences. For example, elevated plasma triglyceride levels are a risk factor for coronary artery disease. LPL must thus be made available near certain tissues, such as adipose tissue, immediately after nutrients become available. LPL is secreted from adipose tissue in response to insulin, and it has long been hypothesized that LPL is stored in vesicles for secretion in an inactive, ‘cryptic,’ condensed form. This cryptic, condensed form of LPL would allow rapid trafficking of LPL out of the cell and into the circulation without disrupting membrane vesicles within the cell. Using cryo-electron microscopy, we identified a new, helical form of LPL. Resolution of the structure of LPL in helices revealed that LPL adopts an inactive conformation within the helices. We complement these cryo-EM studies with analysis of LPL within cells using super-resolution microscopy to better understand how LPL form affects its function during trafficking and enables whole-body lipid homeostasis.

SG151

**Ceramide Is Directly and Stereospecifically Sensed by the Ormdl/spt Complex to Regulate Sphingolipid Biosynthesis.**

**B. Wattenberg**, D. Davis, J. Suemitsu, C. Oltorik, M. Kannan, U. Mahawar; Virginia Commonwealth University, Richmond, VA.

Sphingolipids compose a lipid family critical for membrane structure as well as intra- and intercellular signaling. *De novo* sphingolipid biosynthesis is initiated by the enzyme serine palmitoyltransferase (SPT), which resides in the endoplasmic reticulum (ER) membrane. In both yeast and mammalian species, SPT activity is homeostatically regulated through small ER membrane proteins, the Orms in yeast and the ORMDLs in mammalian cells. These proteins form stable complexes with SPT. In yeast, the homeostatic regulation of SPT relies, at least in part, on phosphorylation of the Orms. However this does not appear to be the case for the mammalian ORMDLs. Here, we accomplished a cell-free reconstitution of the sphingolipid regulation of the ORMDL-SPT complex to probe the underlying regulatory mechanism. Sphingolipid and ORMDL-dependent regulation of SPT was demonstrated in isolated membranes, essentially free of cytosol and ATP. We found that this system is particularly responsive to the pro-apoptotic sphingolipid ceramide and is strictly stereospecific, indicating that ceramide regulates the ORMDL-SPT complex via a specific binding interaction. Our results indicate that ORMDL/Orm-mediated regulation of SPT involves a direct interaction of sphingolipid with the membrane-bound components of the SPT-regulatory apparatus.

SG152

**Endoplasmic Reticulum Stress Sensor Ire1 Deploys a Divergent Transcriptional Programme in Response to Lipid Bilayer Stress**

**G. Thibault, 637551**[1], N. Ho[1], H. Wu[1], J. Xu[2], J. Koh[1], C. Chen[1], W. Yap[1], S. Taubert[2]; [1]Nanyang Technological University, Singapore, SINGAPORE, [2]The University of British Columbia, Vancouver, BC, CANADA.

The unfolded protein response (UPR), a complex adaptive stress response of the endoplasmic reticulum (ER), is compromised in disease and aging. Typically activated by the accumulation of misfolded proteins within the ER lumen, the UPR is similarly activated by alterations of fatty acids and lipid composition at the ER. Chronic UPR activation by ER lipid aberrations, which we termed lipid bilayer stress-induced UPR ($UPR^{LBS}$) is associated to the development of the metabolic syndromes. However, most studies to dissect the $UPR^{LBS}$ mechanisms employ exogenous lipids or omit precursors of lipid biosynthesis. To systematically understand pathways that contribute to UPR activation, we performed a genome-wide genetic screen in *S. cerevisiae* and an RNAi screen in *C. elegans* to identify mutations that activate the UPR though LBS. Several unexpected cellular perturbations were identified to induce the UPR through LBS. As one of the strongest hits inducing the UPR, we further characterise the activation mechanism of Ire1 in Δ*opi3* where lack of phosphatidylcholine (PC) synthesis induces LBS. We found that the luminal domain (LD) of Ire1, which senses misfolded peptides, is dispensable to activate the UPR by LBS while LD overexpression was sufficient to uncoupled LBS-induced to proteotoxic-induced UPR. Conversely, we mutated Ire1 residue R537 located at the interface of the amphipathic and transmembrane helices rendering it insensitive to LBS while retaining the capacity to activate the UPR by proteotoxic stress. Furthermore, transcriptomic and ChIP-qPCR data revealed that the UPR programme diverges if activated

by LBS or proteotoxic stress. Together, our data point to the UPR as a broad-spectrum compensatory pathway in which LBS and proteotoxic stress-induced UPR deploy divergent transcriptional programmes.

## Subgroup P: Mechanics of Large Cellular Machines

SG153

**Mechanical Interactions At the Fusogenic Synapse**

**E. Chen**; UT Southwestern Medical Center, Dallas, TX.

Cell-cell fusion is indispensable for the conception, development and physiology of multicellular organisms. Work in Drosophila myoblast fusion revealed that cell fusion is an asymmetric process between an attacking cell and a receiving cell. Similar asymmetry has subsequently been found in both muscle and non-muscle cells undergoing fusion in vertebrates. While the attacking cell drills actin-propelled invasive protrusions into the receiving cell, the latter mounts mechanosensitive responses to resist the invasive forces, such that the plasma membranes of the two fusion partners can be brought into close proximity for fusogen engagement and membrane merger. In this presentation, I will discuss the molecular components that facilitate the mechanical interactions between the two fusion partners during cell-cell fusion.

SG154

**From Ciliate Biology to Physical Models of Mechanically Encoded Cell Behavior**

**S. Coyle**[1,2], E. Flaum[1], D. Krishnamurthy[1], M. Prakash[1]; [1]Stanford University, Stanford, CA, [2]University of Wisconsin — Madison, Madison, WI.

Ciliates are extraordinary single celled eukaryotes that use rapid morphology changes to perform fast animal-like behaviors such as jumping, grabbing and hunting. These behaviors are built from a toolbox of molecular components—motile cilia, cytoskeletal structures, and contractile machinery—that are regulated in space through different cell geometries and in time through signaling controllers that rapidly regulate activity. To understand how these activities can be used to build microscale molecular machines with complex behaviors, we analyzed the hunting dynamics of the predatory ciliate *Lacrymaria olor*, which locates and captures prey using the tip of a slender “neck” that can rapidly extend more than ten times its body length (500 µm from its body) and retract in seconds. Although its hunting behavior appears complex, a detailed quantitative analysis of the cell’s shape dynamics shows that it actually locates prey through rapid, near-random, dense sampling of the area within the neck’s radius. To execute random search in a challenging low-Reynolds number environment, the cell exploits elasto-hydrodynamic buckling responses of its cytoskeleton to repeated cycles of extension and retraction using its ciliary and contractile machinery. Inspired by these observations, we develop a high fidelity elasto-hydrodynamic physical model in which arbitrary cytoskeletal geometries can be coupled to a surface activity to generate emergent dynamics. Using this model we first explore the cell’s hunting behavior by elucidating the design principles and control signals to the underlying molecular components that underlie this behavior. More broadly, we explore how such a model can be used to explain the diversity of forms and functions within the *ciliaphora*.

SG155

**What Makes a Parasite? Exploring Cell Biology with Apicomplexan Parasites**

**K. Hu**; Indiana University, Bloomington, IN.

Apicomplexans, dinoflagellates, and ciliates comprise the three major clades in the superphylum Aveolata. All 6,000 known members of the phylum Apicomplexa are obligate intracellular parasites and many cause devastating diseases in humans and other animals. *Plasmodium* spp. infections cause malaria, responsible for more than half a million deaths per year. Nearly 20% of the global human population are permanently infected with *Toxoplasma*, which is perhaps one of the most successful parasites on earth. To cause disease, these parasites must reiterate their lytic cycle through host cell invasion, replication, and parasite egress, all of which rely on a functional cytoskeleton. Furthermore, the parasite cytoskeleton is richly detailed, easily visible, and architecturally invariant, which makes it a useful model for exploring cytoskeletal biogenesis in general. In this presentation, I will discuss our recent effort in determining how well-defined cytoskeletal structures in *Toxoplasma* are constructed and propagated through generations, and how they might contribute to the mechanics of parasite invasion.

SG156

**LIM Domains From Diverse Proteins Bind to Stressed Actin Filaments Using a Conserved Mechanism**

**J. Winkelman**; University of Chicago, Chicago, IL.

The actin cytoskeleton assembles into different types of load-bearing structures in the cell including stress fibers, muscle sarcomeres and the cytokinetic ring. These structures can generate and respond to intrinsic and external mechanical forces. The LIM (Lin11, Isl- 1 & Mec-3) domain superfamily is functionally diverse but most members are associated with the actin cytoskeleton and proteomic studies have suggested that many are sensitive to mechanical forces present there. In cells, zyxin is known to rapidly localize via its LIM domains to mechanically stressed actin networks where zyxin non-LIM domains recruit repair proteins to maintain mechanical homeostasis. The mechanism by which these LIM domains localize is not known. Additionally, it is unknown how widespread strain sensing is within LIM family proteins. We observe that LIM domains from functionally diverse proteins are sufficient to to sense spontaneous or induced actin network strain sites in mammalian cells. Additionally, LIM domains from the fission yeast protein paxillin like 1 (Pxl1) strongly recognize strain sites in mammalian cells suggesting that the strain sensing mechanism is ancient and conserved. In vitro, purified LIM domains from mammals and yeast bind very weakly or not at all to relaxed actin filaments, but exposure of these actin networks to various types of mechanical stress induces strong binding of LIM to actin filaments. We propose that LIM recognizes an F-actin conformation that is rare in relaxed state but is enriched in the presence of mechanical stress.

SG157

**Regulation and Dynamics of Force Transmission At Cellular Adhesion Complexes**

**A. Dunn**, C. Garzon-Coral, E. Korkmazhan, N. A. Bax, D. L. Huang; Stanford University, Stanford, CA.

The ability to construct organized, multicellular tissues is a defining feature of metazoan (animal) life. Despite this biological centrality, how cell- and tissue-level organization arise from the interactions of individual adhesion proteins and their binding partners remains incompletely understood. Our

laboratory uses approaches borrowed from single-molecule biophysics to understand the molecular mechanisms that underlie cell-cell and cell-matrix adhesion. In this talk, I will discuss our recent efforts to understand how the physical properties of cell-cell junctions reflect binding and force transmission at the level of individual cadherin-based adhesion complexes. In particular, I will describe recent results that suggest how symmetry-breaking events necessary to coordinate force transmission across neighboring cells may arise at the molecular level. These and other projects support an emerging understanding of how cells regulate cell-cell and cell-matrix adhesion in space and time in order to construct tissues that are both dynamic and physically robust.

SG158

**Torque- and Speed-dependent Remodeling of the Bacterial Flagellar Motor**

**N. Wadhwa**[1], R. Phillips[2], H. C. Berg[1]; [1]Harvard University, Cambridge, MA, [2]California Institute of Technology, Pasadena, CA.

Macromolecular protein complexes perform essential biological functions across life forms. The assembly of such complexes is known to be regulated at the level of gene transcription, but little is known about the factors that control their assembly once the mature protein subunits enter their target space (cytoplasm, membrane, or cell wall). Even less is known about how their assembly is regulated by extracellular signals from the environment. The bacterial flagellar motor is a large macromolecular machine that powers motility in bacteria. The torque-generating stator units of the motor assemble and disassemble in response to changes in external load. We used electrorotation (applying high frequency rotating electric fields) to drive tethered cells forward, which decreases motor load, and measured the resulting stator dynamics. No disassembly occurred while the torque remained high, but all of the stator units were released when the motor was spun forward at high speed. When the electrorotation was turned off, so that the load was again high, stator units were recruited, increasing motor speed in a step-wise fashion. A model in which speed affects the binding rate and torque affects the free energy of bound stator units captures the observed stator assembly and disassembly dynamics, providing a quantitative framework for the environmentally regulated self-assembly of a major macromolecular machine.

SG159

**Probing the Local Mechanical Architecture of the Vertebrate Meiotic Spindle**

**Y. Shimamoto**; Physics and Cell Biology, Natl Inst Genetics, Shizuoka, JAPAN.

The spindle is a microtubule-based intracellular structure that is assembled to segregate chromosomes during cell division. At metaphase, this structure maintains overall stability over minutes to hours despite the dynamic microtubule turnover and motility. Studies have identified a multitude of proteins controlling microtubule assembly, crosslinking, and movement. Advanced microscopy techniques are also revealing their localization in the spindle and an intricated internal organization of microtubules. However, our understanding of the forces and micromechanics underlying spindle assembly is rudimentary, and we still cannot explain the way microtubules self-organize, generate force, and oppose deformation during cell division. We have been addressing this by developing quantitative spindle micromanipulation assays. We use *Xenopus* egg extract as a model as it is tractable to quantitative biochemical and physical manipulations. Force-calibrated microneedles can be used to apply and measure forces in the spindle, and the resultant subcellular responses can be quantitatively analyzed

using microscopy images. Combining this setup with fluorescence "speckle" imaging and microrheology, we recently analyzed the force responses of individual microtubules *in situ* and mapped the physical nature (e.g., coupling strength, fluidity) of microtubule-microtubule interaction at different locations in the spindle. Our results show that the microtubule network around the spindle pole is relatively solid-like, whereas the one around the equator is more fluid-like. These filament networks are both tightly crosslinked at individual locations and can maintain their local structural integrity while generating and responding to forces. On the other hand, the microtubule network at the middle of the spindle half (i.e., between the pole and the equator) is more mechanically compliant and can predominantly slide apart in response to a force that perturbs the overall spindle length. Molecular perturbation experiments suggest that kinesin-5 and dynein, two key microtubule motors needed for proper cell division, play roles in establishing the spindle's mechanical heterogeneity. We propose that the spindle is comprised of two mechanically distinct microtubule networks assembling the pole and the equator, which merge at the middle of the spindle half and respond to force. Our previous *in vitro* reconstitution assay measuring forces in overlapping microtubules suggests the underlying motor protein dynamics. Together, our study reveals the cytoskeletal mechanics that allow the spindle to be robust and adaptable to perturbations and suggests the mechanical design ensuring the mitotic fidelity.

SG160

**Microneedle Manipulation of the Mammalian Spindle Reveals Specialized, Short-lived Reinforcement Near Chromosomes**

**P. Suresh**, A. F. Long, S. Dumont; University of California, San Francisco, San Francisco, CA.

The spindle generates force to segregate chromosomes at cell division. In mammalian cells, kinetochore-fibers (k-fibers) connect chromosomes to the spindle. Yet, how the dynamic spindle anchors k-fibers in space and time remains poorly understood since we lack tools to directly challenge this anchorage. Based on Nicklas' pioneering work, we develop a microneedle manipulation assay to exert local forces on the mammalian spindle with spatiotemporal control. Pulling on k-fibers in the lateral axis away from the spindle reveals the spindle's ability to retain local architecture in response to forces applied for seconds. Upon pulling, sister, but not neighbor, k-fibers remain tightly coupled, and the spindle globally shortens rather than k-fibers detaching from poles or chromosomes. The deformed shape of k-fibers reveals that they can freely pivot around poles, but not kinetochores, remaining unbent within the first 2µm near kinetochores. This local reinforcement near kinetochores has a lifetime of ~20 s under force, and requires the microtubule crosslinker PRC1. Together, these observations suggest strong, short-lived, and specialized reinforcement of the k-fiber in the spindle center. Such reinforcement could help the spindle robustly retain its local structure at dynamic sites of force generation, while allowing spindle remodeling on longer timescales.

SG161

**The *Naegleria* Spindle: a Single-purpose Machine Built From Single-purpose Microtubules**

P. Wadsworth, J. Rafferty, K. Velle, **L. Fritz-Laylin**; University of Massachusetts Amherst, Amherst, MA.

Eukaryotic cells generally use the same microtubules to drive mitosis and to organize the cell during interphase, rendering it difficult to determine whether the microtubule cytoskeleton is evolutionarily optimized for either function. In contrast, single celled *Naegleria gruberi* cells have two distinct microtubule networks: one used for mitosis by actively growing amoebae, and a second used for

cytoplasmic functions in differentiated non-dividing cells. We have determined that the cytoplasmic microtubule system of differentiated *Naegleria* cells resembles interphase microtubule networks of other eukaryotes in terms of organization, content, and assembly mechanisms. In contrast, the mitotic microtubule network uses evolutionarily divergent tubulins, whose sequences suggest unique biochemical properties. *Naegleria* mitotic spindles begin as disorganized arrays of microtubules, which lack obvious microtubule nucleation or organizing centers, surrounding a ball of DNA. This disorganized microtubule array eventually reorganizes into evenly-spaced bundles of microtubules with DNA aligned at the spindle midplane. In late metaphase, the persistent nucleoli in these cells segregate in advance of DNA, resulting in two areas devoid of microtubule staining. Cross-sections of Naegleria spindles reveal approximately 12 microtubule bundles surrounding a hollow core. The divergence of both the mitotic tubulin sequences relative to both Naegleria cytoplasmic tubulin and tubulin from other species suggests that non-mitotic functions are the main driver of microtubule evolution across eukaryotes. To test this idea, we are identifying and tracing the evolutionary history of additional spindle components.

SG162

**Rigidity Dependent Spontaneous Epithelial Tissue Rupture**

S. Sonam[1], **L. Balasubramaniam**[1], J. Rupprecht[2], Y. Ivan[3], C. Jebane[1], M. Fardin[1], Y. Toyama[3], P. Marcq[4], J. Prost[5], R. Mège[1], B. Ladoux[1]; [1]Institut Jacques Monod, Paris, FRANCE, [2]CENTURI institute, Centre de Physique Théorique in Aix-Marseille University, on Luminy campus, Marseille, FRANCE, [3]Mechanobiology Institute, Singapore, SINGAPORE, [4]Laboratoire Physique et Mécanique des Milieux Hétérogènes (UMR 7636), ESPCI - SU - UPD, Paris, FRANCE, [5]Institut Curie, Paris, FRANCE.

Epithelial integrity is a primary tissue function which protects the organs from abrasion and environmental exposure. Instability or gaps in this protective epithelial layer is a rare event. Studies in intestinal epithelium have shown gaps in the tissue after an apoptotic event[1]. Remarkably gaps in the epithelial have been observed upon mechanical load in lung epithelium[2] or in drosophila wings upon the loss of cell-cell adhesion[3]. However, the mechanism of this gap formation is still unclear. In our 2D in-vitro epithelial culture system (MDCK) we observe spontaneous gap formation in a substrate rigidity dependent manner. The size of these gaps can vary from 1-10 cell size. These gaps are able to seal themselves below a maximal gap size of about 4000$\mu m^2$. We observed two sources of gap formation: 1) From an edge of a stretching cell causing it to detach from its neighboring cell and 2) After cytokinesis and failure of complete sealing between the two daughter cells. We also observe that these gaps are formed in regions of high isotropic tensile stress characterized by -1/2 nematic defects. In the first case when cells get stretched prior to gap formation, we notice a peak in Von-Mises stress 30-60 minutes prior to gap formation. Von-Mises stress a term borrowed from material science determines the ductile to brittle yielding of a material. This has also been shown to be a leading cause of tissue rupture in *Trichoplax adherens*[4].References1. Watson, A. J., Duckworth, C. A., Guan, Y. and Montrose, M. H. Mechanisms of Epithelial Cell Shedding in the Mammalian Intestine and Maintenance of Barrier Function. Annals of the New York Academy of Sciences, 2009, 1165: 135-142. doi:10.1111/j.1749-6632.2009.04027.x2. John N. Maina, Sikiru A. Jimoh. Structural failures of the blood-gas barrier and the epithelial-epithelial cell connections in the different vascular regions of the lung of the domestic fowl, Gallus gallus variant domesticus, at rest and during exercise. Biology Open 2013 2: 267-276; doi: 10.1242/bio.201336083. Anne-Kathrin Classen, Kurt I. Anderson, Eric Marois, Suzanne Eaton. Hexagonal Packing of Drosophila Wing Epithelial Cells by the Planar Cell Polarity Pathway, Developmental Cell, Volume 9, Issue 6, 2005, Pages 805-817, ISSN 1534-5807,

https://doi.org/10.1016/j.devcel.2005.10.016.4. Vivek N. Prakash, Matthew S. Bull, and Manu Prakash. Motility induced fracture reveals a ductile to brittle crossover in the epithelial tissues of a simple animal. bioRxiv 676866; doi: https://doi.org/10.1101/676866

SG163

**Hydraulic Control of Oocyte Size Selection in *C.elegans***

**A. Mukherjee**[1,2], N. T. Chartier[3], J. Pfanzelter[3], F. Jülicher[1,4], S. W. Grill[2,4]; [1]Max Planck Institute PKS, Dresden, GERMANY, [2]Max Planck Institute CBG, Dresden, GERMANY, [3]BIOTEC Zentrum, TU-D, Dresden, GERMANY, [4]Center for Systems Biology Dresden, Dresden, GERMANY.

The process of making an oocyte starting from a germline tissue is a fundamental cellular process.Oogenesis demonstrates remarkable mechanical as well as hydrodynamic phenomena across organisms. Dynamic size regulation and mechanical symmetry breaking in germ cell population (within a syncytia) leads to heterogeneous growth leading to cell fate decisions. The roundworm *C. elegans* has a tubular syncytial (tissue architecture with connected cytoplasm) germline, which achieves germ-cell growth by hydrodynamic flows that range across 400 microns. By quantitative analysis and theoretical modelling, we discover that germ cells actively generate long-range hydrodynamic flows along the germline, while also locally maintaining their homogenous size. The coupling of cell mechanics and hydrodynamic fields lead to active pressure-tuning, which yields a hydraulic instability setting a critical size for the germ-cells in the absence of active sources. This mechanism ensures selection and growth of germ cells beyond a critical size at the expense of smaller cells and is independent of the apoptotic machinery. We unravel the physical basis of oogenesis and cell elimination by combining cellular mechanics and active hydrodynamics. Our findings elucidate a novel connection of cell fate and mechanics of volume regulation, and proposes a cell death mechanism that is emergent out of cellular competition rather than programmed.

SG164

**How Ballistic Organelles Invade Host Cells**

P. Jaroenlak, M. Cammer, J. Becnel, D. Ekiert, **G. Bhabha**; New York University School of Medicine, New York, NY.

Microsporidia are unicellular, eukaryotic, parasites with a wide host-range, from insects to humans, and are considered opportunistic pathogens as they cause disease in immunocompromised patients. To gain entry into a target cell, microsporidia employ a remarkably unique harpoon-like invasion machinery called the polar tube, which is conserved among microsporidial species. While initially coiled neatly within the spore of the parasite, infection of a new cell begins with the rapid extrusion of the polar tube from the spore, which anchors the spore to the host cell. After it has been fired, the polar tube is thought to act as a conduit for the transfer of the infectious “sporoplasm” into the target cell, where replication can begin. The mechanism of the infection process remains mysterious. Here we use a combination of serial block-face electron microscopy, optical microscopy and cryo EM to address the structure and mechanism of the polar tube in vitro and in the presence of host cells.

## Subgroup Q: Structure and Function of Cilia

SG165

**IFT Train Spotting by CLEM and Cryo-EM**

**G. Pigino**; MPI -Cell Biology/Genetics, Dresden, GERMANY.

Assembly of the cilium requires the rapid bidirectional intraflagellar transport (IFT) of building blocks to and from the site of assembly at its tip [1]. This bidirectional transport is driven by the anterograde motor kinesin-2 and the retrograde motor dynein-1b [2][3]. However, to drive retrograde transport, dynein-1b must first be delivered to the ciliary tip by anterograde IFT trains. In other bidirectional transport processes, the presence of opposing motors leads to periodic stalling and slowing of cargos moving along the microtubule. However, no such braking effect appears to occur in IFT. Here we use the most advanced technologies in cryo-electron tomography and sub-tomogram averaging to reveal the 3D structure of the complex IFT machinery (25 proteins) in Chlamydomonas and MDCK cilia [4]. We show that a tug-of-war between kinesin-2 and dynein-1b is prevented by loading dynein-1b onto anterograde IFT trains in an inhibited conformation and by positioning it away from the microtubule track to prevent binding. Once at the ciliary tip, anterograde trains disassemble and release dynein-1b in an intermediate "open" conformation, which then transitions into an active form to drive the movement of retrograde trains. These findings show how tightly coordinated structural changes mediate the behavior of complex cellular machines.References[1]Kozminski, K. G., Johnson, K. A., Forscher, P. & Rosenbaum, J. L., PNAS 90, 5519-5523 (1993). [2]Cole, D. G. et al., J. Cell Biol. 141, 993-1008 (1998).[3]Porter, M. E., Bower, R., Knott, J. A., Byrd, P. & Dentler, W., Mol. Biol. Cell 10, 693-712 (1999).[4] Jordan, M.A., Diener, R.D., Stepanek, L., Pigino G., Nature Cell Biology 20(11), 1250-1255 (2018).

SG166

**Single-molecule Tracking Reveals Complex Motility of Transmembrane Proteins in the Chemosensory Cilia Of*C. Elegans***

J. van Krugten, N. B. Danné, **E. J. G. Peterman**; Vrije Universiteit, Amsterdam, NETHERLANDS.

Cilia are vital for the cell's ability to sense its environment and rely on a process called intra flagellar transport (IFT) for their development, maintenance and function in signal-transduction. In IFT, motor proteins transport ciliary components along the polarized microtubule axoneme of the cilium. Among the cargoes of IFT are transmembrane proteins involved in signal transduction. As a model system, we study *C. elegans* chemosensory cilia, which we study using fluorescence microscopy with single-molecule sensitivity. First, we demonstrate that IFT machinery and ciliary components, including TRPV transmembrane channel protein OCR-2 are redistributed away from the ciliary tip upon external chemical stimulation, in a robust, extensive and reversible way. To elucidate the dynamics underlying this dramatic protein redistribution, we performed single-molecule imaging of OCR-2 in live *C. elegans*. Advanced analysis of the single-molecule trajectories shows that, in dendrite and transition zone, active transport is the prevailing motility mode of OCR-2. In the proximal and distal segments, however, motility is a much more complex, location-specific interplay between active transport, normal diffusion and sub diffusion. At the tip, confinement of the membrane proteins plays an important role. Together, our data and analysis demonstrate an intricate interplay between modes of transportation that ensure the proper ciliary distribution of OCR-2. These insights in the dynamics of cellular signal-transduction contributes to a wider understanding of IFT dynamics and to cilia as chemosensory organelles.

SG167

**The Molecular Architecture of the BBSome and Its Implications for Transition Zone Crossing**

K. Bahl[1], S. Yang[2], T. Walz[2], **M. Nachury**[1]; [1]UCSF, San Francisco, CA, [2]Rockefeller University, New York, NY.

The unique membrane composition of cilia is made possible by a diffusion barrier at the transition zone (TZ) that is breached when the BBSome escorts signaling receptors out of cilia. How the TZ functions as a selective barrier remains an open question. Here, we present a Cα model of the BBSome obtained by combining cryo-electron microscopy with comprehensive Rosetta-based structural modeling. We find that the BBSome is auto-inhibited in solution and undergoes a conformational change upon recruitment to membranes by the small GTPase ARL6/BBS3. Modeling how the BBSome binds to membranes and the signaling receptor Smoothened reveals that Smoothened's amphipathic helix 8 must be released from the membrane for it to be recognized by the BBSome. We speculate that the fixed curvature of the TZ membrane excludes amphipathic helices and that the BBSome licenses passage through the TZ by extracting structural elements out of the cytoplasmic leaflet of the membrane.

SG168

**Shedding Light on Ciliary Cyclic AMP Signaling**

**J. N. Hansen**[1], D. Wachten[1,2]; [1]Institute of Innate Immunity, University Hospital Bonn, University of Bonn, Bonn, GERMANY, [2]Center of Advanced European Studies and Research (caesar), Department of Molecular Sensory Systems, Bonn, GERMANY.

Cilia are membrane protrusions that come in two different flavors - they can be motile or immotile, so-called primary cilia. A special case of a motile cilium is the flagellum with the most prominent example being the sperm flagellum. Both, cilia and flagella are subcellular compartments, which are functionally distinct from the cell soma. A major challenge is to analyze signaling in a tiny subcellular compartment independent from the rest of the cell. We apply a combination of optogenetics with genetically-encoded biosensors to manipulate and measure ciliary signaling with spatial and temporal resolution *in vitro* and *in vivo*.

SG169

**A Cytoplasmic Protein Kinase in *Chlamydomonas* Links An Adhesion Receptor-activated Ciliary Signal to Cyclic AMP-mediated Cellular Responses, Including Mobilization of More Adhesion Receptors to the Cilia**

**M. Awasthi**, P. Ranjan, W. J. Snell; Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA., College Park, MD.

Use of cilia to respond to environmental cues arose early in evolution. cAMP-mediated ciliary signaling in vertebrates is crucial for development and homeostasis, and disruption of cilia is associated with multiple human disorders. Many cilium-based signalling pathways rely on regulated redistribution of membrane signaling proteins between the organelles and the rest of the cell, yet we know little about the mechanisms that couple ligand binding on the cilium to a cellular responses. During sexual reproduction in the bi-ciliated green alga*, Chlamydomonas*, interactions between adhesion receptors on the cilia of gametes of opposite mating type (SAG1 on mating type *plus* and SAD1 on *minus*) trigger the

sexually quiescent cells to become activated for fusion. cAMP produced by a ciliary adenylyl cyclase activated by SAD1-SAG1 interactions triggers cells to release their extracellular matrix, erect fusogenic membrane protuberances between the cilia; and, in a positive feedback loop, rapidly (< 5 min) mobilize a pool of SAG1 from the plasma membrane to the ciliary membrane to maintain and enhance adhesion. Here, we report identification of a gamete-specific protein kinase (GSPK), present primarily in the cytoplasm, that couples SAG1-based ciliary adhesion to responses in the cell body. Gametes bearing a mutant allele of *GSPK* retain ciliary adhesiveness, but are incapable of gamete fusion. Immunoblotting studies with cells expressing HA-tagged GSPK show that the protein is gamete-specific and present primarily in the cell body. *gspk* mutant gametes are capable of initial SAG1-dependent ciliary adhesion, but fail to mobilize SAG1 from the plasma membrane and thus fail to maintain ciliary adhesiveness. Failure to recruit SAG1 is a reflection of the overall inability of the mutant gametes to become activated by adhesion: fusion organelles fail to form and the extracellular matrix remains intact. On the other hand, *gspk* mutant gametes are fully capable of being activated by a cell-permeable form of cAMP. Moreover, cAMP assays show that ciliary adhesion in the mutant gametes fails to trigger the large, sustained increases in cAMP characteristic of adhering wild type gametes. Our results support the model that GSPK is essential for coupling the adhesion-induced increase in ciliary cAMP to the increase in cAMP in the cytoplasm required for gamete activation, and raise the possibility that cAMP-mediated responses to ciliary signalling in multicellular organisms might also depend on cytoplasmic production of cAMP. Supported by NIH GM122565.

SG170

**Cholesterol Accessibility At the Ciliary Membrane Controls Hedgehog Signaling**

**M. Kinnebrew**[1], E. J. Iverson[1], B. B. Patel[1], G. V. Pusapati[1], J. H. Kong[1], K. A. Johnson[1], G. Luchetti[1], D. F. Covey[2], C. Siebold[3], A. Radhakrishnan[4], R. Rohatgi[1]; [1]Stanford University, Stanford, CA, [2]Washington University School of Medicine, St. Louis, MO, [3]University of Oxford, Oxford, UNITED KINGDOM, [4]University of Texas Southwestern Medical Center, Dallas, TX.

The Hedgehog (Hh) signaling pathway is critical for proper development and adult tissue homeostasis. Hh ligands (like Shh) are received on target cells by their receptor, the transporter-like protein Patched 1 (PTCH1), and then transmitted across the membrane by Smoothened (SMO). The identity of the endogenous SMO ligand and the mechanism by which PTCH1 inhibits SMO are long-standing mysteries. Prior work pointed to a role for sterol lipids as messengers of the signal between PTCH1 and SMO and showed that PTCH1 inhibits SMO at primary cilia, the subcellular location for SMO signaling. To address the unresolved identity of the endogenous lipidic activator of SMO, we conducted a focused CRISPR screen using a custom library targeting ~1200 lipid-related genes, including all genes encoding annotated enzymes that synthesize or metabolize lipids. Consistent with prior work, our screen nominated cholesterol itself as the lipid that mediates the communication between PTCH1 and SMO. But how can cholesterol, an abundant molecule in the plasma membrane, be regulated tightly enough to control a signaling system that can cause birth defects and cancer? We find that SMO activation and Hh signaling are driven not by total membrane cholesterol but instead by a biochemically defined fraction termed accessible cholesterol. Increasing accessible cholesterol levels by depletion of sphingomyelin (SM), which sequesters cholesterol in complexes, markedly potentiates Hh signaling. Using toxin-based sensors to characterize the lipidic composition of the ciliary membrane, we find that the high SM to cholesterol ratio of the ciliary membrane sequesters cholesterol away from SMO, thus preventing transmembrane signaling. PTCH1, perhaps by depleting the ciliary membrane of cholesterol

using its transporter-like activity, blocks SMO activity by maintaining this high SM:cholesterol ratio. Hh ligands, which bind and inhibit PTCH1, increase cholesterol accessibility in the ciliary membrane, thus allowing SMO activation. In summary, our work shows that cholesterol-based regulation of the Hh pathway is made possible by the unique lipid environment of the ciliary membrane, providing a rationale for why this pathway has become associated with primary cilia in vertebrates. We propose that cholesterol accessibility at the ciliary membrane, directly regulated by PTCH1, is the key second messenger that controls SMO activity. More generally, the lipidic composition of the ciliary membrane can be modified by extracellular ligands to control the activity of cilia-localized signaling proteins.

SG171

**Shaping Sensory Signaling**

A. Philbrook, **P. Sengupta**; Brandeis University, Waltham, MA.

Structurally and functionally diverse cilia present on sensory neurons house signal transduction molecules and play important roles in olfaction, hearing, and photoreception. However, the contribution of unique cilia morphologies to sensory signaling is not fully understood. Sensory neurons in *C. elegans* contain cilia of complex morphologies and ultrastructures. Since the functions of individual *C. elegans* sensory neurons are known and sensory responses can be readily quantified, this system provides an excellent opportunity in which to correlate cilia shape with sensory response properties. We and others have found that as expected, mutations in intraflagellar transport (IFT) genes required to traffic structural and signaling components into and out of cilia abolish responses in a subset of *C. elegans* chemosensory neurons containing simple rod-like cilia. Unexpectedly, primary odorant responses in olfactory neurons containing cilia with complex morphologies are largely unaffected in IFT mutants, although these neurons exhibit defects in sensory adaptation and desensitization. To decouple the contributions of IFT proteins to cilia morphology or ciliary protein trafficking, we generated a temperature-sensitive mutation in a *C. elegans* kinesin motor protein. Upon shift to a restrictive temperature, IFT is acutely blocked without immediate disruption of cilia morphology in these mutants, allowing us to distinguish between the contributions of IFT and cilia morphology to the odorant response profile. We will present our ongoing efforts to systematically characterize how specialized cilia morphologies contribute to the unique responses of individual chemosensory neurons in *C. elegans,* illustrating how cilia morphological diversity modulates chemosensory neuron properties.

SG172

**Expanding Model Organisms for Studying the Structures of Cilia and Flagella**

**M. Kikkawa**; The University of Tokyo, Tokyo, JAPAN.

Eukaryotic cilia are complex cell organelles and play important roles in various cell, such as propeller and antenna. They consist of hundreds of different proteins that are precisely organized by self-assembly mechanisms. To study such the complex system, we have been using *Chlamydomonas*genetics and cryo-electron tomography to identify 3D locations of specific proteins. However, it is becoming clear that cilia and flagella in higher organisms are highly diverse, for example primary cilia rotate, while sperm flagella generate symmetric beating. Therefore, it is necessary to establish higher model organisms for structural studies of cilia and flagella. To this end, we have been expanding the model organisms to zebrafish and mice. We applied genome editing techniques to these organisms and knocked out cilia-related genes. For example, to study the roles of dynein assembly factors, we systematically knocked

out PIH proteins (Pih1d1, Pih1d2, Ktu, and Twister) in zebrafish and revealed their distinct roles by correlation the swimming phenotype and structures of spermusing cryo-electron tomography. We have also knocked out ODF2 (outer dense fiber protein 2) in mice and characterized their sperm structures using cryo-STEM tomography.

SG173

**The Role of the Ciliary Base in Cilia Homeostasis and Function**

S. Chandra Jana, P. Onkeve Ramos, **M. Bettencourt-Dias**; Instituto Gulbenkian de Ciência, Oeiras, PORTUGAL.

Cilia are microtubule-based protrusions involved in the integration of external information, fluid flow and movement. The base of cilia has a variety of different functions, providing stability to the cilium, mediating the trafficking of components in and out of it and acting as a cytoskeleton organiser. Defects in cilia cause human disorders, including ciliopathies and tissue-degeneration, affecting tissues at various ages and thus suggesting that these structures are actively maintained. Though the ciliary base role in cilia assembly is well documented, less its known about its role in cilia homeostasis. We investigated the role of the ciliary base in the physiology of the long-lived olfactory ciliated cells in *Drosophila*. We discovered that several components of the ciliary base are dynamic and necessary for ciliary physiology, their specific-loss in adulthood leading to a decline in olfaction. In particular, a set of pericentriolar material components play a crucial role in olfactory cilia maintenance, suggesting they perform novel functions in cellular homeostasis.

SG174

**Omega-3 Fatty Acids Activate Ciliary Ffar4/gpr120 to Trigger Camp-dependent Differentiation of Preadipocytes**

**P. K. Jackson, 94305**; Stanford University School of Medicine, Stanford, CA.

Mesenchymal stem cells including preadipocytes are uniformly ciliated in vitro, and primary cilia are required for adipogenesis. However, how primary cilia regulate mesenchymal differentiation, including adipogenesis, is unclear. Adipose tissue expands via de novo adipogenesis of preadipocytes and adipocyte hypertrophy. In vitro, adipogenesis is induced by insulin, glucocorticoids, and a cAMP elevating agent). However, the physiological signals, including specific hormones, growth factors, and nutrients that activate quiescent preadipocytes in fat pads to undergo adipogenesis are poorly defined. Moreover, the identity and markers of preadipocytes in vivo remain unclear, though functional studies localize preadipocytes along the vasculature in fat tissue, positioned to sense metabolites and respond to nutritional fluxes.Here, we use an engineered mouse model to show that ciliated cells reside along the vasculature in fat pads. We show that the primary cilium marks the preadipocyte in vivo and triggers exit from quiescence and initiation of adipogenesis by organizing adipogenesis-specific receptors and signaling pathways. We find that Tulp3, critical for ciliary GPCR trafficking, is required for adipogenesis. We screened 36 preadipocyte-expressed GPCRs to discover that Ffar4/Gpr120 is localized to cilia in preadipocytes in vitro and in vivo. Addition of Ffar4 ligand, the ω-3 fatty acid DHA, activates ciliary Ffar4 to promote adipogenesis of 3T3-L1 preadipocytes and primary mouse and human preadipocytes. Using a cAMP sensor, we show Ffar4 activation acutely raises localized cAMP levels specifically within the primary cilium. We find cAMP works via its EPAC effector, not protein kinase A. Finally, Ffar4 activation by DHA leads to cell cycle re-entry of quiescent preadipocytes and activation of the adipogenic master

transcription factors Pparγ and Cebpα. We find DHA can replace cAMP raising drugs in the adipogenic differentiation cocktail, and that the primary cilium is critical for adipogenesis in response to this nutrient. Intriguingly, we find that only the Ffar4 ligand DHA, but not saturated or mono-unsaturated fatty acids, can promote adipogenesis, suggesting that w-3 fatty acids direct the creation of new adipocytes whereas saturated fatty acids only increase lipid within existing cells. Knockout of floxed IFT88 using a Cre-PDGRFa driver, expressed on preadipocytes, causes a substantial loss of adipose tissue. These data extend our understanding of the metabolic importance of Ffar4, provide a molecular rationale for the anti-diabetic effects of dietary DHA supplementation by mechanistically linking it to preadipocyte activation, and contribute to the explanation of how ciliary dysfunction in ciliopathies results in obesity and diabetes.

SG175

**Centriole Self-assembly Is Sufficient to Organize Centriole Amplification in Multiciliated Cells**

O. Mercey[1], M. Levine[2], G. LoMastro[2], E. Brotslaw[3], N. Spassky[1], B. Mitchell[3], A. Meunier[1], **A. J. Holland**[2]; [1]Institut de Biologie de l'École Normale Supérieure, Paris, FRANCE, [2]Johns Hopkins University School of Medicine, Baltimore, MD, [3]Northwestern University, Chicago, IL.

Multiciliated cells (MCCs) amplify large numbers of centrioles, which convert into basal bodies that serve as the foundation for producing multiple motile cilia. Most of the centrioles amplified by MCCs grow on the surface of cell-type specific organelles called deuterosomes, while a smaller number grow through the centriolar pathway in association with the two parent centrioles. The deuterosome organelle is thought to have evolved to enable the massive production of centrioles in MCCs. Here we show that contrary to this expectation, MCCs lacking deuterosomes amplify the correct number of centrioles with normal step-wise kinetics. This is achieved through a massive production of centrioles on the surface and in the vicinity of parent centrioles. These findings suggest that deuterosomes evolved to relieve, rather than supplement, the centriolar pathway during multiciliogenesis. Remarkably, MCCs lacking both parent centrioles and deuterosomes also amplify the appropriate number of centrioles inside a cloud of pericentriolar material (PCM). This shows that centriole number is set independently of their growing platforms and that massive centriole production in MCCs is a robust process that can self-organize.

SG176

**Primary Cilia Control Gut Length by Regulating Tissue Mechanical Properties**

Y. Yang[1], P. Paivinen[2], K. Mostov[1], T. Makela[2], **J. Reiter**[1]; [1]University of California, San Francisco, San Francisco, CA, [2]University of Helsinki, Helsinki, FINLAND.

Intestinal length varies to a small degree, but the mechanisms of its longitudinal growth are poorly understood. We found that primary cilia and cilia-mediated Hedgehog signaling are essential for the longitudinal growth of the mouse intestine. Disruption of cilia results in shortened intestine and decreased intestinal cell proliferation from E13.5. Concurrently, mesenchymal cell cilia direct the development of the circumferential gut smooth muscle layer. Similarly, Hedgehog signaling in the stromal cells also directs intestinal growth and development of the smooth muscle. Partial ablation of the smooth muscle attenuates intestinal lengthening, indicating that ciliary Hedgehog patterning of the smooth muscle is critical for gut growth. Disrupting the smooth muscle via inhibiting ciliary function, Hedgehog signaling or smooth muscle integrity all reduces circumferential residual stress in the intestine and the activity YAP, a key transcriptional effector that interprets mechanical forces and promotes

proliferation. Like upstream ciliary signaling, stromal YAP is also essential for intestinal proliferation and lengthening. However, YAP is dispensable for smooth muscle development, further suggesting that YAP is activated downstream of smooth muscle force generation. Together, our results reveal that ciliary signaling controls the formation of the smooth muscle, which is itself critical for the mechanical properties of the developing gut, the activation of YAP and organ growth. These molecular mechanisms reveal how intercellular signaling and mechanical influences cooperate to regulate organ elongation.

## Subgroup R: Tools and Devices for Cell Biology

SG177

**Spatiotemporal Interrogation of Molecular Mechanobiology At the Cell-cell Signaling Interface with Nanotechnology Tools**

**Y. Jun,** University of California San Francisco, San Francisco, CA.

Cell signaling is orchestrated by cooperative actions of multiple nanoscale biomolecular machines. These processes are highly dynamic in space and time, and aberrant spatiotemporal dynamics of cell signaling results in developmental defects and diseases. How cells choreograph these signaling sequences in space and time to regulate cell functions and fates is of a central question in biology. To interrogate spatiotemporal regulation of cell signaling, we have been developing various nanotechnology tools to image and manipulate cell signaling in space and time with single-cell and molecule resolution. In this talk, I will specifically focus on spatiotemporal dynamics of Notch, a key cell communication receptor, and its signaling consequences in cells. By integrating cutting-edge nanotechnology tools including mechanogenetics (i.e. targeted control of genetically encoded mechanosignaling), super-resolution microscopy, and single particle tracking, we mapped dynamic spatial distributions of Notch receptors during the cell surface activation. We discovered that Notch undergoes dynamic spatial changes immediately after its receptor activation, choreographing downstream cell signaling sequences. From these observations, we disentangled a long-standing mystery of how dynamic phase segregation and colocalization of Notch creates enzymatically distinct environments and hence facilitates sequential proteolysis of Notch and signaling.

SG178

**Force-induced Mitochondrial Fission: on Mechanosensing by Intracellular Membranes and How Mitochondria Are Made Aware of Their Environment.**

Q. Feng[1], S. Helle[1], C. Gaebelein[1], T. Zambelli[1], J. Vorholt[1], **B. Kornmann**[2]; [1]ETH Zurich, Zurich, SWITZERLAND, [2]University of Oxford, Oxford, UNITED KINGDOM.

The intracellular environment of eukaryotic cells is highly complex and compact. The limited volume of the cell, usually a few hundred femtoliters, is not only occupied by numerous complicated, diverse membranous and proteinaceous structures, these structures are also highly dynamic due to constant remodelling and trafficking events. Consequently, intracellular interactions are more than just opportunities to exchange molecules; they also involve components physically navigating around each other in a highly confined space. This navigation involves collisions and clashes, leading to both compressive and tensile forces applied to organelles. We surmised that organelles must be able to sense and respond to such stimuli. Here, several experimental approaches were developed to apply defined amount of mechanical force to mitochondria. We show that mitochondria respond to both compressive

and tensile stimuli by undergoing fission, a controlled biochemical reaction that entails the recruitment of a dedicated machinery to sites of mechanical strain. These data explain why mitochondrial fission is observed at sites of contact with other organelles, like ER and lysosomes. It also provides a conceptual framework to explain the observed relationship between mitochondrial fission and mitochondrial DNA replication, as well as the between mitochondrial fission and ion homeostasis.

SG179

**Understanding the Mechanosensitivity of YAP - and Beyond.**

**P. Roca-Cusachs**; Institute for Bioengineering of Catalonia, Barcelona, SPAIN.

The cell nucleus is increasingly recognized as a mechanosensitive structure, but the underlying mechanisms remain unclear. Here, I will discuss on the different tools we employ to assess nuclear mechanosensitivity, including cell stretch, Atomic Force Microscopy, optogenetic tools, and different molecular perturbations. I will address how we have employed these tools to characterize the mechanosensitivity of the transcriptional regulator YAP, which is regulated by nucleocytoplasmic transport. Further, I will introduce current work in the lab exploring the general mechanisms behind the mechanosensitivity of nucleocytoplasmic transport, regardless of the specific cargo.

SG180

**Cortical Pulling Force Drives Pronuclear Migration and Rotation, and Spindle Positioning and Oscillation**

**H. Wu**[1], E. Nazockdast[2,3], R. Farhadifar[1,2], C. Yu[1], H. Chang[4], M. J. Shelley[2,5], D. J. Needleman[1]; [1]Harvard University, Cambridge, MA, [2]Simons Foundation, New York, NY, [3]University of North Carolina, Chapel Hill, NC, [4]Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei, TAIWAN, [5]New York University, New York, NY.

In the *C. elegans* early embryo, the pronuclei complex migrates to the cell center and rotates such that the mitotic spindle forms along the anterior-posterior axis. Subsequently, the spindle undergoes transverse oscillations as it moves toward the posterior. This asymmetric spindle positioning is crucial for asymmetric cell division and proper development. The contributions of pushing, cortical pulling, and cytoplasmic pulling forces to those dynamic centrosome positioning events are not fully understood. To study these processes, we constructed a novel laser ablation system capable of creating nearly arbitrary 3D cuts of astral microtubules at any desirable timing. We used this system to dissect the relative contribution of pushing and pulling forces throughout pronuclei and spindle motions. Our results suggest that all of these motions are dominated by net pulling. We used microinjected fluorescent nanodiamonds to track cytoplasmic fluid flow, which indicates that cortical pulling forces dominate over cytoplasmic pulling at all stages. We used computer simulations and mathematical modeling to interpret our experimental data. Taken together, our results strongly argue that cortical pulling drives pronuclear migration and rotation, metaphase spindle positioning, asymmetric spindle positioning and all aspects of spindle oscillations.

SG181

**A Novel Molecular Tool, ActuAtor, Generates Force to Deform Intracellular Structures in *Situ***

**H. Nakamura**, E. Rho, T. Inoue; Johns Hopkins University, Baltimore, MD.

Mechanical force underlies every phenomenon in our lives, and living organisms are no exceptions. Cells sense and generate mechanical forces in response to a wide variety of biological contexts, a topic of intense study in the field of mechanobiology. Actin cytoskeleton is a major player in biological force generation through multiple mechanisms. Among them, polymerization-dependent force generation is driven by the polymerization process itself, without any involvement of motors. It has been elucidated in relation to *Listeria monocytogenes*, bacteria that moves around in the cytosol of the host cell. To generate the force that is required for propulsion, *Listeria* uses ActA to hijack the host cell's actin polymerization machinery. ActA protein expressed on the posterior surface of the bacterium leads to local actin polymerization at the site, generating the pushing force necessary to propel the bacteria through the cytosol. To directly observe the diverse outcomes of physical force at work in living cells, we developed a technique, ActA-based actuator (ActuAtor), in which we manipulate the accumulation of an actin polymerizing peptide derived from ActA at desired locations within living cells. Using ActuAtor, we succeeded in inducing actin polymerization at arbitrary subcellular places in an inducible manner, leading to a surprisingly diverse phenomena observed at the sites. As a result, various intracellular structures including mitochondria, Golgi apparatus, and nucleus were significantly deformed, strongly supporting successful force generation. ActuAtor thus provided a means to synthetically deform intracellular objects through actin polymerization-dependent force generation. The novel method with spatial and temporal control of intracellular force generation can potentially pave the way toward direct investigation of mechanobiological events inside living cells. To demonstrate the promise of ActuAtor in cell biology, we evaluated functional effects of mitochondria deformation by combining the tool with single-cell fluorescence imaging. Although the deformation was strikingly significant, functional parameters of mitochondria were not significantly affected. These results question a long-lasting hypothetical assumption in the field that morphology and function of mitochondria are closely correlated. We also confirmed that ActuAtor can induce dispersion of stress granules, a well-established example of biomolecular condensates, which are attracting much attention as a novel class of non-membrane-bound intracellular structures. The novel molecular tool, ActuAtor, can thus be a useful tool in cell biology, revealing the roles of intracellular physical forces in regulated deformation or disassembly of diverse intracellular structures.

SG182

**Genepi: Piezo1-based Fluorescent Reporter for Visualizing Mechanical Stimuli with High Spatiotemporal Resolution**

**P. Pantazis**; Imperial College London, Department of Bioengineering, London, UNITED KINGDOM.

Mechanosensing is a ubiquitous process to translate external mechanical stimuli into biological responses during development, homeostasis, and disease. However, non-invasive investigation of cellular mechanosensing in complex and intact live tissue remains challenging. Here, we developed GenEPi, a genetically-encoded fluorescent intensiometric reporter for mechanical stimuli based on Piezo1, an essential mechanosensitive ion channel found in vertebrates. We show that GenEPi has high specificity and spatiotemporal resolution for Piezo1-dependent mechanical stimuli, exemplified by

resolving repetitive mechanical stimuli of spontaneously contracting cardiomyocytes within microtissues, in a non-invasive manner.

SG183

**Probing Cytoskeletal Dynamics and Fluctuations with Active Micropost Arrays**

Y. Shi[1], C. L. Porter[2], J. C. Crocker[2], **D. H. Reich**[1]; [1]Johns Hopkins University, Baltimore, MD, [2]University of Pennsylvania, Philadelphia, PA.

The actomyosin cytoskeleton is critical to a wide range of cellular mechanobiology and mechanotransduction. However, the understanding of the connections between molecular-scale processes and cell-scale mechanical phenomena is not complete. We have developed an approach using poly(dimethylsiloxane) active micropost array detectors to probe the dynamics and local mechanical properties of cells' actomyosin stress fiber network and cortex in detail. Using high-resolution tracking techniques to measure the microposts' motion enables measurements of local cell-generated forces and cytoskeletal rearrangements with millisecond time resolution and nanometer precision. By driving embedded nanomagnetic actuators within selected microposts with external magnetic fields, we can simultaneously measure the cytoskeleton's local mechanical response to applied force. In studies of 3T3 fibroblasts, we find that stress-fiber associated posts display motion that is both highly and persistently anisotropic, and is aligned with the average traction force acting on each post. In contrast, the motion of cortically adhered posts resembles weakly polarized, two-dimensional random walks with intermittent large steps. Both populations of posts report weakly viscoelastic local mechanics resembling earlier studies. Notably, the step-like motions of the cortically adhered posts exhibit a "fat tail" extending to large amplitudes resembling what is found in physical systems that exhibit avalanches and earthquakes. The regular array of microposts enables measurement of the symmetry and extent (up to several micrometers) of the largest of these cortical rearrangements, revealing spatiotemporal dynamics resembling that seen in plastic solids. These results are not readily described by existing biophysical models of the cortex and suggest that the physics of jammed systems and plastic deformation may be at the origin of cytoskeletal active mechanics, with implications for efforts to link molecular-scale machinery to cellular-scale behavior.

SG184

**Kinetochore-fibers Locally and Slowly Dissipate Force to Maintain Robust Mammalian Spindle Structure**

**A. F. Long**, P. Suresh, S. Dumont; University of California San Francisco, San Francisco, CA.

At cell division, the mammalian kinetochore binds many spindle microtubules that make up the kinetochore-fiber. To segregate chromosomes, the kinetochore-fiber must be dynamic, and must both generate and respond to force. How the kinetochore-fiber responds to force remains poorly understood: it cannot be reconstituted in vitro, and exerting forces on it in vivo remains challenging. Based on Nicklas' classic work, we developed a microneedle manipulation assay to pull on mammalian kinetochore-fibers close to kinetochores and probe how force regulates their dynamics and structure. We show that force lengthens kinetochore-fibers by persistently, and reversibly, favoring their growth versus shrinkage, rather than by increasing growth rate. Using photomarking, we demonstrate that lengthening occurs by preventing depolymerization at both microtubule plus- and minus-ends, and not by microtubules sliding within the bundle. Finally, even under forces sufficient to rupture kinetochore-

fibers we do not observe detachment from kinetochores or poles. Together, this work suggests strategies for spindle structural homeostasis under force: kinetochore-fibers locally dissipating force at both ends, behaving mechanically as a single unit, and having strong spindle connections at force dissipation sites. These simple engineering principles may be helpful for understanding a broad range of dynamic, force-generating cellular machines.

SG185

**Rational design of a chemically inducible t*rimerizaton* system**

**D. Wu**[1], O. Dagliyan[2], N. V. Dokholyan[3], T. Inoue[1]; [1]Johns Hopkins University, Baltimore, MD, [2]Harvard Medical School, Boston, MA, [3]The Pennsylvania State University, State College, PA.

In response to environmental cues many cell signaling events occur rapidly on the order of seconds to minutes. Chemically inducible dimerization (CID) strategies such as the FKBP - FRB system can heterodimerize proteins of interest (POIs) inside cells at a similar timescale upon addition of small molecule drug rapamycin. CID is commonly used to translocate POIs towards or away from desired subcellular locations by using protein localization tags and can elucidate local protein function or synthetically actuate or perturb a downstream event. Other similar systems using chemical or light stimulation also cause dimerization or homo-oligomerization. However, no inducible trimerization system has ever been developed. We engineered a rapamycin-dependent system which we term chemically inducible trimerization (CIT). To our knowledge, this is the first such system which brings together three distinct proteins, perhaps because a trivalent chemical approach confers an extra degree of freedom and is thus challenging. With computational aid, we rationally designed split sites for FKBP and FRB. Positive split sites were identified by ability of plasma membrane (PM)-localized split FRB (sFRB) and FKBP (sFKBP) pairs to recruit FKBP or FRB respectively upon rapamycin administration. Trimerization still occurred when sFRB or sFKBP were localized to different places in the cell, indicating that proximity is not a limiting factor in split pair reconstitution. In-cell kinetics of FKBP recruitment by an sFRB pair was determined, with exponential recruitment rate coefficient of -0.055 ± 0.01 1/s compared to full length FRB at -0.076 ± 0.01 1/s. Using CIT, cytosolic proteins could be conditionally targeted to inter-organelle membrane contact sites such as ER-PM and ER-mitochondria junctions. ER-PM junctions can be sites of lipid transfer and metabolism, which includes the regulation of $PI(4,5)P_2$ (or PIP2). While translocation of phosphatases or kinases to the ER or PM is possible with CID to perturb PIP2 levels, translocation contingent upon both ER and PM presence has not been achieved. Using CIT, we recruited PIP2 phosphatase INP54P to ER-PM junctions and locally depleted PIP2 at these sites - far beyond recruitment of FKBP alone or phosphatase-dead versions. This suggests that CIT can be used as a general paradigm to perturb ER-PM and other junctions. Altogether, we combined concepts of inducible dimerization and rational protein splitting to generate an inducible hetero-trimerization system, which can be useful for probing biological questions requiring three signals.

SG186

**ATP-Independent Bioluminescent Imaging Probes**

**H. Ai**; University of Virginia, Charlottesville, VA.

Bioluminescence, a phenomenon of production and emission of light as the result of a biochemical reaction, has broad applications in biology, biotechnology, and biomedical sciences. Bioluminescent reporters used in laboratories are mostly derivatives of two major luciferase families: ATP-dependent

insect luciferases and ATP-independent marine luciferases. Despite that ATP-dependent luciferase-luciferin pairs, such as firefly luciferase (FLuc)-D-luciferin, have been widely usedfor in vivo bioluminescence imaging (BLI), they consume ATP for photon production and this metabolic disruption issue cannot be addressed by simply improving these reporters. Moreover, any bioluminescent biosensors derived from them are intrinsically ATP-dependent. On the other hand, ATP-independent marine luciferase-luciferin pairs, such as NanoLuc-furimazine (FRZ), have found broad applications in vitro, but they are far from optimal for in vivo BLI due to low photon penetrationdepth in tissue caused by their blue emission, poor substrate solubility and stability, and/or low substrate permeability through the blood-brain barrier (BBB). By integrating synthetic/medicinal chemistry, protein engineering, and in vivo validation, we are adressing the aforementioned caveats of ATP-independent bioluminescent reporters. In this talk, I will discuss our recent progress in developing ATP-independent, bioluminescent imaging modalities with greatly enhanced biocompatibility, robustness, and in vitro and in vivo sensitivity.

## Subgroup S: Tunneling Nanotubes and Other Cell Protrusions: Structure, Composition, and Role in Inter-Cellular Communication and Disease

SG187

**Intercellular Messenger RNA Transfer through Tunneling Nanotubes in Mammalian Cells**

**G. Haimovich**, S. Dasgupta, J. E. Gerst; Weizmann Institute of Science, Rehovot, ISRAEL.

RNA transfer between mammalian cells was first observed in 1972, but the mechanism for intercellular transfer was not investigated until more recently. Contrary to the diffusion model suggested for small RNAs, we have demonstrated that full-length mRNA molecules transfer from donor to acceptor cells via tunneling nanotubes (TNT; membrane nanotubes) - long, thin, actin-based cellular protrusions that are distinct from filopodia.We used single-molecule fluorescent *in situ* hybridization (smFISH) to show that mRNAs, such as those encoding β-actin, HER2, cyclin D1, BRCA1 and GFP are transferred in cultures of mouse embryonic fibroblasts (MEFs), human cell lines, and mouse/human co-cultures. We found that the transfer of either endogenous or tagged mRNAs occurs in both immortalized and primary cells, and that it is modulated by stress conditions and specific inhibitors. The mechanism and the biological significance of intercellular transfer of mRNA remain unknown.In this talk, I will describe several of our approaches to study the scope, mechanism, and the physiological significance of mRNA transfer. I will discuss the technical difficulties we've encountered and present preliminary results that provide clues about the mechanism and function.

SG188

**Tunneling Nanotubes: Structural Identity, Mechanism of Formation and Role in Neurodegenerative Disease**

**C. ZURZOLO**; Pasteur Institute, Paris, FRANCE.

Tunneling Nanotubes (TNTs) are filopodia-like protrusions that play a pivotal role in long-range intercellular communication. TNTs are also implicated in cancer and neurodegenerative diseases, making them promising therapeutic targets. Understanding the mechanism of their formation, and their relation/difference with filopodia is of fundamental importance to uncover their physiological function. By using live and quantitative imaging we have analysed 1) the mechanism of TNT formation and 2) their

role in « prion-like » protein transfer. We found that actin modifiers have opposite roles in TNT and filopodia formation, indicating that these are two different structures. Furthermore, we demonstrated that TNT mediate the intercellular transfer of misfolded proteins involved in neurodegenerative diseases. Thus we proposed that TNTs contribute to the progression of the pathology in these incurable diseases. Furthermore, we have set up advanced Electron Microscopy (fluorescence correlative cryo electron microscopy and tomography (cryo-CLEM) and focused ion beam scanning EM tomography (FIB-SEM) to elucidate the unique native TNT structure in different cellular and molecular contexts. I will discuss our novel data on the mechanism of TNT formation, on their structural details and on their role in the spreading of amyloid protein aggregates.

SG189

**Correlative Light and Electron Microscopy (tomography, FIB-SEM) of TNTs between Leukaemia and Bone Marrow Stromal Cells**

**W. Dudka**[1], M. Kolba[1], P. Ronchi[2], A. Kominek[1], L. Turos[1], Y. Schwab[2], K. Piwocka[1]; [1]Nencki Institute of Experimental Biology, Warsaw, POLAND, [2]EMBL, Heidelberg, GERMANY.

Intercellular communication within the bone marrow niche significantly influences leukemogenesis and the sensitivity of leukemic cells to therapy. Tunnelling nanotubes (TNTs) are a novel mode of intercellular cross-talk. They are long, thin membranous protrusions that enable the direct transfer of various cargo between cells. We found that TNTs are formed between leukaemia and bone marrow stromal cells and enable the cellular membrane vesicles transfer from stromal to leukemic cells, mediating resistance to therapy. We performed morphological characterization of TNTs using live-cell imaging, correlative light electron microscopy followed by tomography, FIB-SEM as well as scanning electron microscopy. We found the plasma membrane at the emerging site to be highly convoluted, with several invaginations and protrusion of different length. Interestingly, the thickness of the emerging TNTs, which are continuous with the plasma membrane of the cell body, is remarkably consistent (~150 nm), with actin present. The specific fluorescent tracking of organelles, followed by confocal microscopy and 3D reconstruction, allowed to identify mitochondria and cytoplasmic vesicles inside TNTs. These data were supported by correlative light electron microscopy (CLEM) followed by tomography of TNTs bulges. Within the lumen of the TNTs bulges, we found vesicles with an average diameter of 111 ± 33 nm that corresponded to the typical size of cellular vesicles. Using vesicle fluorescent tracking with DiD and flow cytometry analysis we observed their transfer between both cell types. Altogether, we found that TNTs are involved in the leukaemia-stroma cross-talk and the intercellular transfer of membrane vesicles correlating with cytoprotection. For more detail: "Tunneling nanotube-mediated intercellular vesicle and protein transfer in the stroma-provided imatinib resistance in chronic myeloid leukaemia cells" (BioRxiv doi.org/10.1101/425041 & Cell Death & Disease, in press).

SG190

**Mechanism and Role of Rhes-mediated Tnt Like Protrusions**

**S. Subramaniam**; SCRIPPS Florida, Jupiter, FL.

**Abstract:** The role of tunneling nanotubes (TNT)-like membranous protrusions in selective neuronal vulnerability in the neurodegenerative disease remains unknown. For example, in Huntington disease (HD), a genetic disorder, neurons die selectively in the brain’s striatum affecting motor coordination in the patients. Even though the HD mutant gene, mHTT, is present throughout the body and equally

distributed, it remains unclear why striatal neurons degenerate in HD. We discovered that Rhes, a striatal-enriched protein, makes membranous tunneling nanotubes which interact with neighboring cells. Through these membranous tunnels, Rhes can now rapidly transported from cell-to-cell, and also carry mHTT. We propose that such cell-to-cell transport of disease-causing protein in the striatum may lead to dysfunction and death of neurons. Blocking the Rhes-mediated mHTT transport may prevent or delay HD. A recent study by Hernandez et al links Rhes in Tau-mediated toxicity. I will discuss these results of Rhes role in TNT-like structure, transport potential in brain and its implications in neurodegenerative disorder.

SG191

**Laser Capture Microdissection and Microproteomics: Uncovering the Proteomes of Diverse Cellular Protrusions**

**K. Gousset**; California State University, Fresno, CA.

Cellular protrusions are important structures that play key roles in cell differentiation, migration and invasion. In 2004, a novel type of cellular communication was described, where cargoes were directly transferred from one cell to another via specialized cellular protrusions called tunneling nanotubes (TNTs). Since then, TNTs and specialized filopodia such as cytonemes have emerged as important intercellular communication mechanisms with possible roles in disease. Cellular protrusions are composed of cytoskeletal components such as actin and/or tubulin. However, the detailed structural differences between the different subtypes of cellular protrusions are still unknown. It is also not clear how these differences might contribute to intercellular transport and/or disease propagation. Thus, the goal of our study was to combine laser capture microdissection (LCM) and microproteomics to specifically isolate TNTs and filopodia and identify their individual proteomes. Using this method we were able to identify, thus far, 1254 proteins in TNTs and 443 in filopodia—with 313 and 42 found exclusively within their respective structures. What's more, using various bioinformatic approaches our study points to major differences between the protein composition of TNTs and filopodia. For instance, TNTs were found to be enriched in proteins involved with membrane rafts, PIP3/PI3K activity, the regulation of actin filament length, Rab GTPase activity, mitochondrial respiration, focal adhesions, and membrane fusion. On the other hand, filopodia are enriched in proteins related to TNF alpha activity, vesicle fusion, Rho GTPase activity, RAL signal transduction, and receptor clustering. Thus, using our LCM/MS method, we are starting to unravel major differences in the protein composition, possible mechanisms of formation and the function of these subtypes of protrusions.

SG192

**Novel Models and Approaches to Study the Formation and Function of Membrane Tube Connections in Brain Tumors**

**E. Jung**[1], D. Hausmann[2], M. Mall[2], P. Koch[3], W. Wick[1], F. Winkler[1]; [1]University Clinic Heidelberg/German Cancer Research Center, Heidelberg, GERMANY, [2]German Cancer Research Center, Heidelberg, GERMANY, [3]Central Institute of Mental Health, Mannheim, GERMANY.

Membrane tube connections between brain tumor cells have been discovered in our group some years ago (Osswald et al., Nature 2015) and were mainly studied *in vivo* so far. Those so-called tumor microtubes (TMs) play a role in invasion and therapy resistance in malignant glioma. The name was chosen to differentiate them from tunneling nanotube *in vitro* characteristics, based on their different

size (length but to 1 mm, mean diameter 1.7 µm) and stability (up to several months), although their relatedness with membrane tubes described in other cell types still has to be elucidated. Neurodevelopmental molecules, namely Gap43 and Ttyh1, were identified as key drivers of TM formation. In the past, glioma cells were cultured under differentiating conditions to study the formation of cellular protrusions, which leads to the downregulation of molecules involved in TM formation. Furthermore, first data indicates that the brain microenvironment strongly promotes network formation and is essential for network functionality. Hence, we established novel *in vitro* models that preserve the stem-like expression profile associated with TM-proficiency, are based on coculture with induced human neurons and astrocytes or on brain organoids. These models provide novel insights into molecular mechanisms involved in TM formation, mechanisms of network resistance against therapies, calcium communication between tumor cells and interactions with neurons and astrocytes, which will be presented. Preliminary results underline the relatedness of TM and neurite outgrowth, with neurodevelopmental pathways being exploited for the formation of tumor cell networks. The investigation of calcium signaling between tumor cells, including its molecular determinants, its impact on proliferation and invasion as well as cellular hierarchies and communication patterns within the networks, is highlighted as an example of the benefits of these models. In addition, results from drug screenings will be presented, that demonstrate their application in translational biology. Taken together the preliminary results substantiate the intersections between neurodevelopment, neurite extension and TM formation.

SG193

**Machine Learning-based Workflow for in *Vitro* Characterization and Quantification of Tnt-like Structures/membrane Tubes Connections: Towards a Medium-throughput Image-based Drug Screen**

**D. D. Azorín**, E. Jung, M. Osswald, D. Hausman, W. Wick, F. Winkler; Neurology Clinic and National Centre for Tumour Diseases, University Hospital Heidelberg and Clinical Cooperation Unit Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Centre (DKFZ), Heidelberg, GERMANY.

A couple of years ago, our group introduced the term “Tumor Microtubes” (TMs) for long (up to several hundreds of micrometres) and thin (less than 2.5 µm) cellular extensions protruding from malignant glioma cells. It was demonstrated that TMs facilitate cellular invasion, development of radiation resistance and furthermore may correlate with prognosis. Hence, the development of anti-TM therapies emerges as a novel therapeutic approach. Interestingly other groups have described thin and interconnecting membrane protrusions in different cell types *in vitro* (Tunneling Nanotubes (TNTs)). It has been shown that some structural and functional similarities exist between TMs and TNTs, though their unequivocal discrimination is not yet possible. Only a couple of molecular drivers of TNT or TM formation are known until today and the identification of specific markers seems to be prerequisite to better differentiate different membrane protrusions. Here, we present a medium-throughput image-based workflow using confocal microscopy and subsequent machine learning-based image analysis to quantify and morphometrically characterize different cellular protrusions. This streamlined workflow can be carried out using freely available and open-source software. Preliminary data demonstrate the robustness of the workflow in terms of accurate differentiation of the membrane tube connections from other cellular structures. In summary, we present a novel machine-learning method which enables us to characterize different membrane tube protrusions and to retrieve parameters about their shape such as the size, the diameter or the length. These insights could be of considerable relevance for better

understanding the fundamental biology, and discovering novel targets with respect to the family of interconnecting membrane tubes. The application of the analysis tools in a systematic morphometric drug screening approach, as well as first discoveries, will be presented that demonstrate the feasibility for the identification of membrane tube-targeting therapeutics.

SG194

**Potential Role of TNTs in Astrocytic Gliosis**

**V. M. Ayres**[1], V. M. Tiryaki[2], I. Ahmed[3], D. I. Shreiber[3]; [1]Michigan State University, East Lansing, MI, [2]Siirt University, Siirt, TURKEY, [3]Rutgers, The State University of New Jersey, Piscataway, NJ.

Astrocytic gliosis induced by direct cell-cell communication(s) has been long suspected but its mechanism(s) have not been clearly identified. We present our recent research that indicates a potential role for Tunneling Nanotubes (TNTs) in A1 phenotype astrocyte reactivity. TNT formation has been reported in several cell systems including astrocytes and neurons. A nanoscale-diameter tube originates on one cell and extends freely without substrate interactions for multiple cell body lengths, to establish a physical or secretory connection with another cell [1], as opposed to gap and adherens junctions formed between adjacent somata. TNTs appear to function as communicating junctions, and represent a mechanism that can be hijacked to communicate a diseased state at a level of cell-to-cell granularity.During our studies of astrocyte responses modulated by external nanophysical cue sets [2], culture surface: poly-l-lysine-functionalized Aclar® (PLL Aclar) was investigated. When primary P1-3 cerebral cortical astrocytes (rat model) were stimulated with dibutyryladenosine cyclic monophosphate (dBcAMP) while modulated by PLL Aclar cues, they became reactive in ways that mimicked scar formation during gliosis, thus providing a model system for the study of A1 phenotype astrocyte reactivity. We now report that atomic force microscopy investigation of dBcAMP astrocytes modulated by PLL Aclar cues at time point 24h revealed extensive coverage of cell surfaces by TNT-like structures. Structures were typically y-shaped with cell attachments on the short branches and a long ~ 80-90 nm diameter branch that extended over one or more cell body lengths. Immunocytochemistry was performed using super-resolution microscopy, revealing long-distance cell-cell connections. The protein constituents were demonstrated to be actin, tubulin and also GFAP. GFAP has not be previously reported as a TNT constituent protein but is well known to be upregulated during astrocytic gliosis. Current investigations using Fluorescent Recovery after Photobleaching (FRAP) to investigate cell-to-cell transmission of Ca2+, up-regulated by mechanical stimulation of one cell in a connected pair, are designed to test if all or only some TNT candidate structures are capable of transmission.[1] HH Gerdes, et al., Mech. Devel. 130:381-387 (2013)[2] VM Tiryaki, et al., Nanomed. 10(4):529-545 (2015)

SG195

**Macrophages Enhance 3D Invasion of Breast Cancer Cells by Induction of Tumor Cell Tunneling Nanotubes Via Egf/egfr**

K. P. Carter[1], A. Genna[1], S. Hanna[2], **D. Cox**[1]; [1]Albert Einstein Coll Med-Jack & Pearl Resnick Campus, Bronx, NY, [2]Joan & Sanford I. Weill Medical College of Cornell University, New York, NY.

Recently novel mechanisms for intercellular communication, such as exosomes and tunneling nanotubes (TNTs), have been identified to play important roles in cell-cell communication. Tunneling nanotubes (TNTs) are thin, F-actin containing, cellular protrusions that mediate intercellular communication. TNTs are present in many different cell types ranging from neuronal to myeloid cells and are found in many

tumors. However, very little is known about the mechanism and structure of TNTs formed by different cell types and whether TNTs between cells of the same type (homotypic) TNTs are different from those between two different cell types (heterotypic) TNTs. While macrophages are essential components of innate immunity, when present in large numbers in breast tumors they appear to play a major role in promoting tumor progression towards an invasive, metastatic phenotype. We have recently identified that communication between macrophages and tumor cells, mediated by Epidermal Growth Factor (EGF) and Colony Stimulating Factor-1 (CSF-1) can occur through interactions via heterotypic TNTs. We have also identified that macrophages stimulate the ability of tumor cells to form homotypic TNTs amongst themselves through both direct contact as well as by secreted factors. Macrophage Conditioned Media (CM) induced an increase in TNTs in a number of breast cancer cell lines as imaged by live cell microscopy. We found that EGF was both necessary and sufficient for tumor cell TNT formation by CM. Since M-Sec (TNFAIP2) is known to be involved in TNT formation in some cell types including macrophages we determined a role for M-Sec in breast cancer cells. We found that CM increased protein levels of M-Sec in MTLn3 mammary adenocarcinoma cells. Reduction of endogenous M-Sec levels via shRNA in MTLn3 cells inhibited the formation of homotypic TNTs and blocked tumor cell invasion *in vitro* using a 3D invasion assay. Overall, these data support the hypothesis that macrophages in the tumor microenvironment stimulate the formation of tumor cell TNTs and these TNTs, in addition to macrophage tumor cell heterotypic TNTs, play a role in enhancing tumor cell invasion *in vivo*.

SG196

**Tunneling Nanotube Formation and Intercellular Trafficking Is Impacted by Macrophage Polarization**

**S. Goodman**, M. Khan, S. Nepahde, J. Sharma, S. Cherqui; University of California, San Diego, La Jolla, CA.

Tunneling nanotubes (TNTs) are cellular extensions enabling cytosol-to-cytosol intercellular interaction between numerous cell types including macrophages. Previous studies of hematopoietic stem and progenitor cell (HSPC) transplantation for the lysosomal storage disorder cystinosis have shown that HSPCs integrate within tissues, differentiate into macrophages that form TNTs delivering cystinosin-bearing lysosomes to disease cells leading to organ preservation. Intercellular lysosomal trafficking via TNTs was confirmed using an *in vitro* co-culture system between IC-21 macrophages and primary cystinotic fibroblasts. Here, we explored if macrophage polarization to either pro-inflammatory M1-like M(LPS/IFNγ) or anti-inflammatory M2-like M(IL-4/IL-10) affected TNT-like protrusion formation, intercellular transport, and ultimately the efficacy of cystinosis prevention.We designed new automated image processing algorithms to demonstrate that pro-inflammatory LPS/IFNγ polarization decreased protrusion formation by macrophages. Some automatically detected protrusions displayed characteristics of TNTs including cytoskeletal structure and 3D morphology. In contrast, co-culture of macrophages with cystinotic fibroblasts yielded more frequent protrusions as well as increased lysosomal and mitochondrial intercellular trafficking to the diseased fibroblasts compared to co-culture with wild-type cells. However, we observed normal transport efficacy and protrusion formation following disruption of anti-inflammatory IL-4/IL-10 polarization *in vivo* by transplantation of HSPCs isolated from the *Rac2*$^{-/-}$ mouse model. Our results demonstrate that despite suppression of TNT formation and function by LPS/IFNγ *in vitro*, pro-inflammatory cells appear necessary for therapeutic HSPC transplantation for cystinosis. This discrepancy between mice and co-cultures highlights the well-known dangers of relying solely on *in vitro* polarization models where cytokine stimulation pushes macrophages to non-physiological polarization phenotypes. That said, cystinotic cells or tissues across both models elicit increased macrophage-derived TNT formation and trafficking activity and so we can

use both co-cultures and transplanted mice to better understand various aspects of TNTs. Altogether, we developed unbiased image quantification systems that probe mechanistic aspects of TNT formation and function *in vitro,* while HSPC transplantation into cystinotic mice provides a complex *in vivo* disease model. Ultimately, we can use both approaches to expand the utility of TNT-like protrusions as a delivery system for regenerative medicine.

SG197

**Tks5 and Dynamin-2 Enhance Actin Bundle Rigidity in Invadosomes to Promote Myoblast Fusion**

**Y. Liu**[1], M. Chuang[1], S. Lin[1], R. L. Ohniwa[2], G. Lee[3], Y. Su[1], Y. Chang[1], M. Tang[3]; [1]National Taiwan University, Taiepi, TAIWAN, [2]University of Tsukuba, Tsukuba, JAPAN, [3]National Cheng Kung University, Tainan, TAIWAN.

Skeletal muscle development requires the cell-cell fusion of differentiated myoblasts to form muscle fibers. The actin cytoskeleton is known to be the main driving force for myoblast fusion; however, how actin is organized to direct inter-cellular fusion remains unclear. Here we show that an actin and dynamin-2 enriched protrusive structure, the invadosome, is required for the fusion process of myogenesis. Upon differentiation, myoblasts acquire the ability to form invadosomes through isoform switching of a critical invadosome scaffold protein, Tks5. Tks5 directly interacts with and recruits dynamin-2 to the invadosome and regulates its assembly around actin filaments to strengthen the stiffness of dynamin-actin bundles and invadosomes. These findings provide a mechanistic framework for the acquisition of myogenic fusion machinery during myogenesis and reveal a novel structural function for Tks5 and dynamin-2 in organizing actin filaments in the invadosome to drive membrane fusion.

SG198

**Mechanism of Cytoneme-mediated Fgf Signaling During *Drosophila* Tracheal Morphogenesis**

**S. Roy**, L. Du, A. Sohr; University of Maryland, College park, MD.

Cytonemes are actin-based signaling filopodia that bridge signal-producing and recipient cells and communicate signals through their contact sites. The mechanisms mediating this contact-dependent signaling are still poorly understood. My lab investigates the mechanisms of cytoneme-mediated intercellular communication of Fibroblast Growth Factors (FGF) using *Drosophila*as a model. To visualize FGF dispersion at its physiological levels, we generated genome-edited flies expressing fluorescently-tagged FGF and FGF-receptor (FGFR) under their own genomic control. Live imaging analyses showed that recipient tracheal cells extend cytonemes containing FGFR to reach towards the cells that produce the FGF, collecting the signal directly from the source. FGF molecules bind to FGFR at cytoneme-contact sites, move along the cytoneme surface, and are endocytosis into the tracheal cells to form a long-range gradient. High-to-low levels of FGF signaling within the tracheal epithelium differentially feedback on cytoneme formation, regulating their signaling levels and specificity. However, for initiating and sustaining this self-regulatory transport mechanism, cytonemes first need to establish contacts with the source cells, and the source cells need to release the signal only at the cytoneme contact sites. We discovered that FGF is synthesized in a precursor form and is endoproteolytic cleaved by a Furin1 protease in the Golgi of source cells. The cleavage activates polarized intracellular sorting of the truncated signal to the FGF receiving cytoneme contact sites. Thus, enzymatic cleavage ensures polarized intracellular sorting of FGF to the signaling site, thereby modulating its cytoneme-dependent

tissue-specific signaling from within the source cells. On the other hand, live imaging analyses revealed that FGF-expressing cells also extend short dynamic FGF-containing cytonemes to contact the recipient cytonemes transiently. Genetic knockdown of cytonemes either in source or recipient cells, led to the removal of cytonemes from the other side. These results showed how source and recipient cytonemes might reciprocally guide each other by establishing signaling contacts. Altogether, our findings provide a novel mechanistic insight on how signaling cells employ cytonemes to find each other and modulate cytoneme-mediated signaling to create tissue patterns.

SG199

**Localized Intercellular Transfer of Ephrin-As by Trans Endocytosis Provides a Memory of Signaling**

J. I. Valenzuela, **F. Perez**; Institut Curie / CNRS, Paris, FRANCE.

Alterations in different biosynthetic trafficking routes lead to losses in cellular polarity, which represent the earliest stages of carcinogenesis. We focused on the trafficking pathways of the tyrosine kinase receptor EphA2 and its membrane-bound ligand ephrinA1. EphA2 is frequently overexpressed in melanoma, gliomas, and around 10 different types of carcinomas where its levels are associated with poor prognosis. The colocalization of EphA2 with the basolateral protein E-cadherin and the presence of an usually apical sorting motif (GPI anchor) in ephrinA1 strongly suggest that both proteins are segregated from each other in their journey to the plasma membrane. To be able to study specifically, quantitatively and in real-time specific trafficking pathways our laboratory has developed a system called RUSH (Retention Using Selective Hooks), which allows to synchronize the secretion of virtually any protein. By using the RUSH system, we demonstrated that EphA2 and ephrinA1 have different kinetics of transport and are partially sorted from each other at the level of the Golgi apparatus. Upon arrival to the plasma membrane, we observed that ephrin A1/EphA2 complexes are locally formed at the tip of filopodia, at cell-to-cell contacts. Clusters of ephrin A1 from donor cells surf on filopodia associated to EphA2-bearing sub-domains of acceptor cells. Full-length ephrin A1 is transferred to acceptor cells by trans endocytosis through a proteolysis-independent mechanism. Trans-endocytosed ephrin A1 bound to its receptor enables signaling to be emitted from endo-lysosomes of acceptor cells. Localized trans-endocytosis of ephrin-A1 sustains contact-mediated repulsion on cancer cells. Our results uncover the essential role played by local concentration at the tip of filopodia and the trans-endocytosis of full-length ephrin to maintain long-lasting ephrin signaling.

SG200

**Differential Regulation of Protrusive Behavior During Collective Cell Migration**

**H. Olson**[1], H. McGraw[2], A. Nechiporuk[1]; [1]Cell, Development, and Cancer Biology, Oregon Health and Science University, Portland, OR, [2]Division of Cell Biology, University of Missouri-Kansas City, Kansas City, MO.

Cells migrate individually or in groups in a process known as collective cell migration. These cohorts of cells maintain cell-cell contact, group polarization and exhibit coordinated behavior. Collective cell migration is important for numerous processes during development including blood vessel branching and neural crest migration as well as in adulthood in wound healing and cancer invasion. During individual and collective cellular migration, cells must extend protrusions to interact with the extracellular environment, sense chemotactic cues, and act as points of attachments. The mechanisms and regulators of protrusive behavior have been widely studied in individually migrating cells; however,

how this behavior is regulated throughout collectives is not well understood. To study protrusive behavior during collective cell migration, we use the zebrafish posterior lateral line primordium (pLLP) as a model. The pLLP is a cluster of ~100 cells that migrates along the zebrafish trunk, depositing groups of cells that will become sensory organs. To define protrusive behavior during pLLP migration, we performed mosaic analysis to sparsely label cells within the pLLP with a transgene marking filamentous actin. This approach revealed an abundance of brush and filopodia-like actin-based protrusions throughout the pLLP. Further, we found that these two types of protrusions behave differently within the collective when comparing the leading to the trailing region. Filopodia-like protrusions are more numerous and less persistent in the leading region, whereas brushes are larger and more dynamic in the leading versus the trailing region. Furthermore, inhibition of branched actin networks prevents the formation of brushes and inhibits pLLP migration, suggesting that brush protrusions are made up of branched actin networks and are necessary for migration. In addition, mosaic labeling of both cellular actin-based protrusions and cell membranes within the pLLP show that these protrusive structures interact with cellular membranes of cells within the pLLP. These results suggest that protrusions in the pLLP could be playing a role in inter-cellular communication among the cells of the pLLP. To identify genes that regulate this protrusive behavior, we examined expression of candidate genes known to regulate actin dynamics. From this, we found a number of candidate genes that show regional specific expression patterns in the pLLP. Our results suggest that protrusive behavior is spatially regulated throughout the pLLP by region specific expression of genes known to regulate actin dynamics.

## Subgroup T: Using Advanced Imaging to Redefine the Cell and Tissue Biology

SG201

**Heterogeneity and Intrinsic Variation in Spatial Genome Organization**

**E. H. Finn**, T. Misteli; National Cancer Institute, NIH, Bethesda, MD.

The genome is organized in 3D space in the form of chromosomes, chromatin types, and megabase-scale domains such as TADs which all form self-associating structures within the nucleus. This organization is altered in development and differentiation, but the processes controlling spatial genome organization are not fully understood. Furthermore, while studies of transcriptional regulation and nuclear organization have demonstrated stochastic effects, the extent and nature of cell-to-cell and cell-intrinsic variability in genome architecture is only poorly characterized. We aimed to enrich the data yielded by population-based biochemical methods and systematically probe heterogeneity in genome organization at the single-cell level. To this end, we used high-throughput imaging to determine the 3D distances between individual locus pairs at genomic distances between 0.25 and 250 Mbp in individual human skin fibroblasts. We observe overall low association frequencies, influenced by genomic distance, higher-order chromatin architecture, and chromatin environment. These trends were also evident within individual TADs: pairwise associations within a TAD varied between cells and interactions between TADs were relatively common. Furthermore, allele-specific analyses revealed very low covariance between alleles in the same cell or adjacent pairs on the same chromosome. Our observations reveal extensive variability and heterogeneity in genome organization at the level of single cells and alleles. They suggest that folding of the DNA fiber is a highly stochastic process, and they demonstrate the coexistence of a broad spectrum of genome conformations in a cell population.

SG202

**Modern Tools for the Systematic Profiling of Intracellular Architecture in Space and Time**

**M. Leonetti**; Chan Zuckerberg Biohub, San Francisco, CA.

As Richard Feynman (in)famously stated in 1959: “it is very easy to answer many [...] fundamental biological questions; you just look at the thing!”. 60 years later, this still very much applies: there is still so much to discover from simply “looking” at how cells are organized in space and time. One way modern tools can accelerate this process is by enabling live cell imaging in high throughput, so that cellular architecture can be examined in a systematic manner. Here we will describe our latest scalable methods that facilitate two major parts of the cell biology workflow: endogenous labeling with genetically encoded fluorescent proteins, and long-term 3D imaging. First, we will present optimized CRISPR-based methods for the rapid generation of endogenously tagged human cell lines, as well as automated tools for the design and analysis of CRISPR editing experiments. Second, we will show how a recently developed inverted light-sheet instrument enables imaging of cells in 3D with high temporal resolution and over extended periods (days), all in multi-well culture format. Together, these high-throughput tools enable us to describe how proteins in a cell are organized at genome-wide scale.

SG203

**The Forest and the Trees — Whole Cell Correlative Cryogenic Super-resolution Microscopy**

**D. Hoffman**; Janelia Research Campus, Ashburn, VA.

Electron microscopy (EM) continues to reveal a baroque world inside eukaryotic cells, spatially organized at all length scales from nano-scale proteins to cell-spanning stress fibers. However, even within a single organelle there are notable structural differences across a cell; e.g. the endoplasmic reticulum (ER) is convoluted and compact in the perinuclear region, yet sparsely reticulated in the lamellae. Thus, a comprehensive picture of cellular organization requires nanometer-level three-dimensional (3D) imaging of whole cells. However, EM produces grayscale images where the unequivocal identification and 3D segmentation of complex subcellular structures can be challenging, and where the distribution of specific proteins can seldom be measured. Here we describe a pipeline for cryogenic super-resolution correlative (cryo SR-CLEM) imaging of entire cells designed to address these issues. It combines 3D structured illumination (SIM) and single molecule localization (SMLM) microscopy for SR protein specific contrast with 3D FIB-SEM for global contrast of subcellular ultrastructure. The SR modality highlights features not readily apparent from the EM data alone, such as exceptionally long or convoluted endosomes, and permits the unambiguous classification of vesicles, such as endolysosomes and peroxisomes. Cell-wide 3D correlation also reveals unexpected localization patterns of proteins, including intranuclear vesicles positive for an ER marker, intricate web-like structures of cell-cell adhesions, and heterochromatin colocalized with transcriptionally-associated H3.3, packaged alongside euchromatin colocalized with HP1α, a marker of transcriptionally inactive heterochromatin, in the nuclei of neural progenitor cells. More generally, whole cell cryo SR-CLEM can reveal compartmentalized proteins within subcellular components of known EM morphology and help discover new subcellular components of non-canonical EM morphology and their roles in cellular metabolism.

SG204

**Prototyping Multiscale Cellular Visualization & Modeling Techniques for Hypothesis Generation, Communication & Learning**

**G. T. Johnson**[1,2]; [1]Allen Institute for Cell Science, Seattle, WA, [2]UCSF, San Francisco, CA.

The visual analysis, assembly, and communication of molecule-scale events has benefited greatly from an explosion of available tools and data that has been provided and evolved by the structural biology community over the past 40 years. Compared to molecular visualization, however, the data, tools, and techniques available for cell-scale visualization remain in a relative infancy. In structural biology, the details of a molecule can commonly be summarized with a few megabytes of data, which is heavily constrained by nanoscale physics. Could we ever uncover patterns and rules that will allow us to extract and communicate an analogously efficient understanding of cellular anatomy and behavior when faced with massive microscopy files that are difficult to share and analyze? Can visualization capture both the diversity and the commonality among cells, which often seem chaotic and random at first glance? An overview of the field will describe available tools and current challenges to advancement. The presentation will elaborate on approaches we have taken to annotate 3D light microscopy data with mesoscale structures modeled using information from higher-resolution information that spans biology. It will further describe whole cell and multiscale visualization tools and data that function directly in a web browser that we develop for allencell.org.

SG205

***In Situ* Measurement of Protein and Lipid Mass by Normalized Raman Imaging**

**S. Oh**[1], C. Lee[1], D. Fu[2], W. Yang[3], A. Li[1], C. Ran[4], W. Yin[4], C. J. Tabin[1], S. Xie[5], M. W. Kirschner[1]; [1]Harvard Medical School, Boston, MA, [2]University of Washington, Seattle,, WA, [3]Harvard University, Cambridge, MA, [4]Massachusetts General Hospital, Boston, MA, [5]Peking University, Beijing, CHINA.

Cell growth is the basis for myriad biological processes ranging from development, regeneration and tumor growth. Diverse extracellular signals such as growth factors, hormones, nutrients, and cell-to-cell contact are transduced by the signaling pathway to regulate growth. Yet it is still an open question how the integration of diverse inputs results in cell growth or maintains cells at the right size in the face of massive protein turnover. Accurate measurement of cell size is critical for probing these questions. State-of-art techniques such as quantitative phase microscopy, suspended microchannel resonator, and novel fluorescence reporter made it possible to measure cellular dry mass in higher precision and throughput than ever. Nevertheless, the existing methods require suspended cells or in vitro cell cultures by which the contextual information of the tissue is lost. While the cell volume can be measured by confocal microscopy, there is no existing method to measure cellular dry mass or protein mass *in situ*. Here we present Normalized Raman Imaging (NoRI) that fills the technology gap by providing the absolute concentrations of total protein, total lipid, and water of cells and subcellular compartments *in situ*. NoRI achieves the absolute quantification by combining Stimulated Raman scattering (SRS) microscopy with a novel computational algorithm that removes the effect of light scattering in thick tissue samples. NoRI is a label-free technique that can measure live or fixed tissue sections, cultured cells and small model organisms without any use of staining. The single cell protein mass and lipid mass can be acquired by integrating the respective concentrations over the cell volume. NoRI reveals that cell types and tissue types have characteristic protein and lipid density, which may

change with developmental or disease processes, and opens a door to many interesting questions about the regulation of cell growth in tissue context.

SG206

**Using Focused Ion Beam - Scanning Electron Microscopy to Identify a Novel Membrane Structure, a 3-way Sheet Junction, Required for Pronuclear Fusion in *C. Elegans***

M. Rahman[1], A. Harned[2], I. Chang[2], R. Maheshwari[1], K. Narayan[2], **O. Cohen-Fix**[1]; [1]National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, [2]NCI, NIH, Fredrick, MD.

After fertilization, the maternal and paternal genomes are initially enclosed in two separate nuclei, called pronuclei. Subsequent nuclear envelope (NE) break-down allows mixing of the two paternal genomes and the generation of diploid nuclei. NE proteins such as lamins and nuclear pore subunits dissociate from the NE after pronuclear meeting. However, the process by which the membranes of the two pronuclei becomes fenestrated is not known. Specifically, once the two pronuclei meet, the parental genomes are separated by 4 membranes that do not disperse. To address this, we used Focused Ion Beam - Scanning Electron Microscopy (FIB-SEM) to analyze individual embryos that were high-pressure frozen at different stages of the first mitosis. This allowed us to examine membrane configuration at near-native state and at nm-scale resolution. This type of analysis has not been done before for fertilized embryos in any organism. The FIB-SEM data revealed that at metaphase, the majority of the interface between the two pronuclei was composed of only two membranes that contained holes ranging from tens of nanometers to several microns in diameter. The chromosomes of the two pronuclei intermingled through one of the large holes. 3D reconstructions of these fenestrated membranes revealed that at they are flanked by a novel membrane structure, a 3-way sheet junction, where the 4 original pronuclear membranes are reduced to 2 membranes. Confocal microscopy of fluorescently tagged inner and outer nuclear membrane proteins revealed that the two remaining membranes contain both inner and outer nuclear membrane components, suggesting that the fenestrated 2-memrbane region originated from one of the two pronuclei. Our analysis also revealed the presence of membrane structures within the pronuclear lumens, suggesting that a reduction in the number of membranes from four to two is achieved, at least in part, by dispersal of membranes into the pronuclear lumen. Furthermore, the 3-way sheet junctions are essential for parental chromosome mixing, as these junctions are absent in a mutant in which the parental chromosomes do not mix. Taken together, our FIB-SEM analysis uncovered a novel membrane structure, the 3-way sheet junction, that is required for parental chromosome mixing during the first mitosis after fertilization in *C. elegans*. A similar process may occur in other organisms.

SG207

**Connecting Chromosome Structure and Dynamics through High-precision Microscopy, Genetic Perturbations and Stochastic Simulations**

**I. V. Surovtsev**[1], J. F. Williams[1], M. L. Bailey[2], H. Yan[2], S. G. J. Mochrie[2], M. C. King[1]; [1]Yale School of Medicine, New Haven, CT, [2]Yale University, New Haven, CT.

Inside cells, the DNA polymers are compacted into a spatially ordered, yet dynamic, 3D-structure. Recent genome-wide chromatin conformation capture (Hi-C) experiments reveal a hierarchical organization of the DNA structure, with DNA domains nested within bigger domains (or compartments). This structure underlies chromosomal dynamics, e.g. the probability that distant loci encounter each

other, with a higher probability for intra-domain contacts relative to inter-domain contacts. To gain insight into the structure-dynamics relationship of chromatin, we combine high-precision microscopy in living *Schizosaccharomyces pombe* cells with systematic genetic perturbations and stochastic modeling of DNA looping. First, we investigate the model that the activity of SMC proteins, which play crucial roles in chromosome structure, drive chromatin dynamics by loop extrusion. Surprisingly, inhibiting the activity of SMC complexes (either condensin or cohesin) using temperature-sensitive alleles increases chromatin mobility. These results suggest that SMC complexes normally constrain rather than agitate chromatin motion. Next, we interrogate how other DNA-organizing protein complexes, including RNAP, nucleosome remodelers or histone modifiers, impact chromosome dynamics. Interestingly, we found that perturbation of the INO80 nucleosome remodeling complex, but not the SWI/SNF, RSC or FFT complexes, reduces the mobility of chromosomal loci. Taken together with prior studies in budding yeast, we suggest that INO80 uniquely couples the act of transcription and chromatin motion. Stimulated by these experimental data, we built and simulated minimalistic stochastic models that include transcription- and nucleosome-dependent dynamics of SMC proteins and compare it to models of CTCF-dependent or random (diffusion-capture) SMC dynamics. As modeling outputs strongly depend on the parameter values, we developed a polymer-physics-inspired metric that allows a quantitative comparison of the model outputs to experimental Hi-C data. Surprisingly, a simple transcription-nucleosome-dependent model can reproduce the major features of Hi-C maps in mammalian cells without invoking positions of CTCF binding. Our work suggests that the underlying architecture of transcriptional units plays a foundational role in establishing DNA structure that is further influenced by sequence-specific factors.

SG208

**Cracking the Nucleus: Visualizing the Higher Order Structures of Dna At Nucleosome Resolutions and Megabase Scales**

**C. O'Shea**; Salk Institute, San Diego, CA.

The local and global organization of chromatin are integrated in the nucleus to determine gene expression and cell fate. Understanding the many scales of organization in the nucleus remains one of the most important challenges in modern biology. A fundamental question is what is the mechanistic and structural basis that determines local and global gene expression programs are activated and silenced in the nucleus? Until now, it has not been possible to visualize chromatin across scales within an intact cell nucleus. To address this, we developed 'ChromEMT', which exploits a membrane permeable fluorescent DNA binding dye that photo-oxidizes DAB on chromatin in the nucleus and enables it to be visualized and reconstructed at using multi-tilt EM tomography. Contrary to textbook models, these technologies revealed that chromatin does not progressively fold into higher order fibers to achieve compaction in the nucleus. Instead, we show that chromatin is a flexible disordered 5-24 nm chromatin chain that packs together at different concentrations in interphase nuclei and mitotic chromosomes. These studies raise an outstanding question, namely, if chromatin chains are all 5-24 nm, what is the structural basis for gene silencing and activation in the nucleus? To address this requires new technologies that enable the chromatin structure of a specific genomic locus and chromatin modulating proteins to be labeled and visualized together with chromatin in both correlated light and ChromEMT datasets. This drove our development of the FIREnano family of probes. Ferritins are 20-21 kDa proteins that self-assemble into 24 mer cages that channel and store iron in their hollow cores. Using synthetic biology, structure-based design and the natural properties of ferritins from different kingdoms/species,

we created genetically encoded synFerritins that assemble in the nucleus, store iron in their cores that are visible in EM, have 24 fluorescent proteins on their surface that are visible by light and interchangeable targeting moieties to label different molecular features. As a proof of principle, we have used FIREnano to visualize the dynamics and chromatin structure of telomeres as well as a heterochromatic locus in silent state and upon transcriptional activation using live-cell imaging and ChromEMT. FIREnano enables the chromatin structure of a specific genomic locus to be visualized in the nucleus at unprecedented resolutions and scales, revealing the structural basis for gene activation and silencing.

## Subgroup U: The Cellular and Molecular Basis of Invasive Metastatic Cancer ASCB 2019

SG209

**Cytoskeletal Dynamics and Metabolism During Tumor Cell Invasion**

**M. McNiven**, G. Razidlo; Mayo Clinic, Rochester, MN.

Metastatic invasion of tumor cells involves membrane remodeling and re-organization of the actin cytoskeleton to direct cell migration through the extracellular matrix. In this presentation, we will provide new insights into the migration, stromal remodeling, and metabolism exhibited by pancreatic ductal adenocarcinoma cells (PDAC).Tumor cells degrade the extracellular matrix using invadopodia, actin-based, degradative protrusions that are targeting sites for matrix metalloproteinases. Here, we report two different protein complexes that regulate invadopodia stability, and consequently, matrix degradation and tumor cell invasion. First, we have found that the large GTPase Dynamin 2 and the actin crosslinking protein α-actinin 4 interact directly to support tumor cell migration and degrade the underlying matrix. Specific disruption of the Dynamin 2/α-actinin4 interaction significantly attenuates cell migration and matrix remodeling, and results in markedly reduced invadopodial stability. High expression of Dynamin 2 or α-actinin 4 leads to poor outcome in PDAC patients, highlighting the role of this complex in cancer. Second, we have found that the protein MT1-MMP Cytoplasmic Tail Binding Protein 1 (MTCBP-1) negatively regulates MT1-MMP by displacing it from invadopodia. A direct interaction between MTCBP-1 and MT1-MMP disrupts the interaction between MT1-MMP and actin, thereby leading to decreased invadopodia stability and function. Consistent with these findings, MTCBP-1-expressing cells show a marked decrease in the ability to invade *in vitro* and metastasize *in vivo*. These findings implicate MTCBP-1 as a natural inhibitor of the metastatic process.Migration and invasion are energy-intensive processes. As obesity and excess lipids are correlated with increased metastasis, we hypothesized that tumor cells utilize lipids stored as lipid droplets as a fuel source for invasive dissemination. Interestingly, we have found that pancreatic tumor cells shift their metabolic program towards lipid storage through the downregulation of the lipase HSL (hormone sensitive lipase) as part of a metabolic rewiring by the oncogene KRas, yet still require residual lipase activity for invasion. Metabolic analysis and live cell imaging revealed that tumor cells catabolize lipid droplets and transiently upregulate oxidative phosphorylation during invasive migration. Inhibition of cytoplasmic lipases to block lipid droplet catabolism, or depletion of stored lipids by lipase overexpression, both inhibit invasion, migration, and metastasis. These data provide new insights into how metabolic plasticity drives tumor cell invasion.

SG210

**Rho Gtpase Signaling in Cancer Cell Invasion and Metastasis**

**A. Ridley**; University of Bristol, Bristol, UNITED KINGDOM.

Rho GTPases coordinate cell migration through their effects on the cytoskeleton and cell adhesions. Cell migration is important for cancer progression, including invasion of tissues and entry into and exit from blood vessels. Most of the 20 human Rho GTPases cycle between a GTP-bound active form and a GDP-bound inactive form, and are also regulated by a variety of post-translational modifications. We have carried out RNAi screens of Rho GTPase signaling networks and found that different subsets of Rho GTPases and their interacting partners contribute to prostate and breast cancer cell invasion and transendothelial migration. I will describe how we have used the results of these screens to analyse how Rho GTPases and their downstream effectors contribute to different steps of cancer cell metastasis.

SG211

**Crosstalk between Mechanosensing and Metabolism in Pancreatic Cancer Cells**

V. Papalazarou[1], T. Zhang[2], M. Cantini[3], M. Salmeron-Sanchez[3], O. Maddocks[2], **L. M. Machesky**[1]; [1]CRUK Beatson Inst for Cancer Research, Glasgow, UNITED KINGDOM, [2]Institute of Cancer Sciences, University of Glasgow, Glasgow, UNITED KINGDOM, [3]University of Glasgow Centre for the Cellular Microenvironment, Glasgow, UNITED KINGDOM.

Pancreatic ductal adenocarcinoma is particularly metastatic, with poor survival rates and is a cancer where very few treatment options are available. Stiff fibrotic matrix and cancer cell mechanosensing regulate cell proliferation, migration and invasion and contribute to the aggressiveness of pancreatic cancer by yet unexplored mechanisms. The remodelling of matrix and breaching of mechanical barriers by invasive cells are highly dependent on energy availability and are thus thought to be fuelled by metabolic adaptations to tumorigenic microenvironments. However, it is still unknown how matrix mechanics influence cellular energetics and metabolism or how cells meet their ATP requirements during invasion and metastasis. By recreating matrix conditions for pancreatic cancer cells in soft and stiff environments, we found that stiff matrix induced enhanced adhesion, migration and mitochondrial fusion. Cells on stiff matrix had larger focal adhesions and appeared elongated and polarised as they migrated. Metabolomic analysis on cells in these environments revealed a previously unknown connection between mechanosensing and regulation of ATP recycling through the creatine phosphagen system. We used a candidate approach to decipher the mechanism for engagement of the phosphagen system, whereby we connect these changes to mechanosensing. Collectively our results indicate that matrix mechanics positively regulate tumour invasion by favouring ATP production and sharpening of the gradient of ATP vs ADP. We highlight the creatine phosphagen system as a potentially interesting target in pancreatic cancer and in general as a regulator of invasion and dissemination of cancer cells.

SG212

**Powering Cell Invasion through Basement Membrane Barriers**

**D. Sherwood**; Duke University, Durham, NC.

Cell invasion through basement membrane barriers allows cell dispersal during development, immune cell trafficking, and is the defining step that initiates metastatic cancer. To understand the mechanisms that promote invasion, my group has pioneered studies using the in vivo model of anchor cell invasion in C. elegans. We recently discovered that during invasion, mitochondria polarize to the site of basement membrane breach and deliver ATP (visualized with the biosensor ATeam) to promote F-actin formation

within invasive protrusions. Strikingly, in the absence of all the matrix metalloproteinases (MMPs, MMP- worms), a class of enzymes that was thought to be crucial in dissolving basement membrane, more mitochondria polarize to the invasive front to fuel the formation of a large protrusion that breaches the basement membrane through physical force. This adaptive ability may explain the failure of MMP inhibitor clinical trials in metastatic patients and suggests that therapeutically targeting adaptive mechanisms, such as mitochondria, will be required to block invasion in cancer. To better understand mitochondria and how energy is acquired to fuel them during invasion, we generated an anchor cell transcriptome. We found that components of Complex I of the electron transport chain (ETC), which oxidizes NADH (~2.5 ATP/molecule versus Complex II FADH2 ~1.5 ATP) are enriched in the AC. This suggests that the anchor cell generates specialized invasive mitochondria to provide a robust supply of ATP to breach the basement membrane. Through a lipid and carbohydrate import screen, we also discovered that loss of FGT-1, the only known worm ortholog of mammalian GLUT2/3 (glucose transporter) slows invasion and in MMP- worms reduction of FGT-1 dramatically blocks invasion. Notably, a genome edited knock-in mNeonGreen FGT-1 reporter (FGT-1::mNG) revealed that FGT-1::mNG polarizes to the invasive front of the anchor cell and that FGT-1 polarization increases in MMP(-) animals. Taken together, these observations reveal how energy acquisition and usage is a vital and dynamic aspect of cell invasive behavior that could be exploited to potentially halt invasive tumors.

SG213

**Regulation of Tumor Cell Invasion by Oncogenic Signaling**

**W. Guo**; University of Pennsylvania, Philadelphia, PA.

Oncogenic signaling controls tumor cell invasion through their regulation of the cytoskeleton and membrane trafficking. The exocyst complex consists of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84, and is involved in exocytosis and plasma membrane remodeling. Recently, the exocyst is implicated in cell migration and tumor invasion. The exocyst subunit, Exo70, is a direct phospho-target of ERK, the principle kinase in the MAPK signaling cascade. ERK phosphorylation of Exo70 promotes the assembly of the exocyst, leading to the tethering and fusion of secretory vesicles carrying MMPs for ECM degradation and invasion. Constitutive activation of the MAPK signaling is observed in the majority of melanoma patients with $BRAF^{V600E}$ mutation. In a $BRAF^{V600E}$ murine melanoma model or in patient tumor biopsies, the number of invadopodia-like structures decrease upon inhibitor treatment. Mechanistically, $BRAF^{V600E}$ induces the phosphorylation Exo70 by ERK, thus promotes cell migration and invasion. Together, our study in culture, in mice, and in patients delineates a molecular pathway that mediates oncogenic signaling during tumor invasion.

SG214

**The Role of Stromal Tissue Architecture in Metastasis**

**K. Tanner**; NCI/NIH, Bethesda, MD.

In the event of metastastic disease, emergence of a lesion can occur at varying intervals from diagnosis and in some cases following successful treatment of the primary tumor. Is there a difference in strategy to facilitate outgrowth? Why is there a difference in latency? Genetic factors that drive metastatic progression have been identified, such as those involved in cell adhesion, signaling, extravasation and metabolism. However, organ specific biophysical cues may be a potent contributor to the establishment of these secondary lesions. Here I discuss using optical tweezer based active microrheology to measure

the mechanical cues that may influence disseminated tumor cells in different organ microenvironments. I further discuss in vitro and in vivo preclinical models such as 3D culture systems and zebrafish in efforts of understanding the role of the biophysical properties of the stromal architecture on the earliest stage of organ colonization.

SG215

**Exosomes in Filopodia Formation**

**C. McAtee**[1], D. Hoshino[2], N. Hong[3], B. Sung[1], A. Maldonado[1], A. Von Lersner[4], A. Zijlstra[4], A. Weaver[1,4]; [1]Vanderbilt University, Nashville, TN, [2]Kanagawa Cancer Center Research Institute, Kanagawa, JAPAN, [3]Fred Hutchinson Cancer Research Center, Seattle, WA, [4]Vanderbilt University Medical Center, Nashville, TN.

Exosomes are small secreted vesicles that carry a variety of cargoes and have been shown to promote tumor cell motility and metastasis. Cell motility is influenced by dynamic formation and stability of filopodia: actin-rich protrusions that extend from the leading edge and control directional movement. Furthermore, filopodia regulators such as fascin are upregulated in multiple epithelial cancers and can also promote invasive phenotypes. However, how filopodia are induced and controlled by extracellular factors is poorly understood. Here, we describe a role for exosomes in regulating filopodia formation and tumor cell motility. Inhibition of exosome secretion in multiple cancer cell lines, via Rab27a or Hrs knockdown, led to decreased numbers of filopodia. Specificity to exosomes was demonstrated by rescue experiments in which purified exosomes, but not microvesicles, rescued the filopodia phenotypes of exosome-inhibited cells. Live imaging of Hrs-KD cells revealed that exosome secretion regulates formation and/or stability of filopodia. Proteomics data and molecular validation experiments identified the TGF-beta coreceptor endoglin as a key exosome cargo regulating filopodia formation, cancer cell motility, and metastasis. These data are relevant to cancer as endoglin expression is altered in many cancers. In addition, endoglin is the disease gene for hereditary hemorrhagic telangiectasia, and may influence angiogenesis. Overall, our data implicate exosome-carried endoglin as a key cargo regulating filopodia.

SG216

**Tracking Extracellular Vesicles in Breast Cancer Metastasis**

**J. G. Goetz**; INSERM U1109 - Tumor Biomechanics, STRASBOURG, FRANCE.

Tumor extracellular vesicles (EVs) promote tumor progression. However, their behavior in body fluids remains mysterious. In addition, further understanding of molecular mechanisms driving their biogenesis is needed to develop strategies aiming to impair their tumorigenic potential.We recently showed that the zebrafish embryo can be used to track and assess the function of circulating tumor EVs *in vivo* and provide a high-resolution description of their dissemination and uptake (Hyenne et al., Dev Cell, 19). We combined chemical and genetically encoded probes with the zebrafish embryo as an animal model and provide a first description of tumor EVs' hemodynamic behavior and intravascular arrest. We also show that circulating tumor EVs are rapidly taken up by endothelial cells and blood patrolling macrophages and subsequently stored in degradative compartments.We recently investigated the molecular mechanisms of EV release in a tumorigenic context and observed that depletion of either RalA or RalB decreases levels of EVs' secretion (Hyenne et al. JCB 15) and perturbs the protein and the RNA content of EVs. Interestingly, RalA and B colocalize with PLD1 on MVBs and their depletion

mislocalizes PLD1 from MVBs that disrupts these compartments. Furthermore, Rala and RalB depletion significantly impairs lung metastasis in a syngeneic model of breast carcinoma suggesting that RalA/B controls lung metastatis by tuning the levels and contents of tEVs.Overall, our recent works proves the usefulness and prospects of zebrafish embryo to track tumor EVs and dissect their role in metastatic niches formation *in vivo*. It further provides new mechanistic information as to how RalA and RalB control the biogenesis of potent tumor-promoting EVs.

## Subgroup V: Redrawing the Cellular Map: Cytoskeletal Forces, Organelles and the Crossroads

SG217

**Sub-organellar Actin Dynamics Revealed by Organelle-targeted Actin Chromobodies**

C. R. Schiavon[1], T. Zhang[1], B. Zhao[2], J. Feng[3], L. R. Andrade[1], M. Wu[1], T. Sung[1], O. A. Quintero[3], Y. Dayn[1], R. Grosse[2], **U. Manor**[1]; [1]Salk Institute for Biological Studies, La Jolla, CA, [2]University of Freiburg, Freiburg, GERMANY, [3]University of Richmond, Richmond, VA.

The actin cytoskeleton has long been known to regulate organelle dynamics. However, the small and transient actin structures regulating organelle dynamics are difficult to detect with fluorescence or electron microscopy. Live cell fluorescence imaging of organelle-associated actin is particularly hindered by the huge amounts of “background” signal caused by an unfortunate combination of (i) the ubiquity of actin throughout the cytoplasm, (ii) the relatively large and stable actin structures in the cell cortex of adherent cells, and (iii) the resolution limit optical microscopy. To circumvent this issue, we developed an approach using fluorescent protein-tagged actin nanobodies fused to organelle membrane targeting sequences. We validated the probes using pan-actin probes including phalloidin and cytoplasmic actin nanobodies. Fluorescence recovery after photobleaching measurements showed that these probes are highly diffuse on the organelle membrane unless bound to actin, which immobilizes them. Live cell imaging of these probes revealed previously undetected sub-organellar actin structures on mitochondria, the ER, and the nucleus. Simultaneous imaging of both ER- and mitochondria-associated revealed that actin first accumulates on mitochondria prior to the ER during mitochondrial fission events. Finally, we found ER-associated actin marks constriction or fission sites for all the organelles we tested, including mitochondria, endosomes, lysosomes, peroxisomes, and the Golgi. Overall, these results show that using organelle-targeted nanobody probes for studying organelle-cytoskeleton dynamics can reveal previously undetected structures in the cell with very high spatiotemporal resolution.

SG218

**Co-expressed Actins Regulate Biogenesis of the Microtubule-based Cilium Via Intracellular Trafficking and Organelle Gating**

B. Jack, B. M. Bigge, N. E. Rosenthal, **P. Avasthi**; University of Kansas Medical Center, Kansas City, KS.

Actin regulates cell morphology, membrane dynamics, intracellular transport and more, but is involved in many processes attributed primarily to other cytoskeletal systems. One such process is in assembly of the microtubule-based organelle, the cilium. Trafficking of proteins needed for cilium function is largely attributed to minus-end directed microtubule motors. These proteins, when accumulated at the ciliary base, also enter cilia using plus-end directed microtubule motors. However, using the green alga

*Chlamydomonas reinhardtii*, we found that normal actin function is required for synthesis of ciliary proteins at normal levels, transport of Golgi-adjacent vesicles, and organization of a gating region at the base of cilia called the transition zone. The transition zone houses many proteins important for the selective entry and export of ciliary proteins including a variety of disease gene products such as NPHP-4. NPHP-4 is mutated in the childhood kidney disorder nephronophthisis. We find that NPHP-4 is mislocalized when a *Chlamydomonas* actin that is ~90% identical to mammalian actins, IDA5, is disrupted with latrunculin B (lat B). However, under these conditions, a divergent actin, NAP1, is upregulated. Upon expression of this lat B-insensitive second actin, NAP1 (63% identical to IDA5), NPHP-4 localization to the transition zone is restored. In contrast, blocking branched actin formation via ARP2/3 inhibition with the small molecule CK-666 prevents the rescue of NPHP-4 localization by NAP1. As NAP1 function (NPHP-4 re-positioning at the transition zone) is disrupted by the ARP2/3 inhibitor, we propose that the branched actin nucleator is capable of interacting with NAP1 filaments. Further, inhibition of *Chlamydomonas* myosins with blebbistatin (previously shown to cause impairments in recruitment and entry of ciliary proteins) also caused loss of NPHP-4 from the transition zone suggesting a role for actin-based transport in positioning or turnover of this key ciliary protein trafficking regulator. Finally, by transmission electron microscopy, we find that transition zones are more dramatically disorganized upon actin disruption compared to what was seen previously for NPHP-4 mutants, suggesting that additional transition zone proteins are dependent upon actin integrity. Our data support a model in which the NPHP-4 ciliary gating protein is trafficked by myosin and regulated by actin networks that can be comprised of IDA5 or branched NAP1 filaments.

SG219

**Spire1C and the Arp2/3 Complex Drive a Wave of Filamentous Actin that Promotes Mitochondrial Fission and Motility**

**S. M. Coscia**[1], A. S. Moore[2], C. L. Simpson[1], E. L. Holzbaur[1]; [1]University of Pennsylvania, Philadelphia, PA, [2]Janelia Research Campus, Ashburn, VA.

Mitochondria are dynamic organelles that undergo fission and fusion and move throughout the cell. The main driver of organelle dynamics is the cytoskeleton. While it is well established that mitochondria are trafficked along microtubules, interactions between mitochondria and actin are less well understood. Novel results from our lab expand on the role of actin in mitochondrial dynamics. Using spinning disk and lattice light-sheet microscopy our lab identified in both immortalized and primary cell types a wave of filamentous (F-) actin that propagates through the mitochondrial network cyclically assembling and disassembling on adjacent subpopulations of mitochondria. In interphase the F-actin wave promotes mitochondrial fission; once actin disassembles daughter organelles often fuse with distinct neighbors leading to mitochondrial mixing. During metaphase the F-actin wave promotes mitochondrial motility via the formation of actin comet tails. To investigate the mechanism underlying the F-actin wave we used both pharmacological inhibitors and RNAi and determined that the wave depends on a diverse set of proteins that facilitate F-actin assembly and disassembly. Specifically, the F-actin wave is regulated by CDC42 and requires the Arp2/3 complex, VASP, and Spire1C. Actin filaments within the wave recruit the stabilizing factor, Filamin A. Active depolymerization of F-actin is also required to maintain the integrity of the wave as siRNA-mediated depletion of ADF, Gelsolin, or Cofilin resulted in significant enlargement of wave size. We propose that the function of the F-actin wave is to allow spatial mixing of mitochondrial contents, including mitochondrial DNA.

SG220

**The MyMOMA Domain of MYO19 Encodes for Distinct Miro-dependent and Miro-independent Mechanisms of Interaction with Mitochondrial Membranes**

J. L. Bocanegra[1], B. M. Fujita[1], N. R. Melton[1], J. M. Cowan[1], E. L. Schinski[1], T. Y. Tamir[2], M. B. Major[2], **O. A. Quintero**[1]; [1]University of Richmond, Richmond, VA, [2]University of North Carolina, Chapel Hill, NC.

MYO19 interacts with mitochondria through a C-terminal membrane association domain (MyMOMA). The specific mechanisms for localization of MYO19 to mitochondria are poorly understood. Using new promiscuous biotinylation data in combination with existing affinity-capture databases, we have identified a number of putative MYO19-interacting proteins. We chose to further explore the interaction between MYO19 and the mitochondrial GTPase Miro2 by expressing mchr-Miro2 in combination with GFP-tagged fragments of the MyMOMA domain and assaying for recruitment of MYO19-GFP to mitochondria. Coexpression of MYO19$^{898\text{-}970}$-GFP with mchr-Miro2 enhanced MYO19$^{898\text{-}970}$-GFP localization to mitochondria. Mislocalizing Miro2 to filopodial tips or the cytosolic face of the nuclear envelope did not recruit MYO19$^{898\text{-}970}$-GFP to either location. To address the kinetics of the Miro2/MYO19 interaction, we used FRAP analysis and permeabilization-activated reduction in fluorescence (PARF) analysis. MyMOMA constructs containing a putative membrane insertion motif but lacking the Miro2-interacting region displayed slow exchange kinetics. MYO19$^{898\text{-}970}$-GFP, which does not include the membrane-insertion motif, displayed rapid exchange kinetics, suggesting that the MYO19 interacting with Miro2 has higher mobility than MYO19 inserted into the mitochondrial outer membrane. Mutation of well-conserved, charged residues within MYO19 or within the switch I and II regions of Miro2 abolished the enhancement of MYO19$^{898\text{-}970}$-GFP localization in cells ectopically expressing mchr-Miro2. Additionally, expressing mutant versions of Miro2 thought to represent particular nucleotide states indicated that the enhancement of MYO19$^{898\text{-}970}$-GFP localization is dependent on Miro2 nucleotide state. Taken together, these data suggest that membrane-inserted MYO19 is part of a larger complex, and that Miro2 plays a role in integration of actin- and microtubule-based mitochondrial activities.

SG221

**Spatial Organization of Glycolytic Enzymes on Stationary Mitochondria**

**G. Pekkurnaz**, H. Wang; UCSD Division of Biological Sciences, La Jolla, CA.

Cells harness energy through a stepwise breakdown of more complex substances, such as glucose, into simpler ones via a plethora of highly interconnected metabolic reactions. Polarity, non-uniform nutrient access and energy needs are fundamental feature of many cell types. However, our understanding of the molecular mechanisms that spatially partition metabolic biochemistry within the cytoplasm, is cursory at best. Here, we investigate how metabolic enzymes work coherently and efficiently within the complex cellular cytoarchitecture of large polarized cells such as neurons. Glycolysis is the first step in the breakdown of glucose, which takes place in the cytosol of a cell and starts with the activity of the first-rate limiting step enzyme Hexokinase (HK). In this study, we report a new molecular mechanism which regulates HK1 activity and mitochondrial localization via the metabolic sensor enzyme O-GlcNAc transferase (OGT). OGT catalyzes a reversible posttranslational modification by adding a GlcNAc sugar moiety to serine and threonine residues (O-GlcNAcylation). The catalytic activity of OGT is regulated by intracellular UDP-GlcNAc concentrations, which fluctuate proportionally in response to glucose flux through the hexosamine biosynthetic pathway. In this study, we show that HK1 is dynamically modified

with O-GlcNAcylation at its regulatory domain. O-GlcNAcylation of HK1 is elevated when OGT activity is upregulated. We further characterize that O-GlcNAc modification increases mitochondrial HK1, and also enhances both glycolytic and mitochondrial ATP production rates. We demonstrate that HK1 is specifically enriched on the stationary mitochondrial pool in neurons. The positioning of HK1 on mitochondria is critical because it couples glucose metabolism with energy generation pathways in mitochondria. Our findings may reveal key molecular pathways which couple glycolysis to mitochondrial function via OGT, and how spatial metabolic organization is used for energy homeostasis.

SG222

**Septin 9 (SEPT9) Promotes the Retrograde Transport of Endolysosomes by Scaffolding Dynein-Dynactin Complexes**

**I. Kesisova**, B. K. Doyle, E. T. Spiliotis; Drexel University, PHILADELPHIA, PA.

Spatiotemporal coordination of membrane traffic is paramount for cell development and survival. Long-range transport of membrane cargos is driven by the microtubule motors kinesin and dynein. However, how cargos selectively associate with and activate motors is not well understood. Here we find that membrane-associated SEPT9, a member of the septin family of GTPases, regulates dynein-dependent transport of lysosomes by direct association with dynein and dynactin. We examined the abundance of septins on cellular membranes by density gradient fractionation and we found that SEPT9 is enriched in lysosomal fractions. Super-resolution structured illumination microcopy (SIM) showed that SEPT9 localizes on domains of Lamp1-positive endolysosomes. In the axons of cultured hippocampal neurons SEPT9 co-migrates with Lamp1-positive endolysosomes, which move with a retrograde bias toward the cell body, and SEPT9 overexpression in COS-7 fibroblasts results in perinuclear repositioning of lysosomes. Membrane relocation assays showed that ectopic targeting of SEPT9 to mitochondrial membranes is sufficient to induce perinuclear clustering of mitochondria, which is reversed by nocodazole treatment and p50/dynamitin overexpression. Similarly, coupling of SEPT9 to peroxisomes through a rapalog-induced heterodimerization assay resulted in perinuclear clustering of peroxisomes. Biochemical *in vitro* binding assays showed that recombinant SEPT9 interacts directly with native dynein/dynactin isolated from HEK-293 cells. We mapped dynein interaction to the conserved GTPase domain of SEPT9, while the N-terminal domain of SEPT9 interacts with dynactin. Interestingly, recombinant SEPT9 interacts with the N-terminal adaptor-binding region of dynein intermediate chain (DIC) (108-268aa in mouse IC1A), which does not associate with SEPT2/6/7. Strikingly, this interaction is sensitive to the nucleotide state of SEPT9 as SEPT9 association with DIC is diminished in the presence of the non-hydrolyzable GTP analog GTPγS. Collectively, our findings suggest that SEPT9 functions on the membranes of endolysosomes as a scaffold for the recruitment and potential activation of dynein. On-going work is exploring this hypothesis and testing the role of SEPT9 in lysosome positioning under conditions of cell stress.

SG223

**Isoform-specific Differences in the Function of Hereditary Spastic Paraplegia-associated Protein Spastin in Endoplasmic Reticulum Morphogenesis and Cellular Homeostasis**

**C. A. Lee**, C. Blackstone; National Institutes of Health, Bethesda, MD.

Hereditary spastic paraplegias (HSPs) are a genetically diverse group of inherited neurodegenerative disorders selectively affecting the longest corticospinal motor neurons; they are characterized most

prominently by the progressive spasticity of the lower limbs. Molecular etiologies of HSPs are complex and caused by mutations in one of more than 60 identified genes. However, the most common forms, representing about half of all affected patients, are due to autosomal dominant mutations in one of three tubular ER-shaping proteins: spastin, atlastin-1, and REEP1. Therefore, ER-shaping defects appear to be a common pathogenic mechanism, however, it is not known how ER morphology defects contribute to degeneration and dysfunction of corticospinal motor neurons. Mutations in the *SPAST* gene are the most common causes of HSP, and the *SPAST* gene product spastin is a microtubule-binding and -severing AAA ATPase protein. A single *SPAST* mRNA transcript produces two isoforms (M1 and M87) with different subcellular localizations and functions. The full-length M1 isoform contains an N-terminal 86-amino acid domain that is absent in the shorter M87 isoform. This N-terminal region harbors a membrane-bound hydrophobic hairpin that confers ER localization and generates membrane curvature to shape the tubular ER. The more abundant M87 spastin is largely cytosolic and has been functionally implicated in endosomal trafficking and cytokinesis. While the overwhelming majority of mutations in *SPAST* will affect the expression of both isoforms, it is not known which isoform contributes to HSP pathogenesis. Here, we have used CRISPR/Cas9 gene editing to generate isoform-specific knockout cell lines. Knockout of M1 spastin results in aberrant peripheral ER morphogenesis and loss of tubular ER polygonal networks, indicating that M1 is necessary for proper tubular ER formation and organization. Isoform-specific roles of spastin in ER morphogenesis and dynamics were also examined in cells specifically expressing each spastin isoform, as well as spastin isoforms harboring the ATPase defective, HSP-associated mutation K388R. Expression of M1 spastin K388R but not M87 spastin K388R results in ER membranes trapped on microtubules. Furthermore, fluorescence recovery after photobleaching (FRAP) and single-molecule imaging approaches were used to examine the specific dynamic nature of each spastin isoform. Understanding the functional contributions of each isoform will be crucial for understanding the etiology of HSP and for finding new therapeutic targets.

SG224

**Mitochondria-Lysosome Contact Site Regulation of Mitochondrial Dynamics and Dysfunction in Charcot-Marie-Tooth Type 2.**

**Y. Wong**, W. Peng, D. Krainc; Northwestern University, Chicago, IL.

Both mitochondria and lysosomes are essential for maintaining cellular homeostasis, and dysfunction of both organelles has been observed in multiple neurodegenerative diseases. We recently found that mitochondria-lysosome contact sites formed dynamically and were distinct from damaged mitochondria targeted into lysosomes for degradation. Contact formation was promoted by active GTP-bound lysosomal RAB7, and contact untethering was mediated by recruitment of the RAB7 GTPase-activating protein TBC1D15 to mitochondria by FIS1 to drive RAB7 GTP hydrolysis and thereby release contacts. Functionally, lysosomal contacts marked sites of mitochondrial fission, allowing regulation of mitochondrial networks by lysosomes, whereas conversely, mitochondrial contacts regulated lysosomal RAB7 GTP hydrolysis via TBC1D15. In addition, mitochondria undergo fission and fusion events, but the dynamics and regulation of a third event of inter-mitochondrial contact formation remain unclear. Using super-resolution imaging, we demonstrated that inter-mitochondrial contacts frequently formed and played a fundamental role in mitochondrial networks by restricting mitochondrial motility. Inter-mitochondrial contact untethering events were marked and regulated by mitochondria-lysosome contacts which were modulated by RAB7 GTP hydrolysis. Moreover, inter-mitochondrial contact formation and untethering were further regulated by Mfn1/2 and Drp1 GTP hydrolysis respectively.

Surprisingly, endoplasmic reticulum tubules were also present at inter-mitochondrial contact untethering events, in addition to mitochondrial fission and fusion events. Importantly, we found that multiple disease mutations linked to the peripheral neuropathy Charcot-Marie-Tooth Type 2 such as Mfn2 (CMT2A), RAB7 (CMT2B) and TRPV4 (CMT2C) converged on prolonged inter-mitochondrial contacts and defective mitochondrial motility, highlighting a role for mitochondria-lysosome contact regulation of inter-mitochondrial contacts in mitochondrial network regulation and neurodegeneration.

SG225

**Opening Windows Into the Cell: Bringing Structure to Cell Biology Using Cryo-electron Tomography**

**E. Villa**; University of California San Diego, La Jolla, CA.

To perform their function, biological systems need to operate across multiple scales. Current techniques in structural and cellular biology lack either the resolution or the context to observe the structure of individual biomolecules in their natural environment, and are often hindered by artifacts. Our goal is to build tools that can reveal molecular structures in their native cellular environment. Using the power of cryo-electron tomography (CET) to image biomolecules at molecular resolution in situ, we are building tools to make compatible with, and directly comparable to, biophysical and cell biology experiments, capturing the structural behavior of macromolecules in action under controlled conditions. I will show how we used these techniques to reveal the structure of LRRK2, the greatest known genetic contributor to Parkinson's disease, and to reveal the molecular architecture of bacterial cells.

## Subgroup W: Maintenance of Genome Integrity in Health and Disease

SG226

**A Novel Chromatin Directed Vulnerability in Brca Mutated Cancers**

P. Verma, J. Shi, **R. A. Greenberg**; University of Pennsylvania, Philadelphia, PA.

A central aspect of the DNA damage response is the induction of myriad homology directed DNA repair mechanisms that use templated DNA synthesis to execute either high fidelity restoration of lesions to their ground state or inherently error prone mechanisms that result in loss of genome integrity. This prominence of repair mechanism utilization is illustrated in the setting of homologous recombination deficiency due to mutation within a network of genes centered around the breast and ovarian cancer suppressor proteins. This BRCA network is required for high fidelity DNA repair by homologous recombination. While deficiency in this canonical homology directed DNA repair pathway is confers cancer susceptibility, it also creates vulnerability to agents that target orthogonal repair mechanisms. Poly(ADP) Ribose Polymerase (PARP) inhibitors are approved to treat homologous recombination deficiency breast and ovarian cancer, with demonstrated improvements in progression free survival in tumors with BRCA mutations. The efficacy of PARP inhibitors depends on expression of the PARP1 enzyme, which becomes trapped on chromatin. In the absence of BRCA1, toxic use of nohomologous endjoining repair mechanisms creates dicentric chromosomes that result in a loss of viability upon passage through mitosis. The intersection of these repair pathways in PARPi response leads to resistance mechanisms that entail either loss of PARP1 expression or deficiency in nohomologous endjoining repair. These are thought to account, at least in part, for failure of ~50% of BRCA mutated cancers to respond to PARPi. Ideally, additional targets could be identified that circumvent PARPi resistance regardless of mechanism and restore efficacy in BRCA mutated cancers.This presentation will describe

our unpublished results that implicate the chromatin remodeling protein CHD1L as a novel therapeutic target in BRCA mutated cancers. Functional domain directed CRISPR-Cas9 screens were used to identify CHD1L as a vulnerability in BRCA1 and BRCA2 mutated cells. Our findings demonstrate that CHD1L loss is synthetic lethal in combination with mutation to either BRCA1 or BRCA2, while conferring extreme PARPi hypersensitivity regardless of resistance mechanism. Models to conceptualize these findings will be presented as well as their implications for harnessing DNA damage responses to enhance therapy in homologous recombination deficient cancers.

SG227

**Redefining Therapy Response**

**S. Cantor**; UMASS Medical School, Worcester, MA.

BRCA1 or BRCA2 (BRCA)-deficient tumor cells have defects in DNA repair by homologous recombination and DNA replication fork stability that is thought to underlie poly(ADP) ribose polymerase, PARP inhibitor (PARPi) sensitivity. However, new findings indicate that PARPi do not initially generate DNA breaks or pause replication forks, but rather accelerate DNA replication forks (Maya-Mendoza, A. et al Nature 2018). Thus, we tested the hypothesis that sensitivity results from combined replication dysfunction. Consistent with this interpretation, here we demonstrate that PARPi-induced replication acceleration is exacerbated in BRCA deficient cells, but avoided in cells that are not sensitive to PARPi. Furthermore, in BRCA deficient -tissue culture and -patient tumors and in known and de novo models of PARPi resistance, we find that this replication dysfunction is suppressed. Collectively a molecular link between PARPi sensitivity and replication dysfunction provides a new paradigm for understanding synthetic lethal interactions in BRCA cancer.

SG228

**Homologous Recombination Repair Domains: Formation and Impact on Genome Stability**

**J. Zagelbaum**[1], B. R. Schrank[1], J. Zhao[1], A. Schooley[2], R. Rabadan[1], J. Dekker[2], J. Gautier[1]; [1]Columbia University, New York, NY, [2]University of Massachusetts Medical School, Worcester, MA.

DNA double-strand break (DSB) repair is spatially organized into nuclear repair domains that specifically facilitate DSB repair by homologous recombination (HR). Our lab demonstrated that upon DSB formation by induction of a restriction endonuclease (RE) or treatment with neocarzinostatin (NCS), WASP activates ARP2/3, which polymerizes nuclear actin into branched filaments (1). This enhances the mobility of DSBs destined for HR and their subsequent clustering into HR domains. Although DSB clustering is crucial for HR, little is known about how repair domains are formed and their local and genome-wide implications. For example, we do not fully understand the crosstalk between movement (actin, WASP) and repair (HR machinery) in mammalian cells.Using live-cell imaging, mean squared displacement (MSD), and clustering analysis, we are elucidating mechanisms by which nuclear actin polymerization and HR proteins regulate repair domain formation and the genome-wide impact of DSB mobility. We have documented the mobility of etoposide-induced (ETO) breaks. We show that ETO-induced breaks move in an ARP2/3-dependent manner in G2. Notably, ETO also generates DNA DSBs that cluster in G1. Whereas motion of DSBs induced by NCS in G1 is independent of actin, breaks formed from ETO in G1 require ARP2/3 for mobility. Additionally, ETO breaks in G1 load the ssDNA binding protein RPA, indicating that these breaks undergo resection, the initial step of HR.We are also examining the role of HR factors, including Mre11 and BRCA2, in repair domain formation following the generation

of DSBs by RE, NCS, and ETO. By using different damaging agents with distinct repair requirements (ETO, RE and NCS), genetic inactivation, mutated cell lines, and small-molecule inhibitors, we will provide mechanistic insight into the role of individual HR machinery in DSB clustering. Finally, we are integrating high-throughput genomic technologies that assess gene-gene localization (Hi-C) and translocation events (High-Throughput Genome-Wide Translocation Sequencing - HTGTS) to determine the genome-wide implications of DSB mobility. The combination of live-cell imaging and genomics approaches provides critical information on the spatial regulation of DSB repair, including its normal functions as well as its pathological consequences such as chromosome rearrangements.1. Schrank BR*, et al.* (2018) Nuclear ARP2/3 drives DNA break clustering for homology-directed repair. *Nature* 559(7712):61-66.

SG229

**Investigation of Break-induced Replication**

**A. Malkova, 52245**; University of Iowa, Iowa City, IA.

Break-Induced Replication (BIR) is a DSB repair pathway responsible for the repair of one-ended DSBs, including those resulting from replication fork collapse or telomere erosion. Using our yeast experimental system, we have previously demonstrated that an HO-induced DSB introduced at *MAT*a, located in a truncated copy of chromosome III, leads to efficient BIR that proceeds via invasion of the broken end into another (intact) copy of chromosome III followed by DNA synthesis continuing for 100kb to the end of the chromosome. Instead of a normal replication fork, BIR is carried out by a migrating bubble proceeding with asynchrony between leading and lagging strand synthesis and results in conservative inheritance of newly synthesized DNA. This unusual type of DNA synthesis is highly mutagenic and often leads to chromosomal rearrangements, which makes it important to understand how BIR is carried out and regulated. While initial steps of BIR and its final products have been previously characterized, detection of transient molecular intermediates of BIR remains problematic. Recently, we detected intermediates of BIR synthesis, which allowed us to determine that progression of BIR synthesis is significantly slower as compared to S-phase DNA replication. We also characterized BIR progression in several BIR-defective mutants including *pif1Δ* and *pol32Δ*. Further, we tested the effect of transcription on the progression of BIR and observed that transcription in both directions (head-on and co-directional) interferes with BIR progression, with head-on orientation having a more drastic effect on BIR elongation. Finally, we investigate the role of BIR in alternative lengthening of telomeres (ALT) in yeast. We report that the absence of Srs2 helicase, which according to our previous results is lethal for cells undergoing BIR is also lethal for cells undergoing ALT. We speculate that similar to the case of BIR, initiation of ALT in *srs2Δ* cells leads to the formation of toxic recombination intermediates, thus killing cells undergoing ALT and precluding formation of ALT survivors. We also identify the frequency of ALT survivors in *tlc1Δ* cells, as well as the frequencies of specific types of ALT survivors, Type I and Type II, and characterize the structure of chromosomal ends in ALT survivors by whole genome sequencing.

SG230

**Regulation of Genome Stability At Replication Forks**

J. Huang[1], A. Taglialatela[1], A. Acharya[2], G. Leuzzi[1], R. Cuella-Martin[1], D. Billing[1], G. Brunette[3], N. Clark[3], K. Bernstein[3], R. Baer[1], P. Cejka[2], **A. Ciccia**[1]; [1]Columbia University Irving Medical Center, New York, NY, [2]Università della Svizzera italiana, Bellinzona, SWITZERLAND, [3]University of Pittsburgh, Pittsburgh, PA.

During DNA replication, DNA lesions can lead to the arrest or collapse of replication forks, thus impairing replication fork progression and causing replication stress. To ensure the completion of DNA replication and the maintenance of genomic stability, DNA repair factors protect stalled replication forks in response to DNA damage. Previous studies have identified a critical role for the tumor suppressors BRCA1 and BRCA2 in protecting stalled replication forks from MRE11-dependent degradation. Here I will show that SMARCAL1, a DNA translocase that associates with the ssDNA-binding complex RPA, remodels stalled forks and induces fork degradation in BRCA1/2-deficient cells. I will additionally describe a novel RPA-associated protein that binds the MCM8/9 helicase to stimulate DNA synthesis after replication stress and promote the repair of DNA double-strand breaks by homologous recombination. These studies provide greater insights into the processes that maintain genome stability during DNA replication.

SG231

**Sirt6 Is Responsible for More Efficient Dna Double-strand Break Repair in Long-lived Species**

**V. Gorbunova**, X. Tian, A. Seluanov; University of Rochester, Rochester, NY.

DNA repair has been hypothesized to be a longevity determinant, but the evidence for it is based largely on accelerated aging phenotypes of DNA repair mutants. Here, using a panel of 18 rodent species with diverselifespans, we show that more robust DNA double-strand break (DSB) repair, but not nucleotide excision repair (NER), coevolves with longevity. Evolution of NER, unlike DSB, is shaped primarily by sunlight exposure. We further show that the capacity of the SIRT6 protein to promote DSB repair accounts for a major part of the variation in DSB repair efficacy between short- and long-lived species. We dissected the molecular differences between a weak (mouse) and a strong (beaver) SIRT6 protein and identified five amino acid residues that are fully responsible for their differential activities. Our findings demonstrate that DSB repair and SIRT6 have been optimized during the evolution of longevity, which provides new targets for anti-aging interventions.

SG232

**Pathological Trans-lesion Synthesis (TLS): a Mutagenic Driver and Molecular Vulnerability in Cancer**

**C. Vaziri**; University of North Carolina, Chapel Hill, NC.

There are fundamental gaps in our understanding of how neoplastic cells tolerate intrinsic DNA replication stress and DNA damage while simultaneously accumulating the mutations that fuel cancer progression. Trans-Lesion Synthesis (TLS) is a specialized mode of DNA replication that employs damage-tolerant and error-prone DNA polymerases. Therefore, TLS provides a potential explanation for how neoplastic cells endure diverse genotoxic stresses while mutating their genomes during multi-step tumorigenesis. We have tested the hypothesis that TLS facilitates carcinogenesis by conferring DNA damage tolerance and mutability. We show that: (1) The TLS pathway is pathologically activated by an aberrantly-expressed germ cell protein (Melanoma Antigen A4, or MAGE-A4) in many cancer cells. (2) TLS maintains ongoing DNA synthesis and sustains viability in cells experiencing oncogene-induced DNA replication stress. (3) The TLS pathway promotes tumorigenesis and shapes cancer genomes *in vivo*. Taken together these results show that TLS endows neoplastic cells with two important tumorigenic phenotypes namely DNA damage tolerance and mutability. The DNA damage tolerance and mutability acquired during carcinogenesis also allows cancer cells to resist therapy-induced genotoxicity. The identification of pathological TLS as a cancer-specific DNA damage tolerance pathway provides

opportunities for new treatments that selectively sensitize tumors to genotoxic therapies yet are innocuous to normal cells.

SG233

**Chromosome Segregation Errors Generate a Diverse Spectrum of Structural Genomic Rearrangements**

S. F. Brunner[1], O. Shoshani[2], P. J. Campbell[1], D. W. Cleveland[2], **P. Ly**[3]; [1]Wellcome Sanger Institute, Hinxton, UNITED KINGDOM, [2]Ludwig Institute for Cancer Research, University of California San Diego, La Jolla, CA, [3]University of Texas Southwestern Medical Center, Dallas, TX.

Alterations in chromosome number and structure are genomic hallmarks of human cancer. Chromosome segregation errors during cell division are established to cause numerical aneuploidy, yet how these errors shape the structural genomic landscape remains unclear. Here we developed CEN-SELECT, a strategy that combines a centromere-specific inactivation approach with selection for a conditionally essential gene, to interrogate the structure of mis-segregated chromosomes in diploid human cells. We show that chromosome mis-segregation during mitosis can directly trigger a broad spectrum of genomic rearrangement types that mechanistically arises from DNA damage within micronuclei and chromosome fragmentation. Cytogenetic profiling revealed that micronucleated chromosomes exhibit 120-fold higher susceptibility to developing seven major categories of structural aberrations, including translocations, insertions, deletions, and complex reassembly through chromothripsis. These rearrangements are formed through DNA double-strand break repair by LIG4-mediated classical non-homologous end joining. Whole-genome sequencing of single cell-derived clones identified random patterns of clustered breakpoints and DNA copy number alterations resulting in interspersed gene deletions and extrachromosomal gene amplification events. We conclude that chromosome segregation errors during mitotic cell division are sufficient to drive extensive structural variation that recapitulate genomic features commonly associated with human disease.

SG234

**Role of Deubiquitinases in the Mammalian Replication Stress Response**

**T. T. Huang**; NYU School of Medicine, New York City, NY.

DNA replication stress is often defined by the slowing or stalling of replication fork progression leading to local or global DNA synthesis inhibition. Failure to resolve replication stress in a timely manner contribute toward cell cycle defects, genome instability and human disease, however, the mechanism for fork recovery remains poorly defined. Here, I will present our recent work regarding how deubiquitinase(s) (DUBs) regulate replication fork progression and genome stability in human cells. We will also show that protein turnover on the replisome is a key determinant for normal fork progression. Understanding the role of DUBs in the replication stress response could represent an attractive therapeutic target against cancers.

## Subgroup X: Cell Dynamics and Matrix Interactions in Three-Dimensional Environments

SG235

**Mechanical Tension in Syndecan-1 Is Responsive to Extracellular Mechanical Cues and Fluidic Shear Stress**

V. Le, **A. Baker**, L. Mei, P. Voyvodic, C. Zhao, D. Busch, J. Stachowiak; University of Texas at Austin, Austin, TX.

Syndecan-1 (SDC-1) is a transmembrane cell surface proteoglycan that controls the shear stress-induced signaling in the glycocalyx of arteries and mediates inflammatory phenotype of endothelial cells. While SDC-1 is involved in mechanically mediated behavior of cells it is unknown how SDC-1 responds to forces on the molecular level. We designed a set of FRET-based SDC-1 tension sensors including full length SDC-1 (SDC1-TS), SDC-1 with deleted ectodomain (SDC1-TSΔE), SDC-1 with deleted glycosylation sites (SDC1-TSΔGAG) and SDC-1 with deleted cytoplasmic domain (SDC1-TSΔC) and used lentiviral transduction to produce stable endothelial cell lines expressing these constructs. We validated the constructs using sequencing, western blotting and FLIM-FRET imaging. We found that the baseline tension in SDC1-TS was significantly higher than that in the SDC1-TSΔE and SDC1-TS ΔGAG. Using micropatterned and nanopatterned substrates, we demonstrated that the tension in SDC-1 is modulated by adhesion and by the presence of nanotopology of the substrate. In regions of adhesion there was a significant increase in tension in SDC-1 in the full-length construct. In addition, we found that substrate compliance could modulate the tension in SDC-1. Full length SDC1-TS showed higher tension on 25 kPa substrate compared to 0.2 kPa while SDC1-TSΔGAG showed no change of tensions between stiff and soft substrates. Under fluidic shear stress, the tension in SDC-1 was found to be higher at the upstream region of the cell while SDC-1 was under compressive forces at the downstream, substrate region of the cells. Using peptide and small molecule inhibitors, we found regulation of SDC-1 molecular tension was consistent with a model in which SDC-1 binds to the substrate in an extended state and then is reduced by association with alphav/beta3 or alphav/beta5 integrins. In addition, immunoprecipitation studies demonstrated that SDC-1 rapidly increases its association with src, actin and FAK on application of fluidic shear stress to endothelial cells while decreasing association with integrin beta3 and beta5. Together, our results demonstrate that SDC-1 is directly responsive extracellular forces and substrate properties, leading to altered association with integrins and focal adhesion components.

SG236

**Proteomic Profiling of the Extracellular Matrix of Mammary Tumor Microenvironments Identifies Novel Metastasis Promoters**

**A. Naba**; University of Illinois At Chicago, Chicago, IL.

The extracellular matrix (ECM) is a complex and dynamic assembly of hundreds of proteins that provides mechanical and biochemical signals to the cells it surrounds. The ECM tightly controls various cellular processes including cell proliferation, motility, and invasiveness. Imbalance in the amount, composition, or mechanical properties of the ECM have been associated to many diseases including fibrosis and cancer.We have previously developed a proteomic pipeline to characterize the composition of the ECM - or "matrisome" - of tissues and tumors. Using this pipeline, we compared the matrisomes of poorly metastatic (MDA-MB-231) *vs* highly metastatic (MDA-MB-231_LM2) mammary tumors and identified a 43-ECM-protein signature characteristic of metastatic potential of breast cancers. Among these proteins, we found that the novel ECM protein SNED1 was uniquely produced by tumor cells. We further showed that knocking down SNED1 inhibited the metastatic dissemination of cells implanted orthotopically in the mammary gland. However, SNED1 knockdown cells were able to metastasize when directly injected in the circulation. Based on these results, we hypothesized that SNED1 regulates metastasis by promoting the acquisition by tumor cells of enhanced migratory and invasive properties.In

this talk, I will discuss our efforts aimed at deciphering the mechanisms by which SNED1 promotes metastasis. Using intravital imaging and *in-vitro* cell-based assays, we have observed that SNED1 knockdown cells have a decreased ability to degrade a collagen-rich ECM. Molecularly, SNED1 knockdown cells have decreased levels of expression of several ECM-degrading enzymes, including MMP2, 7, 9, and 17. Interestingly, the examination of the architecture of the ECM of control *vs* SNED1 knockdown tumors, using second harmonic generation microscopy, revealed that the absence of SNED1 alters the organization of the collagen network of the tumor microenvironment. Moreover, we have shown, using quantitative proteomics, that SNED1 controls the composition of the tumor ECM. Altogether, our results suggest that SNED1 acts as a master organizer of the architecture and composition of the tumor ECM and, by doing so, contributes to create an environment promoting tumor cell invasiveness and metastatic dissemination.

SG237

**The Energetic Costs of Migrating in Confined Spaces**

**C. Reinhart-King**[1], J. Zhang[1], M. Zanotelli[1], J. VanderBurgh[2], A. Rahman-Zaman[2], P. Taufalele[1], F. Bordeleau[3]; [1]Vanderbilt University, Nashville, TN, [2]Cornell University, Ithaca, NY, [3]Laval University, Quebec, QC, CANADA.

Cell make and utilize energy to perform basic life functions. While it is known that energy is needed for migration, little is known about energy production, utilization, and maintenance needed to sustain cellular movement during tumor metastasis. Moreover, it is not clear how the terrain of the microenvironment affects the energetic requirements of the cell. Using experimental measurements of cellular energy synthesis and consumption at the single cell level coupled with computational modeling, we have characterized and measured the energetic requirements of cell migration. Our data indicate that the energetic requirements of migratory cells depend on the migratory state of the cells. Additionally, the microenvironment plays a key role in dictating migration behavior and energetic needs. Cells in confined and/or dense scaffolds require more energy to move than those in porous and/or unconfined matrices. Notably, the path a cell takes can be predicted based on its energy levels: when confronted with a choice of paths of varying size, cells with less ATP are more likely to take a wider path that requires less cell deformation to move. These data suggest that cellular metabolism can be tuned to control cell migration, and metabolic inhibition to limit energy synthesis may be a promising approaching to preventing metastatic cell migration.

SG238

**Actomyosin Contractility-dependent Matrix Stretch and Recoil Induces Rapid Cell Migration**

**B. Baker**, W. Wang, C. Davidson, D. Lin; University of Michigan, Ann Arbor, MI.

Cell migration, a fundamental biological process in development, wound healing, and metastasis, has traditionally been studied on flat surfaces coated with extracellular matrix (ECM) proteins. However, it is now appreciated that physical attributes of the ECM strongly influence cells in selecting from a range of potential cell migration strategies. In this work, we implemented a recently established synthetic material system with independent control over alignment and stiffness to decouple their respective effects on cell migration through fibrillar ECMs. We found that increasing matrix alignment, independent of stiffness, increases the speed and directionality of cell migration, likely via the effect of contact guidance. Interestingly, varying the stiffness of aligned matrices resulted in a biphasic response with

respect to migration speed, where the highest speeds occurred at an intermediate stiffness. Towards identifying the cause for these variations in migration speed, high spatiotemporal imaging studies revealed two distinct cell migration modes. Traditional mesenchymal cell migration ('continuous'), consisting of elongation and adhesion of the leading edge followed by contraction and retraction of the trailing edge, was observed in all stiffness conditions. In contrast, cells only in deformable matrices demonstrated an elongated contraction phase where active protrusions recruited and stretched local matrix fibers. Following this phase of elastic energy storage, failure in the cell's adhesion to the matrix resulted in sudden recoil of the matrix and simultaneous rapid forward translation of the cell body in the direction of matrix alignment. We termed this migration mode 'slingshot migration' (SSM), given the clear role of matrix stretch and recoil. In additional studies, we further found that SSM resulted in higher speeds than continuous migration and occurred most frequently at an intermediate stiffness. Additionally, SSM was found to be actomyosin contractility dependent, as inhibiting or enhancing myosin II activity respectively decreased or increased the frequency of SSM events. In summary, this work introduces and characterizes a previously undescribed migration mode whereby cell contractility-mediated reorganization and stretching of matrix fibrils directly contributes to rapid cellular movement. Given the ubiquity of fibrous tissues throughout the body, an understanding of how matrix structure and mechanics influence cell migration could help improve approaches to recruit repair cells to wound sites or identify methods to inhibit primary cancer cell egress through the surrounding tumor stroma.

SG239

**Real-time Imaging of Intrinsic and Extrinsic Control Mechanisms in Invasive Breast Carcinoma Cells**

**B. Gligorijevic, 19122**; Temple University, Philadelphia, PA.

Tumor cell structures that have long been hypothesized as necessary for metastasis are invadopodia, invasive protrusions rich in structural proteins (Tks5, actin), adhesion proteins (eg. integrin β1), and metalloproteases. Using our unique intravital imaging approaches, we previously demonstrated that invadopodia in vivo are necessary for intravasation and consequent lung metastasis. In primary tumors, we found that cells which assemble invadopodia migrate at slow speeds, in perivascular niches where the ECM is cross-linked. Outside of these niches, no invadopodia were observed and cells migrated at high speeds, via contact guidance along collagen fibers. The invadopodia-driven motility can be switched to contact guidance by reducing the ECM cross-linking or by knocking down Tks5, which in turn reduces intravasation and metastasis. We next deduced that invadopodia-driven motility consists of two oscillating states: i. Invadopodia state, in which a cell is relatively sessile while it assembles invadopodia and degrades ECM; ii. Locomotion state. State balance is regulated by integrin β1 activation levels. Importantly, the Invadopodia state only occurs in early G1, whereas the Locomotion state can be seen throughout the entire cell cycle, suggesting that the cell cycle controls invadopodia assembly. Using FUCCI markers, we next show that Invadopodia state occurs during the G1 phase of the cell cycle. A close look at the regulators of G1 revealed that the cell cycle regulator p27kip1 localizes to the sites of invadopodia assembly and overexpression of p27kip1, but not p21cip1, causes faster turnover of invadopodia and increased ECM degradation. Taken together, these findings suggest that invadopodia function is extrinsically controlled by ECM via integrin β1, as well as intrinsically, via specific cell cycle regulators.

SG240

**Cell-matrix Interactions in Collective Invasion and Tumor Cell Cluster Dissemination: Ex-vivo to In-vivo Mappings**
**K. Cheung**; Fred Hutchinson Cancer Research Center, Seattle, WA.

Recent clinical and experimental evidence indicate that tumor cells invade, circulate, and extravasate as multicellular clusters. An important challenge is to understand how extracellular matrix shapes the dynamics of collective invasion and dissemination. Here we describe new experimental observations in ex-vivo organoid and in-vivo animal models to shed light on this process and the role of clusters. We outline new molecular phenotypes discovered ex-vivo describing tumor cell clusters in transit, and provide evidence correlating these molecular phenotypes with dissemination events in vivo. Our studies dissect the relative contributions of collectively invading and disseminating tumor cell clusters for distant metastasis.

SG241
**Matrix Nanotopography as a Regulator of Cell Function**
**D. Kim**; University of Washington, Seattle, WA.

The ability to create synthetic soft nanostructures that mimic architectural and mechanical features of 3D matrix microenvironment holds a considerable promise for advancing diverse biomedical fields, ranging from fundamental cell biology to tissue engineering and bio-prosthetics. In this talk, I will present nano- and microengineering approaches to control cell-matrix interactions for cell biological applications. Inspired by ultrastructural analysis of the native tissue, I will focus on two different settings in physiological and pathological contexts, in which controlling cell-matrix interactions on the nanoscale can have dramatic consequences: cancer cell migration and cardiac maturation. As novel approaches to address these problems, we have recently developed scalable, nanotopographically-controlled cell culture models. Using these tools in combination with traditional molecular and cell biology approaches, I will highlight how these biomimetic cell culture models enabled by micro and nanotechnologies help to gain better understanding of matrix control of cell structure and function.

SG242
**A Bioengineered Lymphatic Vascular Model Reveals a Lymphedema Treatment**
**E. Lee**; Cornell University, Ithaca, NY.

Lymphatic vessels (LVs), comprised of lymphatic endothelial cells (LECs), play a key role in maintaining tissue fluid homeostasis by draining interstitial fluid. A failure in lymphatic drainage triggers abnormal fluid accumulation in the interstitium causing tissue swelling, which is referred to as “lymphedema”. Lymphedema is the most common lymphatic disease influencing more than 150 million individuals worldwide. Currently, there is no clinically available drug for treating the disease and widely practiced physical therapies are palliative. One of the major obstacles to better understanding and curing the disease is a lack of efficient experimental models for assessing lymphatic drainage. Here, we built a “lymphatics-on-chip" by fabricating a microfluidics-based device that includes a PDMS housing and two parallel μ-channels within three-dimensional (3D) collagen. Employing luminal and interstitial flow in and through the μ-channels, human primary LECs in the channel formed an engineered LV, exhibiting physiologically relevant lymphatic junctions, dynamics, and drainage. Based on this model, we revealed integrin $\alpha_5$ as a new therapeutic target for lymphedema. Integrin $\alpha_5$, normally inactivated, became highly

activated in lymphedema and tightened lymphatic junction, impairing lymphatic drainage. Employing inhibitors targeting integrin $\alpha_5$, we promoted lymphatic drainage in both lymphatics-on-chip and mouse models of lymphedema and lymphatic drainage. This lymphatics-on-chip thus provides an important in vitro platform for revealing previously unappreciated lymphedema mechanisms and targets.

## Education Minisymposium: Biology competency for the classroom and beyond

M1

**ASCB Declaration on Effective and Inclusive Undergraduate Biology Education: From National Vision to Widespread Action**

**V. A. Segarra**[1], M. L. Styers[2], E. L. Dolan[3]; [1]High Point University, High Point, NC, [2]Birmingham-Southern College, Birmingham, AL, [3]University of Georgia, Athens, GA.

It has become routine for instructors to share strategies that "work" for their students and to implement successful methods from their colleagues. This is first order change, which aims to incrementally improve the current ways of operating. In contrast, second-order change aims to substantively transform underlying instructional philosophies in lasting ways. An example would be a deliberate strategy to provide students with more individualized attention and instruction. Given the large body of knowledge about how to teach and mentor effectively and inclusively, the time has come for second-order change in undergraduate biology education. With leadership from the Education and Minority Affairs committees, the American Society for Cell Biology has developed a tool for our community to leverage in achieving second-order change in undergraduate biology education: the Declaration on Effective and Inclusive Undergraduate Biology Education. This presentation will describe the Declaration, including how and why it was developed. The presentation will also highlight concrete, actionable recommendations that faculty members, teaching staff, graduate students, departments, institutions, and other stakeholders in undergraduate biology education can take in moving toward second-order change.

M2

**Using modeling and animation to overcome previously-held misconceptions**

**L. V. Paliulis**; Bucknell University, Lewisburg, PA.

Modeling of biological processes through drawing or animation is a skill that can be key to understanding essential concepts in biology. In fact, the development and construction of models is an essential practice for meeting the Next Generation Science Standards (www.nextgenscience.org) for K-12 science education and a key core competency in Vision and Change in Undergraduate Biology Education (www.visionandchange.org). From early life science classes in elementary and secondary school through graduate education drawing and animation are used effectively to cement key concepts. Unfortunately, early use of incorrect drawings and models can lead to misconceptions and a lack of competency in foundational areas that are often retained. To study ways to overcome misconceptions in biology, I have focused on the intentional construction of new models following observation of videos of an actual cellular process. One foundational cellular process that students frequently model incorrectly is mitosis. Incorrect models of mitosis lead to incorrect understanding of errors made in mitosis and more complex phenomena such as meiosis; two key topics in the upper-level Cytogenetics class offered at Bucknell University. In an effort to replace incorrect models retained from earlier study of mitosis, students started the semester by producing a short animation of mitosis, showing their baseline understanding of the process. Students then watched time-lapse videos of mitosis, carefully comparing their own animations to the videos of the process in living cells. This comparison showed that students' models were often inaccurate. Students then reanimated mitosis based on their view of the process in living cells. A quiz given at the end of the semester (two months later) showed that students retained

what they learned from re-modeling mitosis after watching the process in living cells. I propose that careful reflection on misconceptions and combining observation of processes in life with re-modeling those processes can help students overcome previously-held misconceptions.

M3

**Developing a New Measure of Reasoning in Introductory Biology Education**

**F. E. Nelson**[1], T. Fechter[2], T. Dai[3], J. G. Cromley[4], Y. Du[4]; [1]Temple University, Philadelphia, PA, [2]Sole Proprietor, Monterey, CA, [3]University of Illinois at Chicago, Chicago, IL, [4]University of Illinois at Urbana-Champaign, Champaign, IL.

**Aim:** Over the last 3 years, we aimed to develop and validate a new measure of reasoning in undergraduate biology which we call Inference Making and Reasoning in Biology (IMRB). Previous measures have focused on the reasoning involved in designing experiments; our focus was on the deductive reasoning required when learning new biological principles and applying them to new situations, focusing on introductory gateway biology courses. Previous research suggests that instructors and instructional materials require students to engage in deductive reasoning, beyond what is simply stated during instruction. When students either come to incorrect conclusions (e.g., over-generalize, under-generalize) or simply restate a premise, this is associated with poorer learning. **Method:** Based on students' verbalizations while learning about the immune system, we created multiple-choice reasoning questions based on brief passages, about half of which include a diagram, and requiring no special background knowledge. We interviewed 123 students while they answered one of two IMRB test forms (15 and 21 questions each). The interviews were transcribed and student statements were categorized (i.e., coded) using both prior research and emerging themes and compared between correctly-answered and incorrectly-answered questions. We also administered IMRB questions in sets of 15 to 2428 undergraduate students in introductory gateway biology courses at two universities. Measures were completed using paper and computer formats, and took approximately one hour. **Results:** Analyses of the Interviews suggest that deductive reasoning is indeed what distinguishes correctly answering our reasoning questions, not prior knowledge, vocabulary, or test-taking skills. The two forms were equated, and show excellent reliability, unidimensionality, and predictive validity for course grades. Cronbach's alpha reliability with different samples is $\alpha = .82$ to .86. Confirmatory factor analysis suggests the items all load on one factor, with excellent fit: CFI > .95, SRMR < .09, and RMSEA < .05. Differential Item Functioning analysis suggests the items are free from bias by race, sex, or socio-economic status. **Conclusion:** Our method of identifying correct and incorrect inferences from student verbalizations and creating multiple-choice reasoning questions appears to be a robust method. The test questions show excellent measurement properties, and can be used with undergraduate students to predict course grades, as long as used in combination with SAT or ACT scores. The items are available to interested instructors and departments, together with a user manual that includes detailed technical specifications and directions for administering either or both form(s) of the IMRB.

M4

**Expert-novice Comparison Reveals Pedagogical Implications for Students' Analysis of Primary Literature**

**M. Segura-Totten**, A. Nelms; University of North Georgia, Dahlonega, GA.

Student engagement in the analysis of primary scientific literature is known to increase critical thinking, scientific literacy, data evaluation, and science process skills. However, little is known about the process through which biology undergraduates read scientific articles. For this reason, we decided to compare how faculty experts and student novices engage with a research article. We performed think-aloud interviews of biology faculty (N = 6) and undergraduates (N = 11) as they read through a scientific article. We analyzed these interviews inductively using qualitative content analysis procedures. Our analysis revealed three key themes in how experts and novices went about reading primary literature: Thinking Tools, Scientific Literacy and Process Skills, and Comprehension Difficulties. We also identified 27 subthemes within the 3 key themes. We then grounded the analysis of our themes in cognitive load theory and the ICAP (Interactive, Constructive, Active, and Passive) framework. We found that faculty had more complex schemas than students, and that they reduced cognitive load effectively through two main mechanisms: summarizing and note-taking. Faculty also engaged with the article at a higher cognitive level, described as constructive by the ICAP framework, when compared to students. More complex schemas, lowering cognitive load, and deeper engagement with the text may help explain why faculty encountered fewer comprehension difficulties than students in our study. Finally, faculty utilized certain dimensions of scientific literacy skills, such as the analysis and evaluation of data, more often than students when reading the text. Findings include a discussion of successful pedagogical approaches for instructors wishing to enhance undergraduates' comprehension and analysis of research articles.

M5

**Building a Successful High School Outreach Project with Undergraduate Mentors: the Prince Edward County Environmental Molecular Biology Institute (PECEMBI)**

**M. J. Wolyniak**[1], A. R. Barber[2]; [1]Hampden-Sydney College, Hampden-Sydney, VA, [2]Longwood University, Farmville, VA.

With financial support from an ASCB Public Engagement Grant funded by the Science Sandbox, an initiative of the Simons Foundation, we launched the Prince Edward County Environmental Molecular Biology Institute (PECEMBI), an outreach program connecting the students of Hampden-Sydney College and Longwood University with the students of Prince Edward County High School, Virginia. Prince Edward County is a rural, underserved school system in central Virginia with a significant population of students from groups traditionally underrepresented in the STEM disciplines. In the fall semester, undergraduates from Hampden-Sydney and Longwood visited the high school each week and worked with biology students on a long-term project that explored the microbiome of student-selected locales around the school and introduced students to microbiology and molecular biology techniques. The spring semester featured work on examining the microbial contents and the quality of the watershed around the high school to consider issues more directly related to environmental biology. Both semester's activities culminated with a public presentation in coordination with Longwood's student research symposia. While Hampden-Sydney and Longwood faculty were present at each session, the undergraduates led all activities and discussions with the high school students as much as possible. Assessment of the program conducted via surveys administered to high school students and teachers as well as Hampden-Sydney and Longwood undergraduates and faculty revealed high levels of enthusiasm and satisfaction among all participating parties in the program. While the high school students especially enjoyed the opportunity to participate in an authentic research experience and to interact with the undergraduate students, they expressed their hope that in future iterations of the program that the specific undergraduates working with them would not change from week to week. Likewise, both the

high school and undergraduate faculty expressed praise for the overall work of the undergraduates but saw value to place more training emphasis in future iterations on the cultural aspects of successfully mentoring high school students. Overall, PECEMBI gives high school students insight into how college level research is done, how to summarize and present their research at a university poster session, and how to respond to peer criticism. It is PECEMBI's goal to supplement the scientific educations of the high school students in Hampden-Sydney's and Longwood's backyard and better prepare them for potential careers in science.

M6

**Six New York City (nyc) Medical Schools Will Offer Two Summer-long, Eight Weeks/summer, Professional Development (pd) Programs for New York City'S Secondary Science Teachers Beginning in Summer 2021**

**S. C. Silverstein**[1], J. Wiederhorn[2]; [1]Columbia University Coll Phys & Surg, New York City, NY, [2]Associated Medical Schools of New York, New York City, NY.

The U.S.'s current biomedical research workforce does not mirror the demographics of its population, seriously limiting our nation's scientific enterprise. Research shows that workforce diversity has beneficial outcomes including increased productivity, higher quality, and higher impact work (Valantine, H.A. and Collins, F. S. *PNAS U.S.A.*112, 12240-12242, 2015). However, to increase diversity of the biomedical workforce, we must increase the number of underrepresented (UR) students entering STEM fields. UR students show strong interest in STEM fields upon matriculation into college, but 50-70% of them drop out before graduation (Meyers L.C., *PLoS ONE*13(1), 2018). Of all U.S. bachelor degree recipients, only ~21,000 Black and ~26,000 Hispanic students received STEM degrees, compared to ~195,000 White and ~39,000 Asian students (https://nces.ed.gov/pubs2016/2016007.pdf). This deficit in STEM education of UR students is due, in part, to inadequacies of secondary science teacher preparation in teaching inquiry-based science. The educational skills of most science teachers plateau 3-5 years after their entry into teaching. Columbia University's Summer Research Program (CUSRP) for Secondary Science Teachers was designed to address these deficits. Outcomes studies show that following teacher completion of Columbia's two summer-long, eight week/summer program, 10% more students of participating teachers pass New York State Regents science exams than students in classes taught by non-participating teachers in the same school at the same time. Participating teachers are retained in education at an ~3.5-fold higher rate than non-participating teachers. As such, New York City's (NYC) Department of Education saves educational costs for courses and exams students do not have to repeat and teachers it does not have to replace (*Science*326, 440-442, 2009). The program, its graduates, and their students have been recognized by 75 national, state and local educational awards since 2003 and has been replicated successfully at multiple sites, both in and outside of New York State. The program's success has prompted AMSNY, whose mission is to increase diversity of the biomedical and physician workforce, to engage a consortium of six of its member medical schools, to jointly implement CUSRP-like secondary science teacher professional development (PD) programs in NYC. These PD programs will result in the further professional growth of experienced teachers, higher science teacher retention rates, elevation in the quality of STEM education in NYC's schools and a significant increase in the number of UR students prepared for post-secondary STEM education and careers.

M7

**An Integrated Undergraduate Steam Course: the Art and Science of Cell Death**

**S. Walsh**[1], A. Stewart[2], J. Almond[2], S. Berenguer[2]; [1]Soka University of America, Aliso Viejo, CA, [2]Rollins College, Winter Park, FL.

This fully integrated laboratory-and studio-based course enabled students to visualize apoptosis, design experiments around this basic cell biology process, and then use those experimental data to generate two art pieces. After completing this interdisciplinary course, students fulfilled both their art and science general education requirements. Through short lectures and demonstrations, a mix of thirteen biology and non-science majors were introduced to both the intrinsic pathway of apoptosis and the principles of art and design. Students first learned basic cell culture techniques through a guided experiment expressing Bax-GFP in Omi-RFP HeLa cells. Images were then captured on a confocal microscope and used to thematically inform an exquisite corpse drawing that was digitally rendered using a 3D computer-aided design (CAD) program to create a computer numerical control (CNC) milled wood panel. Following this, students in small groups applied their laboratory experience to design and test their own apoptotic protein using genetic engineering and synthetic biology techniques. Here, we present data generated by two student groups that fused a CARD or a DED domain to GFP to accelerate cell death, as well as another group that engineered caspase cleavage sites into a dual fluorescent protein to serve as an indicator of apoptosis. Finally, using their personally collected confocal images, students digitally designed and fabricated a 3D printed sculptural form. In the context of this class, students practiced critical thinking and problem-solving skills utilizing both hands-on, novel experiments and computer-aided design.

M8

**A Data Analysis and Literature Intensive Undergraduate Course that Positively Impacts Student Ability and Confidence in Scientific Critical Thinking Skills and Increases Post-graduation Success.**

**K. K. Resendes**; Westminster College, New Wilmington, PA.

Bio 404: Nuclear Structure and Function is an advanced cell and molecular biology course taken by junior and senior undergraduates at Westminster College (New Wilmington, PA). While the content of the course focuses on architectural aspects of nuclear cell biology and molecular understanding of gene expression and genome structure, the major objective of the course is to broaden students' skills in content comprehension, data-analysis, modeling, and productive scientific discussion using literature. The design builds upon existing models for using data analysis or individual journal club sessions in order to maximize student gains in ability, confidence, and postgraduate preparation. The four course units (Nuclear Periphery, Nuclear Pore, Chromatin and Nuclear Bodies), are divided into multiple, one-week modules consisting of three one-hour sessions. 1) Secondary article based lecture, introducing a theme. 2) "Work it out Wednesday" where students work in groups of 3-4 answering questions to analyze data and interpret results in order to develop a biological model that links back to lecture. 3) Student-led journal club discussing a theme correlated article. Course evaluations from the 2016 offering indicated that students thought the course was well organized, was a valuable learning experience and that the assignments had instructional value; moreover, exam and course grades in 2016 improved over the 2014 offering, which was structured in a more traditional lecture style. As a result of the positive outcomes with the change in course format a more formalized analysis of student gains and perceptions of the course was performed in the 2018 offering, together with a survey of the 2016 alumni. Student

gains in ability and confidence with critical thinking skills were assessed and compared to the pre-requisite course and alumni. Greater gains across several metrics occurred in the advanced course, with students closing the pre-course gap between ability and confidence. Alumni retained gains and reported advantages over peers in postgraduate programs. This presentation will consist of an example of a course module, data from this initial study and ideas for application of the method to a variety of content areas and course sizes.

M9

**Promoting Biology Literacy through a Non-majors First-year Seminar Focused on Cancer**

**J. Hood-DeGrenier, 01602-2861**; Worcester State University, Worcester, MA.

A basic level of biology literacy is essential for the general public to understand many issues that feature prominently in the media and everyday life, such as risk factors for cancer, the benefits and potential dangers of genetically modified organisms, the importance of vaccination, and the effects of climate change. In the U.S., all undergraduate students are typically required to take some science as part of their general education curriculum, but students who are not majoring in a science often delay taking these classes until the end of their undergraduate education, possibly due to anxiety about the difficulty of science courses or presumed disinterest in science. Shifting the timing of students' exposure to college-level science courses to earlier in their undergraduate careers is likely to increase their overall science literacy by building their confidence, skills, and knowledge at a point where they will be able to apply these to other courses and possibly be stimulated to take additional science courses beyond the minimal requirements. At Worcester State University (WSU), a first-year seminar (FYS) course focused broadly on the topic of cancer was offered in the Fall 2018 semester, without selection based on major. While some students who selected the course were science majors, others were psychology, history, education, and business majors. The course covered the basic biology of cancer, introductory concepts of epidemiological data analysis, and societal issues surrounding cancer while also meeting the general requirements of the WSU FYS program related to written and oral communication, critical thinking, and information literacy. I will describe the course structure and assignments and present assessment data from student surveys. Survey data indicated that the course topic stimulated strong interest in students and increased their interest in science overall, that it increased their confidence in understanding scientific content, and that they believed the skills and knowledge they gained would be useful to them in the future, among other positive outcomes.

## Minisymposium 1: Chromosome Structure

M10

**Matchmaking during Meiosis: How Chromosomes Recognize Their Homologous Partners**

G. V. Caldas[1], F. Wu[1], **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]California Institute for Quantitative Biosciences, Berkeley, CA, [4]Lawrence Berkeley Natonal Laboratory, Berkeley, CA.

During early meiosis, each chromosome must pair with its unique homologous partner. Pairing enables crossover formation, which is required for accurate chromosome segregation. The molecular basis for recognition between homologs remains a major unsolved mystery of meiosis. At meiotic onset,

chromosomes undergo dramatic reorganization, including the reorganization of cohesins to form a linear chromosome core, known as the axis. This linearization, together with attachment and movement of chromosomes along the nuclear envelope, promotes homologous pairing and synapsis. In many organisms, stable pairing between homologs also requires early steps in homologous recombination. However, recombination-independent mechanisms are sufficient to mediate stable homologous pairing and synapsis in some species, including *Caenorhabditis elegans* and *Drosophila melanogaster.* These models thus provide unique opportunities to investigate the basis for recombination-independent recognition of homology.We have established an experimental pipeline to interrogate chromosome architecture and chromatin landscapes in *C. elegans* germ cells. We adapted low-input chromosome conformation analysis (Hi-C) and CUT&RUN methods to map intra- and inter-homolog chromosomal contacts and to define chromatin landscapes in germline tissue. By arresting meiotic cells at the zygotene stage of meiosis, we are elucidating early inter-homolog interactions that mediate pairing and recognition.Consistent with recent findings from mouse spermatocytes, *C. elegans* meiotic chromosomes show pronounced A/B compartmentalization, indicative of preferential contacts between regions of similar epigenetic states. We observe particularly pronounced compartmentalization within meiotic “pairing centers,” specialized regions located near one end of each chromosome that connect chromosomes to cytoplasmic microtubules and dynein to promote pairing. These regions are functionally defined by a family of meiotic zinc finger proteins (ZIM/HIM-8), which bind to sequence motifs enriched in these regions. We observe strong synapsis-independent pairing contacts within these regions. Intriguingly, we also observe meiosis-specific chromatin loops anchored at ZIM/HIM8 binding motifs, suggesting that these proteins may act as boundary elements. Our germline-specific chromatin profiling reveals that these sites preferentially occur within highly expressed genes and are strongly enriched for active histone marks, although they reside on chromosome arms enriched for repressive chromatin. We speculate that ZIM/HIM-8-mediated 3D architecture together with the intercalation of active and repressed chromatin regions along the chromosome arms may form a “bar code” for homolog recognition.

M11

**A Time-Resolved Network of Meiotic Chromosome Associated Proteins**

**S. Ur**, R. Suhandynata, H. Zhou, K. Corbett; UCSD, La Jolla, CA.

Meiosis, a hallmark of sexual reproduction, reduces the ploidy of a cell by undergoing two chromosome segregations after a single round of chromosome replication. In order to accomplish this diploid-to-haploid transition, homologous chromosomes identify one another and physically associate through DNA recombination, before segregating from one another in the first meiotic division. Meiotic recombination initiates with programmed double strand breaks (DSB) on each chromosome, some of which are eventually resolved as inter-homolog crossovers. Many protein complexes are involved in meiotic recombination, and their spatial and temporal interplay is critical to the proper segregation of chromosomes in Meiosis I. The proteinaceous chromosome axis and synaptonemal complex organize chromosomes and control their recombination, while other recombination complexes shuttle a subset of double-strand breaks to a crossover fate. Previous studies have globally interrogated the timing of meiotic protein expression and indeed, many individual meiotic complexes have been studied in detail. However, a comprehensive view of meiotic proteins’ chromosome localization and interactions has not been performed. Here, we present a protocol to assess the localization of proteins to chromosomes throughout a synchronous *S. cerevisae* meiosis using chromatin-enriched fractions coupled with high

resolution tandem mass spectroscopy. We demonstrate the ability of our assay to identify proteins from critical meiotic complexes such as the chromosome axis, synaptonemal complex, ZMM family, recombination complexes, and proteins responsible for resolving key crossover intermediates. By disrupting individual proteins from these stratified groups, we elucidate how meiotic complexes work together to manipulate meiotic chromosomes and promote homolog pairing and segregation during Meiosis I. With this tool we have created a temporal atlas for meiotic proteins, and defined how protein complexes assemble and disassemble from chromosomes to coordinate meiotic recombination.

M12

**Retrotransposons in *Drosophila* Male Germline Stem Cells Maintain Ribosomal DNA Copy Number**

**J. O. Nelson**[1,2], Y. M. Yamashita[1,2]; [1]University of Michigan, Ann Arbor, MI, [2]HHMI, Ann Arbor, MI.

In order to meet the high biosynthetic demands of the cell, the genes encoding ribosomal RNAs are tandemly duplicated hundreds of times, creating a repetitive genomic region called the ribosomal DNA (rDNA) in essentially all eukaryotes. Repetitive regions like rDNA are inherently unstable due to their tendency to create intra-chromatid exchanges that can rearrange and delete portions of the repeats. The progressive loss of rDNA copies due to these exchanges is a major source of replicative senescence. Despite the inherent instability of rDNA loci, rDNA copy number is maintained within a consistent range between individuals of the same species, indicating the presence of mechanisms to prevent the deterioration of these loci. We recently found that the *Drosophila melanogaster* male germline achieves this maintenance by expanding rDNA copy number in germline stem cells (GSCs) to offset rDNA loss, although the underlying factors that create these new rDNA copies are largely unknown. Here we report our surprising discovery that this expansion is mediated by the activity of transposable elements. Approximately half of *Drosophila* rDNA copies are inserted by the rDNA-specific retrotransposons R1 and R2, and like most transposable elements their expression is normally repressed in the germline to preserve genomic integrity. However, we found that R1 and R2 expression becomes derepressed in GSCs when rDNA copy number is reduced. Despite the potentially harmful effect of these retrotransposons, we found that R1 and R2 inhibition by RNAi strikingly causes GSC loss and accelerates rDNA copy reduction during aging, likely due to an inability to efficiently recover lost rDNA copies. R1 and R2 transpose within the rDNA locus via rDNA-specific endonucleases and we found that R1 and R2 contribute to the accumulation of double stranded breaks (DSBs) in GSCs with reduced rDNA copies. Considering that the homologous recombination-mediated DSB repair pathway has previously been shown to be required for rDNA copy expansion, we propose that the rDNA-specific endonuclease activity of R1 and R2 initiates rDNA recovery in GSCs with reduced rDNA. The repair of DSBs at tandem repeats by homologous recombination can result in unequal sister chromatid exchange (USCE) that duplicates the repeats, so we are now directly investigating if these retrotransposons induce USCE between rDNA copies. Uncovering this role for R1 and R2 in rDNA copy expansion reveals how a symbiosis between genomic parasites and a host genome formed to maintain the rDNA locus, and the strong conservation of rDNA-specific retrotransposons may indicate that similar mechanisms occur throughout the animal kingdom.

M13

**Transposable Element-driven Reorganisation of 3D Chromatin During Early Embryonic Development**

**J. M. Vaquerizas**; MPI for Molecular Biomedicine, Muenster, GERMANY.

Transposable elements (TEs) are highly abundant in eukaryotic genomes, affecting processes such as transcription, splicing, and recombination. In particular, recent studies have demonstrated a role for specific families of TEs during the process of zygotic genome activation in mammals. However, despite this association, the molecular mechanisms by which TEs regulate this crucial developmental transition are unknown.Here, using genome-wide chromatin conformation maps, we demonstrate that the Murine Endogenous Retroviral Element (MuERV-L/MERVL) family of transposable elements drives 3D chromatin reorganisation in 2-cell-embryo-like cells as well as in the mouse 2-cell embryo. This reorganisation involves the emergence of hundreds of TAD boundaries across the genome and it is associated to the upregulation of directional transcription from MERVL, which results in the activation of a subset of 2-cell embryo genes. Additionally, we find extensive inter-strain MERVL variation, suggesting multiple non-overlapping rounds of recent genome invasion. Our results have important implications for our understanding of how nuclear organisation emerges during zygotic genome activation in mammals, and how this organisation can be influenced by repetitive elements in early embryonic development.

M14

**Localization of Drosophila Cenp-A to Non-centromeric Sites Depends on the Nurd Complex**

E. Demirdizen[1], **S. Erhardt**[1], M. Spiller-Becker[1,2], A. Förtsch[1], A. Bergner[1], B. Hessling[3]; [1]ZMBH - Center for Molecular Biology Heidelberg, Heidelberg, GERMANY, [2]Active Motif, Carlsbad, CA, [3]DKFZ, Heidelberg, GERMANY.

Centromere function requires the presence of the histone H3 variant CENP-A in most eukaryotes. The precise localization and protein amount of CENP-A are crucial for correct chromosome segregation, and misregulation can lead to aneuploidy. To characterize the loading of CENP-A to non-centromeric chromatin, we utilized different truncation- and localization-deficient CENP-A mutants, and show that the N-terminus of Drosophila melanogaster CENP-A is required for nuclear localization and protein stability, and that CENP-A associated proteins, rather than CENP-A itself, determine its localization. Co-expression of mutant CENP-A with its loading factor CAL1 leads to exclusive centromere loading of CENP-A whereas co-expression with the histone-binding protein RbAp48 leads to exclusive non-centromeric CENP-A incorporation. Mass spectrometry analysis of non-centromeric CENP-A interacting partners identified the RbAp48-containing NuRD chromatin remodeling complex. Further analysis confirmed that NuRD is required for ectopic CENP-A incorporation, and RbAp48 and MTA1-like subunits of NuRD together with the N-terminal tail of CENP-A mediate the interaction. In summary, our data show that Drosophila CENP-A has no intrinsic specificity for centromeric chromatin and utilizes separate loading mechanisms for its incorporation into centromeric and ectopic sites This suggests that the specific association and availability of CENP-A interacting factors are the major determinants of CENP-A loading specificity.

M15

**Centromere Incompatibility as the Basis for Chromosome Segregation Defects in Inviable *Xenopus*Hybrids**

**M. Kitaoka**, R. Heald; University of California, Berkeley, Berkeley, CA.

**Centromere incompatibility as the basis of chromosome segregation defects ininviable *Xenopus* hybrids**Maiko Kitaoka* and Rebecca HealdDepartment of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA*Presenting author: mkitaoka@berkeley.edu**ABSTRACT**Despite the evolutionarily conserved function and importance of centromeres to mediate faithfulsegregation of replicated chromosomes, the underlying DNA sequences and centromeric proteinsare rapidly evolving, even among closely related species. Interestingly, faulty chromosomesegregation has been reported in a number of inviable hybrids, contributing to post-zygoticdevelopmental barriers and speciation. However, the molecular mechanisms underlying specificchromosome loss in hybrids is poorly understood. *Xenopus* frog species provide an ideal systemto study hybridization and genome evolution, as hybrids produced when *X. laevis* or *X. borealis*eggs are fertilized by *X. tropicalis* sperm (*l*e×*t*s and *b*e×*t*s) are viable, but the reverse crosses (*t*e×*l*sand *t*e×*b*s) are not. Both inviable hybrids show chromosome segregation defects, however, wholegenome sequencing revealed that *t*e×*l*s hybrids lose two specific *X. laevis* chromosomes, while*t*e×*b*s hybrids lose regions of four specific *X. borealis* chromosomes. We used *Xenopus* egg extractsto demonstrate that a subset of *X. laevis* and *X. borealis* chromosomes lost CENP-A, the corecentromeric histone variant, as well as Ndc80, the key outer kinetochore component that attachesto spindle microtubules, after one cell cycle. CENP-A loss is cell cycle-dependent, as unreplicatedpaternal sperm retain all CENP-A localization. *X. laevis* chromosome centromere localization isrescued with addition of *in vitro* transcribed and translated CENP-A with its chaperone HJURP to*X. tropicalis* cytoplasmic extracts, although *X. borealis* centromeres are not rescued. Preliminaryresults suggest that transcription affects centromere assembly in these hybrids, as triptolideinhibition of RNA Pol II can also rescue CENP-A localization. Ongoing experiments aim to rescuesegregation defects in inviable hybrid embryos and uncover the mechanisms underlying specificpaternal chromosome mis-segregation and inviability in *Xenopus* hybrids.

M16

**Understanding Unconventional Kinetoplastid Kinetochores**

**B. Akiyoshi**, G. Marcianò, H. Hayashi, M. Ishii, P. Ludzia, O. Nerusheva; University of Oxford, Oxford, UNITED KINGDOM.

The kinetochore is the macromolecular protein complex that drives chromosome segregation in eukaryotes. Although it was widely assumed that the structural core of kinetochores would consist of proteins that are common to all eukaryotes, we identified 20 kinetochore proteins in *Trypanosoma brucei* (an experimentally-tractable kinetoplastid parasite that branched early in eukaryotic history) and discovered that they constitute unconventional kinetochores specific to kinetoplastids. We are currently characterizing these unique kinetochore proteins in vitro and in vivo to understand how they carry out conserved kinetochore functions. By understanding how trypanosomes segregate their chromosomes, we aim to understand fundamental principles of chromosome segregation machinery.

M17

**A New Hi-C Method Reveals the Conformation of Sister Chromatids**

**M. Mitter**[1], C. Gasser[2], Z. Takacs[1], R. Stocsits[3], W. Tang[3], S. L. Ameres[1], J. Peters[3], A. Goloborodko[4], R. Micura[2], D. W. Gerlich[1]; [1]Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna BioCenter, Vienna, AUSTRIA, [2]Institute of Organic Chemistry and Center for Molecular Biosciences (CMBI), Leopold-Franzens University, Innsbruck, AUSTRIA, [3]Research Institute of Molecular Pathology, Vienna BioCenter, Vienna, AUSTRIA, [4]Department of Physics, Massachusetts Institute of Technology, Cambridge, MA.

The three-dimensional organization of chromosomal DNA is key for proper gene regulation, DNA recombination and repair, and chromosome segregation. Throughout interphase, cohesin forms DNA loops to shape topologically associated domains (TADs). Moreover, a subset of cohesin complexes links replicated sister chromatids from S-phase until mitosis to enable DNA damage repair and faithful chromosome segregation. The genomic landscape of chromatin loops and TADs has been studied in great detail by chromosome conformation capture techniques (Hi-C), but available methods cannot distinguish between identical DNA copies of replicated chromosomes. The distribution of sister chromatid linkages has hence remained unclear. We have developed a sister-chromatid-sensitive Hi-C method based on in vivo DNA labeling with nucleotide analogues. Using this methodology, we have generated genome-wide conformation maps of replicated chromosomes from interphase and mitotic cells. We discovered four-way junctions where loops from both sister chromatids are linked at their base, as well as unlinked loop structures on individual sister chromatids. We have also applied our methodology to study reorganization of sister chromatids during cell cycle progression and will discuss the implications for chromosome segregation.

M18

**Reconstitution of Cohesin and Condensin-mediated Dna Loop Extrusion in Xenopus Egg Extracts**

S. Golfier, T. Quail, **J. Brugues**; Max Planck Institute, Dresden, GERMANY.

The structural maintenance of chromosomes (SMC) complexes, cohesin and condensin, organize chromatin in metaphase and interphase. Condensin can extrude DNA loops *in vitro*, which may be responsible for folding chromatin into condensed chromatids during metaphase. Cohesin has been hypothesized to organize interphase chromatin into topological associating domains also via a loop extrusion mechanism. However, direct imaging of loop extrusion in cellular contexts is lacking, and consequently whether loop extrusion is a general mechanism that organizes chromosomes both in interphase and metaphase is unknown. Here we used *Xenopus* egg extract to reconstitute and image loop extrusion in single DNA molecules. We show that loops form in both metaphase and interphase, but with distinct symmetry and processivity. Condensin extrudes non-symmetric loops in metaphase, whereas cohesin extrudes symmetric loops in interphase. This work shows that loop extrusion is a general mechanism for chromosome organization, with dynamical and structural properties that are molecularly regulated to achieve different levels of chromatin organization during the cell cycle.

M19

**Centromere Strength Is Transgenerationally Inherited through the Male But Not Female Germline**

**A. Das**, V. Fu, B. E. Black, M. A. Lampson; University of Pennsylvania, Philadelphia, PA.

Centromeres direct genetic inheritance but are not themselves genetically inherited. Instead, centromeres are defined epigenetically by the presence of a histone H3 variant, CENP-A. According to existing models for centromere inheritance, preexisting CENP-A nucleosomes serve as templates to direct new assembly, which quantitatively maintains centromere chromatin through every cell cycle in cycling somatic cells. To test this model in vivo in the mammalian germline, we created CENP-A hemizygous mice with reduced levels of centromere chromatin in the gametes. We show that genetically wild-type progeny from two *cenpa+/-* hemizygous parents have partially reduced centromere chromatin in somatic tissue and in the male germline and that these levels are transgenerationally inherited. But we find wild-type levels of CENP-A in the female germline, indicating that CENP-A levels reset prior to prophase I arrest in the oocyte. Furthermore, when only one parent is hemizygous, we don't observe any detectable differences between maternal and paternal centromeres in adult progeny, indicating equilibration between maternal and paternal centromeres. Based on these results, we propose two key exceptions to template-dependent centromere inheritance. First, a unique assembly mechanism in the female germline increases CENP-A chromatin, possibly to protect against loss during the prolonged prophase arrest. Second, CENP-A chromatin can differ quantitatively between sperm and egg chromatin in the zygote, but CENP-A redistributes in the early embryo to equalize maternal and paternal centromeres, providing a mechanism to suppress centromere drive.

## Minisymposium 2: Genetic and Environmental Drivers of Cellular Metabolic Phenotypes

M20

**Metabolic Coordination of Cancer Cell Fate**

**L. Finley**; Meml Sloan Kettering Cancer Ctr, New York, NY.

Increasing evidence demonstrates that intracellular metabolic pathways can be critical drivers of tumor progression. Metabolites provide the biomass required for cancer cell proliferation and contribute to antioxidant defenses that promote survival and metastatic dissemination. Moreover, oncogenic mutations in several metabolic enzymes lead to accumulation of pathological levels of metabolites that interfere with histone and DNA demethylation and impose a block to differentiation. However, absent these mutations, whether the metabolic profile of cancer cells contributes to the establishment or maintenance of malignant cell identity remains poorly understood. Combining in vitro culture of cancer cells with mouse models of tumor progression, we define the metabolic changes driven by common oncogenes and tumor suppressors and elucidate how these metabolic alterations affect cancer-associated phenotypes. In particular, we focus on how metabolic alterations influence regulation of the chromatin landscape and differentiation programs that antagonize tumor progression. These studies provide insight into the mechanisms through which tumor cells evade differentiation and sustain malignancy.

M21

**The Nutrient Microenvironment of Tissues and Tumors Affects the Metabolism of Resident Cells**

**A. Muir**; University of Chicago, Chicago, IL.

The metabolism of mammalian cells is influenced both by cell-intrinsic factors and cell-extrinsic factors. There has been substantial effort towards understanding how cell-intrinsic factors, such as disease causing genetic mutations, alter cellular metabolism. However, much less is known about how physiological microenvironmental conditions alter cell metabolism. Nutrient availability is a cell-extrinsic factor that substantially influences cellular metabolism, yet we have relatively little information regarding nutrient availability in different healthy or diseased tissues. To address this, we developed a quantitative metabolomics approach to measure nutrient levels in the interstitial fluid (IF) of tissues and tumors, providing insight into the metabolic substrates available to cells in their local microenvironment. Profiling IF nutrient levels in different tissues and tumors has provided insight into nutrients that are depleted or accumulate in different anatomical sites in the body. To determine how local nutrient availability alters cellular metabolism, we have developed a cell culture medium based on observed IF nutrient levels in murine pancreatic cancers. Pancreatic cancer cells, as well tumor stromal cells, can be grown in IF-based medium in order to study how cells utilize metabolism to support homeostasis and function when constrained by microenvironmental nutrient levels. In initial experiments, we found that freshly isolated murine pancreatic cancer cells grow robustly in IF-based media, but cancer cells initially cultured in standard media lose the ability to grow in IF-based media, suggesting that the adaptations required by cancer cells to cope with tumor nutrient levels are rapidly lost by pancreatic cancer cells in standard culture. We are using a multi-omics approach to identify what metabolic adaptations pancreatic cancer cells use to support their growth in their nutrient environment. Collectively, these experiments provide insight into how nutrient availability acts as a cell-extrinsic factor to alter pancreatic cancer cell metabolism, and provide an experimental approach for determining how nutrient availability impacts cellular metabolism in healthy and diseased mammalian tissues.

M22

**The Creatine Phosphagen System in Mechanoresponsive in Pancreatic Ductal Adenocarcinoma Cells and Fuels Invasive Behaviour**

**V. Papalazarou**[1], T. Zhang[2], N. Paul[1], M. Cantini[3], O. Maddocks[2], M. Salmeron-Sanchez[3], L. Machesky[1]; [1]CRUK Beatson Institute, Glasgow, UNITED KINGDOM, [2]Institute of Cancer Sciences, University of Glasgow, Glasgow, UNITED KINGDOM, [3]Centre for the Cellular Microenvironment, University of Glasgow, Glasgow, UNITED KINGDOM.

Unbalanced cues from the extracellular matrix (ECM) govern pancreatic tumorigenesis and dissemination. Stiff fibrotic stroma and cancer cell mechanosensing regulate cell proliferation, migration and invasion. Such key processes are highly dependent on energy availability and need to be fueled by metabolic adaptations to tumorigenic microenvironments. However, it is still unknown how ECM mechanics influence cellular energetics and metabolism as well as how cells meet their ATP requirements during invasion and metastasis. We found that pancreatic cancer cells tune their metabolic networks to favour ATP production on stiff ECM. This process appeared dependent on mitochondrial fusion and polarization on stiff substrata as well as in pseudopods formed during ECM invasion. In addition, this was accompanied by a mechano-dependent regulation of ATP recycling through the creatine phosphagen system. The cytoplasmic creatine kinase CKB was expressed on stiff

substrata in a YAP-dependent manner providing a critical advantage to the invasion capacity of pancreatic cancer cells. Collectively our results indicate that ECM mechanics can positively regulate tumour invasion by favouring ATP production and sharpening the gradient of ATP vs ADP through CKB activity. Our study highlights the importance of this ATP recycling circuit as a mechanism generating an efficient energy balance within the cytoplasm boosting invasion. Interestingly, targeting creatine phosphorylation hindered migration and invasion in 2D and 3D, revealing CKB as a potentially druggable target against the spread of pancreatic cancer.

M23

**Accumulation of Systemic Succinate Controls Activation of Adipose Tissue Thermogenesis**

**E. Mills**; Dana-Farber Cancer Institute, Boston, MA.

Brown and beige adipose tissue thermogenesis can combat metabolic disease, however this requires activation by extrinsic stimuli. Adipocyte lipolysis through cAMP/PKA signalling activates thermogenic respiration, but this pathway has proven challenging to target pharmacologically in a clinical setting. Here we apply a comparative metabolomic approach and unexpectedly identify an independent metabolic pathway that controls acute activation of adipose tissue thermogenesis *in vivo*. We show that substantial and selective accumulation of the tricarboxylic acid (TCA) cycle intermediate succinate is a unique metabolic signature of thermogenic adipose tissue upon activation by exposure to cold. Succinate accumulation occurs independently of the adrenergic cascade and is sufficient to activate brown adipocyte thermogenic respiration *in vivo*. This selective accumulation is driven by a newfound capacity for brown adipocytes to sequester elevated circulating succinate. We demonstrate that systemic administration of succinate initiates brown adipose tissue (BAT) thermogenesis in mice. Succinate is accumulated from the extracellular milieu and is rapidly taken up by brown adipocyte mitochondria. Here, its oxidation by succinate dehydrogenase (SDH) is required for the apparent activation of thermogenesis. Mechanistically, we find that SDH mediated-succinate oxidation controls reactive oxygen species (ROS) production to drive uncoupling protein 1 (UCP1)-dependent thermogenic respiration, which inhibition of SDH supresses thermogenesis. Finally, we show that this pathway can be activated by pharmacological elevation of circulating succinate to drive UCP1-dependent BAT thermogenesis *in vivo*, which stimulates robust protection against diet-induced obesity and improves glucose tolerance. These findings reveal an unexpected mechanism for control of thermogenesis *in vivo*, utilizing succinate as a systemically-derived thermogenic molecule, and suggests new treatments for metabolic disease.

M24

**Characterization of Mitochondrial Metabolic Oscillations in Live Rodents**

**Y. Ng**[1], D. Chen[1], W. Losert[2], R. Weigert[1]; [1]Laboratory of Cellular and Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, [2]College of Computer, Mathematical, and Natural Sciences, University of Maryland, College Park, MD.

Mitochondria are specialized cellular compartments that function in energy production and calcium homeostasis. Although these organelles have been primarily investigated in cell cultures, very little is known about their morphology, function, and dynamic properties in live multicellular organisms. To address this issue, we used Intravital Subcellular Microscopy (ISMic), an imaging approach that enables the visualization of biological processes in live animals. In rat salivary glands, we previously discovered

that: 1) mitochondrial metabolic activity exhibits rapid and periodic oscillations under basal conditions, and 2) mitochondrial oscillations are synchronized throughout the salivary epithelium via gap junctions. Importantly, dysfunctional mitochondrial oscillations are linked to many metabolic diseases e.g. obesity, cardiovascular disease, developmental defects and cancer, thus prompting us to ask: i) what is the physiological role of mitochondrial oscillations and ii) how oscillations are orchestrated at the whole organismal level. Here, we extended our characterization in rat and mouse tissues under both physiological and pathological conditions. Under basal conditions, we found that mitochondrial oscillations occur in all tissues tested, with substantial differences in their period, amplitude, and coordination, that most likely reflect the metabolic status of the tissues analyzed. Additionally, we identified that the metabolic oscillatory pattern is significantly altered in aged mice and during tumor progression in a mouse model for head and neck squamous cell carcinoma. In conclusion, we provided for the first time a detailed quantitative analysis of the characteristics of the metabolic oscillations under basal conditions, which can be served as a baseline to study these processes during pathological states.

M25

**Branched-Chain Amino Acids Control Mitochondrial Metabolite Carriers Via the Mitochondrial-Derived Compartment Pathway**

**M. Schuler**, A. M. English, J. M. Shaw, A. L. Hughes; University of Utah, Department of Biochemistry, Salt Lake City, UT.

Mitochondria are double membrane-bound organelles that fulfill central tasks in cellular metabolism. Metabolite transport into and out of mitochondria is mediated by carrier proteins of the SLC25A family embedded in the inner mitochondrial membrane. Despite being critical regulatory points in cellular metabolism, it remains unclear how mitochondrial carriers are modulated in response to changes in the intracellular nutrient status. We recently identified a new subcellular structure that forms from yeast mitochondria, called the Mitochondrial-Derived Compartment (MDC). In aging yeast cells, MDCs are formed in response to defects in lysosomal amino acid storage and target select mitochondrial proteins for degradation. However, the function of the MDC pathway and the underlying signal activating MDC formation are not known. Here we present new evidence that MDCs are conserved from yeast to mammals and control the levels of metabolite carriers on mitochondria in response to intracellular amino acid elevation. Using super-resolution live-cell imaging, we demonstrate that MDCs are large (1um), organelle-like structures that selectively sort and remove metabolite carriers and their associated import receptor Tom70 from yeast and mammalian mitochondria. We show that MDCs form from mitochondria in response to intracellular amino acid elevation with branched-chain amino acids (BCAAs) being the most potent inducers of MDC formation. Our data indicates that BCAA catabolism by the branched-chain amino acid transaminases *BAT1* and *BAT2* is a major driver of MDC formation in yeast and that downstream products of BCAA degradation directly activate MDC-dependent remodeling of mitochondria. By contrast, the MDC pathway is unresponsive to other common mitochondrial stressors, including ROS generators, inhibitors of the mitochondrial respiratory chain and membrane potential uncouplers. Since MDCs sort and remove metabolite carriers from mitochondria, we propose that the MDC pathway provides cells with a mechanism to fine-tune mitochondrial metabolite transport in response to BCAA elevation by controlling the level of select SLC25A carriers on mitochondria. In support of this idea, our preliminary data indicate that overexpression of mitochondrial carriers directly activates MDC formation in yeast, whereas deletion of the carrier receptor Tom70 reduces MDC

formation. Our current experiments are focused on testing how MDC formation affects cellular and mitochondrial metabolism and identifying the machinery and metabolic signaling mechanisms that control selective sorting and removal of nutrient carriers from mitochondria via the MDC pathway.

M26

**Intersection of the Golgi Stress Response and Redox Homeostasis in Huntington's Disease**

**B. D. Paul**, S. H. Snyder; Johns Hopkins University School of Medicine, Baltimore, MD.

The Golgi stress response (GSR) is emerging as a vital stress response in cells comparable in importance to the Endoplasmic Reticulum (ER) stress response. However, molecular mechanisms operating during the GSR are relatively unexplored. We show here that the GSR engages the hydrogen sulfide signaling axis to mediate cytoprotective responses in cells. Hydrogen sulfide is a gaseous signaling molecule which participates in diverse physiological processes ranging from stress response to vasorelaxation. One of the enzymes which generates $H_2S$ is cystathionine gamma lyase, CSE, which is induced in response to stress. $H_2S$ signals through a posttranslational modification termed persulfidation or sulfhydration, wherein -SH groups of cysteine residues are modified to -SSH groups. Sulfhydration is substantially prevalent in cells and modulates the structure and/or function of proteins being modified. $H_2S$ metabolism is compromised in neurodegenerative disorders such Huntington's disease (HD), leading to diminished sulfhydration levels in both cell culture and mouse models of HD. HD is caused by expansion of polyglutamine repeats in the protein huntingtin, htt, which causes it to aggregate and cause widespread damage leading to motor and cognitive deficits. Stimulating $H_2S$ production is beneficial in HD and promotes longevity in mouse models of HD. Using striatal progenitor cells from HD mice, we show that Golgi stressors induce CSE, via the PKR-like ER kinase/Activating Transcription Factor 4 (PERK/ATF4) pathway. Golgi stress also induces the Golgi protein, acyl-CoA binding domain containing 3 (ACBD3). ACBD3 interacts with CSE and stimulates its catalytic activity to produce $H_2S$. We demonstrate that mild Golgi stress induces sulfhydration to stimulate cytoprotective responses (Paul et al, unpublished). These findings have implications in development of novel therapeutics that can delay or halt neurodegeneration in not only HD, but other neurodegenerative diseases involving aberrant redox and $H_2S$ signaling. References 1. **Paul BD**, Sbodio JI, Xu R, Vandiver MS, Cha JY, Snowman AM and Snyder SH. Cystathionine γ-lyase deficiency mediates neurodegeneration in Huntington's disease. ***Nature***. 2014; 509(7498):96-100. 2. Sbodio JI[#], Snyder SH* and **Paul BD**[#]*. Golgi stress response reprograms cysteine metabolism to confer cytoprotection in Huntington's disease. ***Proc Natl Acad Sci USA***. 2018; 115(4):780-785. *([#]Equal contribution,*Co-corresponding author).* 3. Sbodio JI, Snyder SH* and **Paul BD*** Redox mechanisms in neurodegeneration: From disease outcomes to therapeutic opportunities. ***Antioxid Redox Signal***. 2019; 30(11):1450-1499 (**Co-corresponding author*).

M27

**Aquaporin-7 Is a Metabolic Sensor that Regulates Response to Cellular Stress in Breast Cancer**

C. Dai, V. Charlestin, M. Wang, N. Dovichi, J. Li, **L. Littlepage, 46556**; University of Notre Dame, Notre Dame, IN.

Misregulation of nutrient signaling contributes to cancer growth and treatment response. Using GC-MS, LC-MS/MS, and capillary zone electrophoresis (CZE)-MS platforms, we quantified and compared the levels of 374 metabolites in breast tumor tissue from normal tissue and transgenic mouse breast cancer models overexpressing a panel of oncogenes (PyMT, PyMT-DB, Wnt1, Neu, and C3-TAg). We then

effectively integrated metabolomics and gene expression data from breast cancer mouse models through a novel unbiased correlation-based network analysis and have identified 35 metabolite and 34 gene hubs with the most network correlations. These hubs have prognostic value and are likely integral to tumor metabolism and breast cancer. We focused on the gene hub Aquaporin-7 (AQP7), a water and glycerol channel, as a novel regulator of breast cancer. AQP7 is prognostic of overall survival in breast cancer patients. In mouse breast cancer models, reduced Aqp7 expression caused reduced primary tumor burden and lung metastasis. Metabolomics and complex lipid profiling of cells and tumors with reduced Aqp7 revealed dramatically altered lipid metabolism, glutathione metabolism, and urea/arginine metabolism compared to controls. These data identify Aqp7 as a critical regulator of the metabolic and signaling responses to environmental cellular stresses in breast cancer, highlighting AQP7 as a potential cancer-specific therapeutic vulnerability. Because breast cancer cells with Aqp7 deficiency are sensitive to these perturbations, Aqp7 is an attractive therapeutic target.

M28

**Metabolic Coordination of Stem Cell Fate Drives Tumor Initiation**

**S. Baksh**[1], P. Todorova[2], S. Gur-Cohen[1], E. Fuchs[1], L. Finley[2]; [1]The Rockefeller University, New York, NY, [2]Memorial Sloan Kettering Cancer Center, New York, NY.

Tissue stem cells are the cell of origin for many malignancies. Metabolites regulate the balance between self-renewal and differentiation, but whether endogenous metabolic pathways predispose stem cells to transformation *in vivo* remains unknown. Here, we tackle this question in epidermal stem cells (EpdSCs), a cell of origin for squamous cell carcinoma (SCC). Using genetics, cell and molecular approaches, metabolic profiling and dietary interventions, we find that *de novo* serine synthesis promotes epidermal differentiation. We show that oncogenic EpdSCs restrict glucose-derived serine synthesis, and in doing so, limit production of alpha-ketoglutarate that drives H3K27me3 loss and epidermal differentiation. When extracellular serine is limiting, enforced serine synthesis induces differentiation and stunts SCC growth, whereas blocking serine synthesis or antagonizing alpha-ketoglutarate-driven demethylation facilitates malignant progression *in vivo*. Thus, metabolic reprogramming that limits serine synthesis supports oncogenic self-renewal and renders SCC initiation sensitive to dietary serine manipulation. These results demonstrate that a stem cell's intrinsic metabolic program dictates its capacity to initiate a neoplasm.

M29

**Metabolic Limitations of Cell Proliferation**

**M. Vander Heiden**; Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA.

Complex regulatory mechanisms enable cell metabolism to match physiological state. The major pathways cells use to turn nutrients into energy and to synthesize macromolecules have been elucidated; however, how cells determine which nutrients to use from their environment and which processes are limiting is less well understood. We find that the ability to produce oxidized biomass can be limiting for the proliferation of some cells. Specific cells may be differentially dependent on how they overcome this redox limitation for biomass synthesis. We are examining which metabolic pathways are most limiting in different contexts as well as ways in which cells support an increased requirement for oxidation reactions. This is providing insight into metabolic phenotypes such as aerobic glycolysis as well as understanding why specific cancers are differentially sensitive to drugs that target metabolism.

## Minisymposium 3: Higher Order Cytoskeletal Structures

M30

**Centriole Motility and Subcellular Positioning Require Pericentrin-like-protein and Kinesin-1**

**M. R. Hannaford**, Z. T. Swider, B. J. Galletta, C. J. Fagerstrom, N. M. Rusan; National Heart, Lung and Blood Institute, Bethesda, MD.

Centrosomes are the major microtubule organizing center (MTOC) of the cell. They comprise a pair of centrioles surrounded by a matrix of proteins term the pericentriolar material. Through microtubule nucleation they organize the mitotic spindle, cilia and flagella. To fulfill these functions, centrosomes must be motile to achieve proper positioning within the cell. Very little is understood about the different mechanisms of centrosome motility. Typically, it is thought to be governed by the activity of microtubule motors, pushing or pulling on the microtubules anchored at the centrosome. In some cell types, centrioles lack PCM and microtubules, and are otherwise known as inactive centrioles. Inactive centrioles must be motile as their intracellular positioning is critical for asymmetric cell division and sensory cilia formation. Despite this the mechanism by which inactive centrioles are able to move around the cell is poorly described and not understood. Here we investigate how inactive centrioles move in interphase cells. Using high resolution live cell imaging coupled with genetic analysis in *Drosophila*, we show that centrioles move on microtubules, in a manner involved Dynein and Kinesin-1. Importantly, structured illumination microscope revealed Kinesin-1 localizes to centrioles in *Drosophila* S2 cells. To identify centriole components required for movement we performed a targeting RNAi screen and found that the centriole protein Pericentrin like protein (Plp) is necessary for centriole movement in interphase cells. Through yeast-2-hybrid analysis coupled with an *in vivo* interaction assay we mapped novel and direct protein-protein interactions between Plp and Kinesin-1. Structural analysis demonstrated that these interactions involve the conserved PACT domain. Our data support a model where Plp acts as a direct adaptor that links the centriole to motor proteins, thereby facilitating their movement. In this work we propose the first detailed mechanism of how centrioles can move independently of their role as an MTOC, in the context of developing tissue. Further understanding of inactive centriole motility has far-reaching implications in studies of asymmetric cell division and sensory ciliogenesis.

M31

***In Vivo* Proximity Labeling of PTRN-1/Patronin with TurboID Reveals Novel Components of Non-centrosomal MTOCs in Epithelial Cells**

**A. D. Sanchez**[1], T. Branon[2], A. Y. Ting[1], J. L. Feldman[1]; [1]Stanford University, Stanford, CA, [2]MIT, Cambridge, MA.

Microtubules have dynamically growing plus ends and comparatively stable minus ends that are localized to microtubule organizing centers (MTOCs). The centrosome is the primary MTOC during cell division, but in differentiated cells, MTOC function is often reassigned to non-centrosomal sites (ncMTOCs). Unlike the centrosome, the components that are necessary to grow and localize microtubules at ncMTOCs are poorly understood. To address this gap in knowledge, we are identifying additional ncMTOC components using proximity labeling, a technique that can identify proximal protein networks *in vivo*. In particular, we developed TurboID, a promiscuous BirA mutant enzyme that rapidly catalyzes the covalent attachment of biotin to proximal proteins. Proteins tagged with biotin are then

isolated and identified by mass spectrometry. To identify novel ncMTOC components, we fused TurboID to the microtubule minus end protein PTRN-1/Patronin and expressed this transgene in *C. elegans* embryonic intestinal epithelial cells. This strategy resulted in spatially restricted biotinylation at the apically localized ncMTOC and a list of 62 candidate ncMTOC components including the spectraplakin VAB-10B, which we verified as a PTRN-1 physical interactor by immunoprecipitation. Using tissue-specific degradation and mutant analysis of VAB-10B, we found that VAB-10B orchestrates microtubule localization during ncMTOC establishment, which is helping us understand the mechanisms by which microtubules are initially targeted to an ncMTOC in vivo. In addition, we find a role for VAB-10B in targeting PTRN-1 to the ncMTOC and are exploring the role of VAB-10B in targeting other microtubule-associated proteins. As the apical polarity protein PAR-3 is properly localized following VAB-10B depletion, we propose a model where VAB-10B serves as a scaffold to couple microtubule organization to polarity determinants. In addition to VAB-10B, we are currently testing the ncMTOC-related functions of other candidates from our proximity labeling dataset and will present our results.

M32

**Volumetric Electron Microscopy Reveals New Details of Primary Cilia Organization and Potential Function in the Brain**

**C. Ott**[1], R. Torres[2], A. Weigel[1], N. da Costa[2], J. Lippincott-Schwartz[1]; [1]HHMI Janelia Research Campus, Ashburn, VA, [2]Allen Institute, Seattle, WA.

Signaling through primary cilia contributes to both development and function of the brain, but we currently have few insights into the roles and spatial organization of cilia on different cell types. Neuropeptides and neurotransmitters emerge from, and pass through, the dense network of axons, dendrites and glial processes known as the neuropil to activate cilia-mediated signaling pathways. For practical reasons, most strategies implemented to study primary cilia visually isolate cilia from this complex network. To elucidate the context in which primary cilia reside in brain tissue, we analyzed cilia on more than 200 mouse visual cortex cells in volumetric transmitted electron microscopy (TEM) data. In addition, we characterized a cilium from the nucleus accumbens in an isotopic focused ion beam-scanning electron microscopy (FIB-SEM) volume. We investigated both the structural features of neuronal and astrocytic cilia and the cellular and extracellular environment surrounding them. In the neuropil, cilia were proximal to glial processes, axons and dendrites. In addition, many neuronal and astrocytic cilia were adjacent to at least one synapse - close enough to theoretically detect spillover signals if they contain relevant receptors. On pyramidal neurons, cilia emerged at a distance from the axon initial segment and either traveled along the edge of the soma for part of their length, or projected directly into the neuropil. In contrast, most astrocyte cilia in the visual cortex were recessed in a deep ciliary pocket. We observed endo/exo-cytic events on the plasma membrane adjacent to the cilium, as expected. However, we also found areas where the plasma membrane hosted extensive endoplasmic reticulum-plasma membrane contact sites adjacent to where the cilium abutted the plasma membrane. Tracking cilia into the neuropil using volumetric TEM also revealed structural differences between neuronal and astrocytic cilia. Together, FIB-SEM and volumetric TEM of primary cilia are providing a new nanoscale understanding of cilia structure and cellular organization. The resolution and large volume of these data allow us, for the first time, to quantify differences between cilia of different cell types, measure changes along the lengths of cilia, and generate hypotheses about the origin of ciliary signals in the brain.

M33

**Ift Trains Fragment Into 'Carts' of IFT-A, -B, and Dynein At the Ciliary Tip**

**J. L. Wingfield**[1], I. Mengoni[1], P. Liu[1], J. Dai[1], Y. Hou[2], W. Zhao[2], G. B. Witman[2], K. Lechtreck[1]; [1]Department of Cellular Biology, University of Georgia, Athens, GA, [2]Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, MA.

Intraflagellar transport (IFT) trains carry building blocks from the cell body into cilia. These trains are assembled at the ciliary base, move by anterograde IFT to the flagellar tip and return by retrograde IFT to the cell body (Wingfield et al. 2017). In *C. reinhardtii*, the anterograde trains possess layers of IFT-A, IFT-B and IFT dynein particles with distinct periodicities whereas the retrograde trains are more amorphous (Jordan et al. 2018). The remodeling of trains at the ciliary tip involves fragmentation of the anterograde trains (Chien et al. 2017). However, this study is based on IFT dynein and the IFT-B1 protein IFT27-GFP. Thus, the degree of train fragmentation, e.g., into "IFT carts" consisting of IFT-A and B or into IFT-A, B1 and B2 subcomplexes or into individual IFT proteins, remains unknown. Understanding of this reorganization could shed light on the mechanisms of cargo unloading and IFT dynein activation at the tip. Here, we analyzed IFT dynamics at the ciliary tip utilizing fluorescent protein-tagged IFT-A, IFT-B1, IFT-B2, and IFT motors subunits. All IFT proteins dwell at the ciliary tip for ~2s and material from one anterograde train is distributed onto several retrograde trains. We estimate that the IFT pool at the tip correspond to 3-5 anterograde trains based off of photo-bleaching experiments. Two-color imaging of IFT140-sfGFP and IFT54-mScarlet indicates that IFT-A and B remain together during the reorganization. IFT-A and B proteins continued to travel together in BBS4-deficient cilia revealing that the BBSome is not critical for A-B association in *C. reinhardtii.* While also dwelling for ~2s, BBS4-GFP mostly diffused at the ciliary tip rather than being stationary as the IFT proteins suggesting that it is released from IFT trains. FRAP analysis of retrograde IFT at the tip revealed that IFT54-mScarlet and the IFT dynein subunit D1bLIC-GFP mostly remained together during turnaround Our data support a model of IFT turnaround in which anterograde trains fragment into carts or short strings of carts while IFT-A, B and IFT dynein remain associated.

M34

**Dynamic Basal Body-associated Striated Fibers Promote Ciliary Array Organization through Basal Body Coupling and Cortical Interactions**

**A. Soh**[1], T. van Dam[2], A. Stemm-Wolf[1], A. Pham[1], G. Morgan[3], E. O'Toole[3], C. Pearson[1]; [1]University of Colorado Anschutz medical campus, Aurora, CO, [2]Utrecht University, Utrecht, NETHERLANDS, [3]University of Colorado at Boulder, Boulder, CO.

Multi-ciliary arrays promote cellular motility and fluid flow using the polarized and coordinated beating of hundreds of motile cilia. Basal bodies (BBs) nucleate and position cilia into longitudinal rows. Cilia beat in an asymmetric pattern and produce forces that are imposed upon BBs and the cell cortex. Even in the face of these forces, BBs maintain their position and polar orientation. To promote BB anchorage and orientation, each BB possesses appendages, including striated fibers (SFs) and bundles of microtubules that establish BB coupling. Mutants resulting in shorter SFs cause BBs to disorient. Here, we show that disoriented BBs in *Tetrahymena* multi-ciliary arrays are reoriented into organized ciliary rows when the length of the SF is restored. This reveals that multi-ciliary arrays possess error correction mechanisms to resolve BB disorientation. EM tomography and super resolution fluorescence microscopy were used to show that BBs within the same longitudinal rows are coupled by interactions between the

SFs and post-ciliary microtubule bundles. In addition, SFs adopt different length states by their shrinkage and growth in response to reduced and elevated ciliary forces, respectively. This promotes dynamic BB coupling and cortical interactions that are responsive to environmental forces. Analogous to vertebrate SFs, *Tetrahymena* SFs are multiprotein macromolecular complexes. They are composed of a network of at least eight protein components, belonging to the SF-assemblin protein family, that exhibit distinct and overlapping localization profiles within the SF structure. Components associated with the base of the SF control both steady state SF length and high ciliary force-induced SF elongation. Thus, the dynamic regulation of SFs promotes BB and cortical interactions to organize and couple cilia within ciliary arrays. This enables efficient generation of hydrodynamic flow to serve cellular and extracellular motility.

M35

**Arp2/3-mediated Branched Actin Networks Play a Role in Ciliary Assembly and Maintenance in *Chlamydomonas***

**B. M. Bigge**, P. Avasthi; University of Kansas Medical Center, Kansas City, KS.

Actin is essential for many cellular processes, including maintaining and assembling cilia. With our increasing number of tools to study actin, including phenotypic screens, phalloidin staining, and genetic and chemical perturbations, we're able to leverage the most powerful ciliary model, *Chlamydomonas reinhardtii,* to study the role of actin in ciliary assembly. *Chlamydomonas* also provides a unique opportunity to study the diversity of potential actin functions due to its two actin genes: *IDA5,* a conventional actin with about 89% sequence homology to mammalian actin; and *NAP1*, a divergent actin with only about 65% sequence homology to mammalian actin. Null mutants of each of these proteins have been created, and using the *nap1* mutant background and LatrunculinB, a drug that depolymerizes IDA5 but not NAP1, we can acutely create *Chlamydomonas* cells with no filamentous actin. With these tools, our lab has previously shown that filamentous actin is needed for normal ciliary protein synthesis, trafficking, and incorporation into cilia. Now, we are taking a closer look at this filamentous actin network in order to determine the nature of the actin filaments important for these functions. The topology of the actin throughout the cell varies with branched actin networks, linear actin networks, and others playing specific cellular roles. Here, we study the branched actin network, mediated by Arp2/3 and its role in our newly identified actin-dependent functions related to the cilia. Our data suggests that Arp2/3 interacts with both IDA5 and NAP1 networks allowing them to maintain and grow cilia and perform other functions in the cell. Genetically or chemically perturbing Arp2/3 results in shorter steady-state and regenerating cilia, as well as altered actin structures visible with phalloidin that may contribute to those phenotypes. This data supports the hypothesis that branched actin networks consisting of IDA5 and NAP1 and mediated by Arp2/3 are necessary for normal cilia maintenance and assembly.

M36

**Generation of Stress Fibers From the Cortical Actomyosin Meshwork**

**J. Lehtimäki**, K. Rajakylä, S. Tojkander, P. Lappalainen; University of Helsinki, Helsinki, FINLAND.

Contractile actomyosin bundles are important for motile and morphogenetic processes in animal cells and tissues. Ventral stress fibers are the most prominent actomyosin structures in many non-muscle cell-types. These thick, contractile bundles are typically connected to focal adhesions at their both ends

and contribute to cell migration, morphogenesis and mechanosensing. Ventral stress fibers can be generated either from the pre-existing network of two other types of actin bundles; transverse arcs and dorsal (radial) stress fibers (Hotulainen & Lappalainen, *J. Cell Biol.,* 2006; Tojkander et al., *Curr. Biol.,* 2011, Burnette et al., *Nat. Cell Biol.,* 2011; Tojkander et al., *eLife*, 2015; Fenix et al., *Mol. Biol. Cell.,* 2016; Hu et al., *Nat. Cell Biol.,* 2017), or via splitting of pre-existing ventral stress fibers (Young & Higgs, *Curr. Biol.,* 2018). In addition to thick ventral stress fibers, which are typically enriched at the lamellum of motile cells, many cell-types also exhibit thinner stress fibers at their posterior region (e.g. Burnette et al., *J. Cell Biol.,* 2014). Also these thin (basal) stress fibers are connected to focal adhesions at their both ends, but whether they are generated from the pre-existing network of transverse arcs and dorsal stress fibers, or through another mechanism, has remained elusive. Here, by utilizing 3D-SIM and live-cell TIRF microscopy on U2OS osteosarcoma cells and mouse embryonic fibroblasts (MEFs), we revealed that basal stress fibers assemble through NMII-dependent reorganization of the cortical actin meshwork at the bottom of the cell. This leads to the formation of an actomyosin bundle, and subsequent enrichment of focal adhesion components at the ends of the bundle. The assembly of basal stress fibers is typically preceded by a NMIIA pulse at the ventral surface of cell, and appears to be dependent on formins but not the Arp2/3 complex. Basal stress fibers display periodic NMII pattern and are contractile, although based on traction-force microscopy they exert weaker forces to the substratum compared to thick ventral stress fibers. Live-cell imaging revealed that basal stress fibers undergo dynamic re-organization and are able to 'exchange neighbors': an event where the *de novo* forming actomyosin bundle interacts with another one and subsequently displaces its attachment from a focal adhesion to another in the near vicinity. Collectively, our work provides evidence that basal stress fibers are dynamic, contractile actomyosin bundles, which are generated through reorganization of the cortical actomyosin meshwork by a novel, NMII-dependent mechanism.

M37

**Reach Out and Touch Fate: Cytonemes in Sonic Hedgehog Signal Propagation**

**E. T. Hall**, D. P. Stewart, S. K. Ogden; St. Jude Children's Research Hospital, Memphis, TN.

The formation and maintenance of morphogen gradients is essential during development, but the mechanisms by which morphogens are transported to form gradients are not yet clear. Evidence is mounting in support of their transport by long specialized filopodia known as cytonemes. Despite experimental evidence demonstrating cytoneme based transport of morphogens in vivo, insight into the mechanisms of cytoneme initiation, regulation, and signal propagation is lacking. This is due to technical difficulties visualizing these fragile structures. We recently overcame this obstacle by developing a fixation technique termed MEM-fix that preserves the cytonemes of cells in culture, allowing investigation of fundamental questions about cytoneme biology, and the role they play in morphogen signal propagation. We find that cytonemes are ubiquitous across cultured cell lines and demonstrate dynamic motile and sensing properties. The expression of the morphogen Sonic Hedgehog (Shh) can affect the occurrence of cytonemes in ligand-producing cells. We show that Shh travels along cytonemes in vesicles with the Shh deployment protein Dispatched. Live cell imaging identified that cytoneme-based Shh deposits can activate the pathway in receiving cells within seconds of release. The use of MEM-fix along with live cell imaging assays, is now providing insight into the functionality and the extent cells use cytonemes for morphogen transport.

M38

**Measured Contractile Ring Component Dynamics Inform Agent-based Models of Animal Cell Cytokinesis**

**D. Cortes**[1], M. DiSalvo[1], N. Allbritton[1], F. Nedelec[2], P. Maddox[1], A. S. Maddox[1]; [1]UNC Chapel Hill, Chapel Hill, NC, [2]University of Cambridge, Cambridge, UNITED KINGDOM.

Cytokinesis, the division of one cell into two, is indispensable for development and homeostasis. The contractile ring is a dynamic cytoskeletal structure composed of actin filaments, myosin motor proteins and a myriad of crosslinkers. The dynamic remodeling of this structure drives the physical invagination of the plasma membrane to divide a cell. Past research has defined the main components of the contractile ring, the regulation of their recruitment to the plasma membrane, and the hierarchy with which they are loaded. Extensive work has shown how tuning levels of crosslinkers and motors can affect global dynamics. However, little is known about the spatiotemporal dynamics of ring components during constriction in animal cells. Combining imaging with high temporal resolution and agent-based modeling, we explored the dynamic structure of contractile rings during constriction. Using custom microfluidic traps, we oriented the zygote of *Caenorhabditis elegans* upright such that a light sheet orthogonal to an inverted imaging objective lens illuminated the division plane and contractile ring. Imaging of functional fluorescently-tagged conserved components including septins, anillin, and myosin and multiple probes for F-actin through constriction allowed us to calculate the relative density of each component as a function of ring perimeter. Conserved structural proteins were generally recruited and compacted early in cytokinesis and lost later in ring closure, with their density peaking at different stages of constriction. We implemented the density dynamics functions in our Cytosim agent-based models of contractile ring constriction to more realistically depict rings *in vivo*. Explicit inclusion of component abundance dynamics improved the realism of some aspects of the kinetics of simulated contractile ring constriction, such as the stage at which maximum speed was reached. Because the division plane was evenly illuminated, we were able to determine the distribution of components along the ring, finding that some, such as septin, were punctate and some, such as anillin, were more uniform. The exceptional spatial resolution afforded by agent-based modeling will allow us to compare component distribution and its dynamics *in vivo* and *in silico*, and define the lengthscale of contractile “units” in the ring. Thus, the pursuit of both quantitative live imaging and mathematical modeling in one research program is advancing our understanding of cytokinetic cytoskeletal remodeling.

M39

**Neurite Morphogenesis Requires Suppression of the Lamellar Protrusions of the Cell Body by Septin 7**

**M. R. Radler**, E. Spiliotis; Drexel University, Philadelphia, PA.

Axons and dendrites, the structures that transmit and receive electrical and chemical signals in the brain, develop from the membrane protrusions of neuronal cell bodies. Formation of neurites, the early precursors of axons and dendrites, involves the formation of actin-rich filopodia and their engorgement with microtubules. How actin and microtubules are coordinated and how neurites mature and grow asymmetrically into axons and dendrites is not well understood. Septins are a family of filamentous GTP-binding proteins, which associate with actin and microtubules, and septin 7 (Sept7) was recently found to determine the sites of neurite formation in neuronal progenitors. Here, we have sought to investigate the role of septins in early neurite formation and asymmetry establishment. We found that that Sept7 depletion in embryonic rat hippocampal neurons (DIV1) resulted in neurons with abnormally enlarged

cell bodies, which lacked pyramidal-shaped morphology and characterized by extensive lamellipodia-like protrusions. Strikingly, several neurites were observed to extend directly from these lamellipodial protrusions rather than directly from the cell body. This phenotype was accompanied by a reduction in the number of neurons that broke neurite symmetry and developed a single elongated tau-positive neurite, the presumptive axon. Expression of the constitutively active myosin II regulatory light chain in Sept7-depleted cells rescued partially the size and shape of neuronal cell bodies, and restored neurite formation from the cell body as well as axonal specification. Taken together, our data suggest that Sept7 functions synergistically with myosin-II in suppressing membrane protrusive activity and possibly branched actin polymerization, restricting neurite growth and maturation to filopodia that are in direct continuum with the cell body.

## Minisymposium 4: Membrane Trafficking: Vesicle Formation, Cargo Sorting and Fusion

M40

**Protein Droplets Catalyze Assembly of Endocytic Vesicles**

**K. J. Day**[1], G. K. Kago[1], J. B. Richter[2], C. C. Hayden[1], E. M. Lafer[2], J. C. Stachowiak[1]; [1]Biomedical Engineering, University of Texas at Austin, Austin, TX, [2]Biochemistry, University of Texas Health Science Center at San Antonio, San Antonio, TX.

During clathrin-mediated endocytosis, dozens of adaptor and coat proteins assemble into a mesh-like network at the plasma membrane, where they work together to drive vesicle formation. Fcho1 and Eps15 are among the earliest adaptors to arrive at endocytic sites. These initiator proteins are responsible for recruiting, concentrating and organizing downstream components at the membrane surface. However, as the budding vesicle grows, the initiator proteins must also allow dynamic rearrangement of the adaptor network. It is unclear how initiator proteins are able to meet these competing demands. Toward explaining this apparent paradox, we have made the exciting discovery that Fcho1 and Eps15 assemble into liquid protein droplets at membrane surfaces. Droplet assembly requires specific protein domains in Fcho1 and Eps15 that contribute to the ability of the two proteins to form a multivalent network. Further, these droplets exhibited liquid-like properties, including coalescence and dynamic exchange of proteins in and out of droplets. Toward characterizing the phase behavior of these initiators over a range of biochemical conditions, we have modulated protein concentrations, temperature, and crowding conditions to develop a set of phase diagrams. The liquid properties of Fcho1/Eps15 protein droplets can explain how the downstream adaptor network is tightly concentrated yet also dynamic. To test the role of Fcho1/Eps15 liquid assemblies in live mammalian cells, we developed an assay that allows us to tune the strength of initiator protein assembly in real time using light. Specifically, we created a chimeric version of Eps15 that contains a light-inducible oligomerizing domain, Cry2. Application of low light levels drove fluid assembly of the initiator proteins. These fluid assemblies resulted in an increase in endocytic initiation events. In contrast, high light levels drove the formation of solid initiator complexes, which stalled vesicle dynamics. These results indicate that solid assemblies of initiator proteins are unable to release endocytic assemblies. In contrast, more fluid assemblies can serve as strong yet dynamic catalysts. These results suggest that the liquid-like behavior of Fcho1/Eps15 droplets is important for effective catalysis of clathrin-mediated endocytosis in cells.

M41

**A Kinetic Analysis of Secretory and Vacuolar Protein Sorting Subdivides the TGN Stage of Golgi Maturation**

**J. C. Casler**, B. S. Glick, PhD; University of Chicago, Chicago, IL.

We recently developed a method to track the passage of cargo proteins through the yeast biosynthetic pathway by video fluorescence microscopy. Individual Golgi cisternae containing cargo molecules can be visualized over time. Our approach is to aggregate a fluorescent protein in the ER, and then solubilize the protein by the addition of a ligand to permit ER exit and synchronous transport through the secretory pathway. This method revealed that a fluorescent secretory cargo remains within a Golgi cisterna throughout the time course of maturation. Surprisingly, we also discovered that a fraction of the secretory cargo molecules recycle within the Golgi in a manner that depends on the clathrin adaptor AP-1 and N-linked glycosylation.How are secretory cargoes recycled by AP-1? This adaptor recognizes a subset of resident TGN proteins and is unlikely to bind a foreign cargo directly. We propose that N-glycosylated cargoes are indirectly recycled by AP-1 via interaction with glycosylation enzymes in the TGN. A strong candidate for a recycling “receptor” is the mannosyltransferase Mnn1, which has been shown to localize to the Golgi in a clathrin-dependent manner. We find that deletion of Mnn1 prevents recycling of the fluorescent secretory cargo. In general, recycling of secretory cargoes may act as a quality control mechanism to ensure that these cargoes are fully processed before secretion. We are now testing whether AP-1- and glycosylation-dependent recycling of secretory cargoes also occurs in mammalian cells.Other proteins moving through the yeast Golgi are trafficked to the vacuole. To address how vacuolar cargo sorting may differ from secretory cargo sorting, we modified the fluorescent cargo to include a tetrapeptide that serves as a vacuolar sorting signal. Trafficking of the modified cargo to the vacuole depends on Vps10, a receptor for vacuolar hydrolases. Kinetic analysis revealed that the modified cargo departs the Golgi about half way through the maturation process. Departure begins when the clathrin adaptor Gga2 arrives at the TGN. Thus, vacuolar and secretory cargoes exit the Golgi at different times. This result challenges the concept that all cargoes fully transit through the TGN before being sorted. Our interpretation is that in yeast, the TGN stage of maturation represents about half of the total Golgi maturation time, and that the TGN can be subdivided into two kinetic sub-stages: an initial sub-stage that supports sorting of vacuolar proteins, and a terminal sub-stage that supports AP1-dependent recycling as well as secretory vesicle formation.

M42

**COPI and COPII Cooperate At ER Exit Sites to Support ER-to-Golgi Protein Trafficking Revealed by 3D Ultrastructure Analyses and Live-Cell Imaging**

**A. Weigel**[1], C. Chang[1], G. Shtengel[1], D. Hoffman[1], M. Freeman[1], C. S. Xu[1], S. Pang[1], E. Betzig[2], H. Hess[1], J. Lippincott-Schwartz[1]; [1]Janelia, Ashburn, VA, [2]University of California, Berkeley, CA.

The endoplasmic reticulum (ER) is the site of many important cellular processes, among them the birth and early steps of the secretory pathway. Cargo synthesized in the ER continues through a series of dynamic compartments before reaching its final destination. This includes specialized sites on the ER called ER exit sites (ERESs), transport intermediates, and the Golgi apparatus. Here, we visualize ERESs using a cryo-correlative light electron microscopy (cryo-CLEM) system. Cells with ERESs fluorescently labeled by Sec23 were high pressure cryo-fixed, visualized by cryo-structured illumination microscopy (SIM), and then directly correlated to focused ion beam scanning electron microscopy (FIB-SEM). FIB-

SEM data provides isotropic, high-resolution (4-8nm), large volume EM while cryo-SIM grants super-resolution (100nm lateral) protein specific localization. We found that ERESs were composed of a highly intertwined nest of tubules with a diameter of 360±60nm (n=110). The tubule diameter within the ERES was more constricted than the ER by 20%, 30±20nm (n=782). Using acargo synchronization and release system, RUSH (Retention Using Selective Hook), we directly studied cargo movement through the secretory pathway. When the ERESs were stressed by overloading with cargo we found that the total volume occupied by each ERES swelled 1.45-fold and the comprising tubule diameter expanded by 2.6-fold. Further investigation of coat protein localization at ERESs revealed that more than 70±10% (n=15 cells) of COPII positive ERESs also contained COPI. Using AiryScan microscopy and point localization techniques we observed a separation between COPI and COPII labeling on ERESs of 136±4nm (n=29), which increased by 1.45-fold with cargo overexpression. This was in agreement with FIB-SEM measurements of ERES sizes. Confocal imaging revealed that upon RUSH cargo release, COPI readily moved with cargo away from the ERES in transport intermediates, which often appeared as tubules, while COPII remained at the ERES. Applying cryo-CLEM in cells overexpressing RUSH cargo that were cryo-fixed soon after cargo release allowed visualization of transport intermediates carrying RUSH cargo. Correlated FIB-SEM of these cells revealed that the transport intermediates existed as pearled membrane tubules running alongside microtubules. Among the pearled membrane tubules observed were those still connected to the ERES, those detached from ERESs, and those that had fused with the Golgi. Examples of the same structures were seen in FIB-SEM of untransfected cells. Together, this data provides a 3D view of the early secretory pathway never before seen with equivalent isotropic resolution, large depth-of-field, and precise protein localization.

M43

**Tango1 Assembles a Machine to Select and Export Collagens From the Endoplasmic Reticulum**

**I. Raote**[1], F. Campelo[2], V. Malhotra[1]; [1]Centre for Genomic Regulation, Barcelona, SPAIN, [2]The Institute of Photonic Sciences, Barcelona, SPAIN.

Secreted collagens compose 25% of our dry body weight and are necessary for tissue organization, skin and bone formation. But how are these bulky cargoes, that are too big to fit into a conventional COPII vesicle, exported from the endoplasmic reticulum (ER)? Our discovery of TANGO1, a ubiquitously expressed, ER-exit-site-resident, transmembrane protein, has made the pathway of collagen secretion amenable to molecular analysis. TANGO1 connects collagens in the ER lumen to cytoplasmic membrane-sculpting machinery, including COPII coat components. We show that TANGO1 forms a linear filament, which assembles into a ring at the ER exit site (ERES), thus corralling and scaffolding a macromolecular complex to form a sub compartment at ERES. Our results show that a filament of TANGO1 stabilizes the neck of a growing carrier and can induce the formation of large carriers by regulating ER membrane tension. The growth of the collagen-containing transport carrier is not by mere accretion of a larger COPII-coated patch of ER membrane, but instead by rapid addition of retrograde COPI-coated vesicles at the ERES. To recruit retrograde vesicles, TANGO1 binds the NRZ (NBAS/RINT1/ZW10) retrograde multisubunit tether complex. This mode of transport carrier formation is fundamentally different from that used to produce small COPII vesicles. The fence formed by TANGO1 extends in the lumen of the ER. A large, predominantly disordered, lumenal domain of TANGO1 contains an SH3-like domain that binds the collagen-specific chaperone HSP47 and presents multiple other sites to selectively recruit cargo and chaperones. Such an unstructured binding platform forms a selective domain within the ER lumen can limit the access to ERES to allow for cargo proteins to be concentrated prior to carrier formation and

packaging, and also actively support retention of ER residents. Our data indicate that TANGO1 generates a semi-stable sub-domain across multiple compartments. We have now mapped the components that work to co-assemble with TANGO1 into a ring. The processes that allow this assembly also coordinately select, partition, and organize export machinery, and membrane for a cargo-export tubule/carrier, thus defining the minimal machinery for collagen export.

M44

**Synthetic Lethality between Vps4a and Vps4b Triggers An Inflammatory Response in Colorectal Cancer**

**M. Miaczynska**[1], E. Szymanska[1], P. Nowak[1], K. Kolmus[1], M. Cybulska[2], K. Goryca[2], E. Derezińska-Wołek[3], A. Szumera-Ciećkiewicz[3], M. Brewińska-Olchowik[4], A. Grochowska[5], K. Piwocka[4], M. Prochorec-Sobieszek[3], M. Mikuła[2]; [1]International Institute of Molecular and Cell Biology, Warsaw, POLAND, [2]Department of Genetics, Maria Skłodowska-Curie Institute-Oncology Centre, Warsaw, POLAND, [3]Department of Pathology and Laboratory Medicine, Maria Skłodowska-Curie Institute-Oncology Centre, Warsaw, POLAND, [4]Laboratory of Cytometry, Nencki Institute of Experimental Biology, Warsaw, POLAND, [5]Department of Genetics, Maria Skłodowska-Curie Institute-Oncology Cent, Warsaw, POLAND.

Vps4A and Vps4B, members of the AAA ATPase family, are the only enzymes of the ESCRT machinery, which mediates membrane remodeling events. By controlling the release of other ESCRT components from cellular membranes, Vps4 paralogs are involved in a series of key cellular processes, such as endocytic cargo sorting, autophagy and cytokinesis. Expression of some ESCRT proteins is changed in several human pathologies, including cancers.Colorectal cancer (CRC), one of the most frequent human malignant tumors, results from an accumulation of genetic changes in colon epithelial cells, which transform them into adenocarcinomas. The majority of CRC have allelic deletions, the four most prevalent are localized on chromosomes 5q, 8p, 17p and 18q, the latter containing the *VPS4B* locus.To assess possible genetic alterations in the *VPS4B* gene in CRC, we performed an analysis of the TCGA dataset that revealed mono-and biallelic deletions of *VPS4B* at a frequency of 67% and 2% of CRC, respectively. Consequently, by testing clinical samples we observed significant downregulation of both mRNA and protein levels of Vps4B. Based on this data, we hypothesized that loss or decreased level of Vps4B make CRC cells more dependent on the Vps4A activity. We confirmed that concomitant depletion of Vps4A and Vps4B (Vps4A+B) generated a synthetic lethal phenotype in CRC cell lines cultured in vitro and as xenografts in immunodeficient mice.Our transcriptomics and biochemical analysis showed that Vps4A+B depletion induced inflammatory response involving NFκB activation and various cell death mechanisms, such as intrinsic and extrinsic apoptosis as well as a caspase-independent, RIP1 kinase-dependent death pathway. Cell death evoked by Vps4A+B synthetic lethality triggered release of damage-associated molecular patterns characteristic of immunogenic cell death (ICD). These immunomodulatory molecules caused the activation of macrophages toward an anti-tumor and proinflammatory M1 state.Cumulatively, our data demonstrate a synthetic lethality between Vps4 paralogs. They further identify a pair of novel druggable targets for personalized oncology and provide a rationale to develop Vps4 inhibitors for precision therapy of *VPS4B*-deficient cancers.

M45

**Tumor Protein D54 Defines a New Class of Intracellular Transport Vesicle**

G. Larocque, **S. J. Royle**, P. J. La-Borde, N. I. Clarke, N. J. Carter; University of Warwick, Coventry, UNITED KINGDOM.

Transport of proteins and lipids from one membrane compartment to another is via intracellular vesicular carriers. Several classes of carrier have been described based on morphology, location and function. For example, clathrin-coated vesicles (50-100 nm diameter) formed at the plasma membrane or trans-Gogi network (TGN), COPII-coated vesicles (60-70 nm) originating at the endoplasmic reticulum (ER), and intra-Golgi transport vesicles (70to90 nm). Whether cell biologists have a complete inventory of all classes of transport vesicle is an interesting open question.We began by investigating the function of Tumor Protein D54 (TPD54/TPD52L2), a member of the TPD52-like family. TPD54 is one of the most abundant proteins in HeLa cells but its function is unclear. We found that TPD54 was involved in multiple membrane trafficking pathways: anterograde membrane traffic, recycling endocytosed proteins back to the cell surface and Golgi integrity. To understand how TPD54 controls these diverse functions, we used an inducible method to reroute TPD54 to mitochondria. Surprisingly, this manipulation resulted in the capture of many small vesicles (30 nm diameter) at the mitochondrial surface. Superresolution imaging confirmed the presence of similarly sized TPD54-positive structures under normal conditions. We demonstrated that INVs meet three criteria for vesicle functionality. They contain specific cargo, they have certain R-SNAREs for fusion, and they are endowed with a variety of Rab GTPases (16 out of 43 tested). We propose that TPD54 defines a new class of transport vesicle, which we term intracellular nanovesicles (INVs).Visible by EM, INVs are small and uncoated; likely escaping previous detection due to their inconspicuousness. The molecular heterogeneity of INVs and the diverse functions of TPD54 suggest that INVs have diverse membrane origins and are likely to have a number of destinations. We conclude that INVs are a generic class of transport vesicle which transfer cargo between these varied locations.

M46

**Analysis of Retromer Complex Dynamics on Supported Lipid Bilayers**

**C. L. Deatherage**[1], J. Nikolaus[2], E. Karatekin[2], C. G. Burd[1]; [1]Yale University, New Haven, CT, [2]Yale University, West Haven, CT.

In the endocytic system, vesicles from the plasma membrane containing lipids and integral membrane proteins fuse with sorting endosomes. There these cargoes are sorted and delivered back to the plasma membrane or to other organelles by the process of retrograde trafficking.The retromer complex, a trimeric (proteins Vps35, Vps26, and Vps29) endosomal sorting device , is a key component of retrograde trafficking and recognizes and sorts specific cargoes to multiple destinations. Despite the unequivocal importance of retromer in retrograde trafficking, the mechanism of how retromer sorts and packages cargo into retrograde sorting vesicles is unknown. It has been proposed that retromer sorting (i.e. cargo concentration/clustering) is facilitated first by intrinsic oligomerization of the retromer complex bound to cargo, which would begin to drive cargo into nascent sorting tubules. Further enrichment/concentration is proposed to be induced through an interaction with the WASH complex member Fam21, which has 21 retromer binding sites.We reconstituted the retromer coat complex assembly on a supported membrane bilayer, which allowed us to systematically probe the role of Fam21 and retromer interaction. In the reconstituted system we have both the membrane and the essential

proteins of the retromer cargo-sorting complex, allowing us to investigate how these components interact and assemble on a supported bilayer using TIRF microscopy. When associated with the membrane, retromer exists as a distribution of oligomers, with a mean of eight subunits. Surprisingly, despite the presence of multiple retromer binding sites in Fam21, direct interaction between Fam21 and retromer on the bilayer does not influence the distribution of retromer oligomers. In addition, the oligomer distribution of retromer was not influenced by the presence of accessory coat factors that bind retromer, including Rab7-GTP, Sorting nexin 3 (Snx3), or cargo. These data suggest that, contrary to long-standing hypotheses, retromer does not substantially oligomerize on a membrane, either by itself, or in the presence of cargo, accessory factors, or the Fam21/WASH complex.

M47

**CMEpi, a Potent and Selective Structure-based Inhibitor of Clathrin-mediated Endocytosis**

**Z. Chen**, R. Mino, M. Mettlen, P. Michaely, M. Bhave, D. K. Reed, S. L. Schmid; UTSouthwestern Medical Center, Dallas, TX.

Clathrin-mediated endocytosis (CME) is a predominant route of entry into cells via the formation of clathrin-coated vesicles. The major coat proteins are clathrin and AP2. Tools to address CME function, especially a specific inhibitor that works by rapid and well-defined mechanism, are in great demand. Here we revealed that overexpression of N-terminal fragments of the clathrin heavy chain encoding the terminal domain (TD) with or without the distal leg potently and specifically inhibit CME and CCP dynamics, primarily through the interference of AP2 and SNX9 functions. Furthermore, we designed small membrane-penetrating peptide mimics of the binding surfaces on the TD and observed differential inhibitory abilities. A peptide corresponding to the W-box motif binding site was identified as the strongest CME inhibitor, also by the mechanism of inhibiting AP2 and SNX9 functions. This peptide inhibitor, which we named CMEpi, does not affect other endocytic pathways and even other clathrin-dependent trafficking event, such as AP1-dependent Golgi trafficking.

M48

**Exocyst Conformational Changes Control Interactions with SNARE and Sec1/Munc18 Proteins**

**D. Lepore**[1], M. Feyder[1], L. Martinez-Nunez[1], L. Kenner[2], A. Czuchra[1], G. Rossi[3], P. Brennwald[3], A. Frost[2], M. Munson[1]; [1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, [2]Department of Biochemistry and Biophysics, University of California, San Francisco, CA, [3]Department of Cell Biology and Physiology, University of North Carolina School of Medicine, Chapel Hill, NC.

Membrane trafficking is an essential eukaryotic process necessary for a variety of cellular functions including, but not limited to, growth, cell division, motility, and signaling. The trafficking and fusion of cargo-containing vesicles with organelles and the plasma membrane is tightly regulated to ensure that proteins and lipids are delivered to their correct cellular destination. SNARE-mediated fusion is regulated by both Sec1/Munc18 (SM) proteins and multi-subunit tethering factors. The exocyst is a conserved, hetero-octameric tethering complex that is essential for exocytosis. Several of the exocyst subunits interact directly with SNAREs and the SM protein Sec1, however it remains unclear how the full complex coordinates these interactions to regulate SNARE complex formation and membrane fusion. Intriguingly, we found that the assembled exocyst octamer interacts only weakly with SNAREs and Sec1, despite previously observed robust SNARE/Sec1 binding to individual recombinant exocyst subunits. We

purified exocyst subcomplexes (modules) and showed that a module of exocyst has increased apparent affinities for SNAREs and Sec1, suggesting that SNARE/SM binding sites are occluded within the full exocyst complex. As we cannot detect the presence of this module in wild-type yeast, we propose that the octameric complex undergoes regulated conformational changes to interact with SNAREs/Sec1. We demonstrate that gain-of-function mutations of the exocyst subunit Exo70, previously shown to rescue the *cdc42-6* allele and *rho3Δ*, "activate" the complex by stimulating SNARE binding without disassembling the complex. These activated exocyst complexes have an altered structure, as shown by negative stain electron microscopy, revealing that the SNARE/Sec1 binding subunit Sec6 is more dynamic and accessible than in the wild-type complex. We conclude that conformational changes of the exocyst complex regulate interactions with SNAREs and Sec1 to control SNARE-mediated membrane fusion during exocytosis.

M49

**Revealing the Mechanism that Controls Fusion Pore Dynamics in Giant Secretory Vesicles**

**T. Biton**, K. Kumari, N. Scher, E. D. Schejter, B. Shilo, O. Avinoam; Weizmann Inst Science, Rehovot, ISRAEL.

Secretion by exocytosis occurs in every living cell and is essential for many cellular processes including metabolism, signaling and trafficking. To accommodate different cargos and cellular needs, exocytic vesicles are produced across multiple scales ranging from synaptic vesicles that are smaller than 50 nm in diameter to giant exocrine vesicles that reach up to a few µm in diameter. The later typically secret viscous cargos, such as digestive enzymes from pancreatic acinar cells and surfactant proteins from type II lung cells. Nevertheless, to enable cargo release these different types of vesicles all dock and fuse with the plasma membrane. Yet, how vesicle size affects docking and fusion is poorly characterized on the mechanistic level. It is well established that during exocytosis of small vesicles, vesicle fusion can proceed in one of two morphological scenarios: In the first a fusion pore opens and expands irreversibly, leading to complete incorporation of the vesicular membrane to the cell membrane. The second is a "kiss-and-run" scenario, when the fusion pore "flickers", opening briefly and collapsing back, separating the two membranes. Here, we aimed to elucidate how fusion of giant vesicles progresses. We used the *D. Melanogaster* salivary gland, which secretes an adhesive protein through vesicles that are 5-8 µm in diameter, as a model. We visualized the secretion process using super resolution live-gland imaging and observed that unlike smaller vesicles, the fusion pore between giant vesicles and the membrane initially expand but then stabilize with a wide opening reaching up to 3 µm and subsequently constricting back down to hundreds of nm or less. Since constricting a membrane tube requires considerable energy input, we hypothesized that a dedicated protein machinery mediates this phenomenon. To identify this machinery we used the enormous power of Drosophila genetics to perform a candidate gene based screen and identified several conserved proteins from the BAR domain superfamily that act as key regulators of pore dynamics. This research has wide reaching implications on our understanding of exocrine secretion, leading to new and exciting insights into the molecular mechanism of membrane fusion and the regulation of membrane homeostasis in secreting cells.

## Minisymposium 5: Quantitative Approaches to Cell Biology

M50

**Mechanism of Mismatch Tolerance Difference between Rad51 and Dmc1 in Homologous Recombination**

**J. Xu**, L. Zhao, R. Liang, C. Chen, H. Wang; Tsinghua University, Beijing, CHINA.

Homologous recombination is an important pathway for double-stranded DNA break repairing to ensure the integrity of a cell's genetic information. In eukaryotic cells, Rad51 and Dmc1 are two homolog proteins that also play central roles in chromatin genetic recombination in meiosis. While the two proteins share similar overall structure, they have distinct properties to tolerant mismatches in DNA pairing. Rad51 can only catalyze recombination of perfect-matched DNA molecules but Dmc1 can catalyze recombination of DNA molecules with certain degree of mismatch. In order to understand the mechanism of the difference between the two recombinases, we employed cryo-EM analysis of the Rad51-DNA and Dmc1-DNA complexes at near atomic resolution respectively and discovered essential structural elements responsible to the mismatch tolerance difference. Further analysis with single molecule FRET to monitor the DNA capture and strand-exchange activities of the recombinases and related mutants revealed distinct kinetic differences among them, providing new insights into the homologous recombination during meiosis of eukaryotic cells.

M51

**Effects of Phase Separation on Dynamics of Polycomb Proteins Revealed by Live-cell Single-molecule Imaging**

**X. Ren**; University of Colorado Denver, Denver, CO.

Liquid-liquid phase separation (LLPS) has been shown to be involved in multiple cellular processes and functions, including transcription. Polycomb group (PcG) proteins, master regulators of development and differentiation, are silencing machineries that act directly on specific chromatin regions. In the nucleus, PcG proteins form liquid-like condensates in a multitude of cell types. PcG condensates are the physical sites for silencing PcG targets. Recently, we demonstrated that CBX2, one of PcG proteins, undergoes phase separation to form liquid-like condensates that concentrate DNA and nucleosomes. Here, we use live-cell single-molecule imaging to unravel that LLPS facilitates the target-search dynamics of PcG proteins while having minimal effects on its binding stability on chromatin, thereby achieving a high chromatin-bound level. The accelerated target-search process is due to a reduction in both the 3D free diffusion time and the number of non-specific interactions. These results reveal fundamental mechanisms underlying that LLPS achieves efficient transcriptional control through accelerating the target-search dynamics.

M52

**Morphologically Discrete ER Subdomains Support Synthesis of Different Types of Protein**

**H. Choi**[1], Y. Liao[1], Y. J. Yoon[2], J. Grimm[1], L. D. Lavis[1], R. H. Singer[1,2], J. Lippincott-Schwartz[1]; [1]Janelia Research Campus, Ashburn, VA, [2]Albert Einstein College of Medicine, Bronx, NY.

The endoplasmic reticulum (ER) has a complex morphology comprised of stacked sheets, tubules, and three-way junctions, which together function as a platform for protein synthesis of membrane and

secretory proteins. It is widely believed that specific ER subdomains are spatially organized for protein synthesis activities, but precisely where these domains occur in relation to the ER's overall architecture is unclear due to lack of functional-spatial resolution. Here, we use single-molecule tracking of ribosomes and mRNAs in combination with simultaneous imaging of ER to assess sites of membrane protein synthesis in the ER. We found that ribosomes were widely distributed throughout the different ER morphologies. However, synthesis of membrane proteins (including type I, II and III) and an ER luminal protein occurred primarily at three-way junctions. Unlike mRNAs coding for membrane proteins, mRNA coding for the translocon-independent, tail-anchored protein Sec61β was found primarily on ER tubules and was actively translated on the ER. Surprisingly, mRNA coding for the cytosolic protein β-actin was also primarily associated with ER tubules. These results support the idea that discrete ER subdomains exist to support specific types of protein synthesis activities, with ER morphological features playing an important role in this organization

M53

**Super-Resolution Microscopy Elucidates Curvature Generation by Endocytic Clathrin Coats in Live Cells and Tissues**

N. Willy[1], J. Ferguson[1], S. Silahli[1], C. Cakez[2], F. Hasan[1], H. Chang[3], A. Travesset[4], R. Zandi[5], S. Li[5], D. Li[6], E. Betzig[7], E. Cocucci[1], **C. Kural**[1]; [1]The Ohio State University, Columbus, OH, [2]University of New Mexico, Albuquerque, NM, [3]Purdue University, West Lafayette, IN, [4]Iowa State University, Ames, IA, [5]University of California, Riverside, Riverside, CA, [6]Chinese Academy of Sciences, Beijing, CHINA, [7]University of California, Berkeley, Berkeley, CA.

Clathrin-mediated endocytosis is the most extensively studied internalization mechanism of membrane lipids and proteins from the cell surface. Over the past decades, a multitude of biophysical and biochemical methodologies have been employed to elucidate structural and dynamic properties of endocytic clathrin coats. However, fundamental aspects of clathrin-mediated endocytosis remain controversial due to the lack of experimental approaches that allow correlation of ultra-structural and dynamic properties of clathrin-coated structures. Using electron micrographs, it was originally proposed that clathrin initially grows into a flat array (i.e., clathrin plaques) on the plasma membrane prior to transitioning into a curved coat. Flat-to-curved transition of clathrin coats during endocytic vesicle formation was rejected by others as it requires a substantial structural rearrangement, which is energetically unfavorable. As an alternative, it was suggested that curved clathrin-coated structures form gradually without a major structural rearrangement. In this study, we used structured illumination microscopy in the total internal reflection mode to monitor curvature formation by clathrin coats during assembly of individual endocytic complexes within cultured cells and tissues of developing metazoan organisms. Our findings very clearly demonstrate that endocytic clathrin coats acquire curvature without a major flat-to-curved transition that requires an extensive reorganization of the clathrin lattice. Altogether, our results signify the importance of employing methodologies comprising high resolution in both spatial and temporal dimensions for constructing dynamic models.

M54

**Self-organization and Load Adaptation by the Mammalian Endocytic Actin Network: Integrating Modeling with Experiment**

**M. Akamatsu**[1], R. Vasan[2], D. Serwas[1], M. Ferrin[1], P. Rangamani[2], D. G. Drubin[1]; [1]UC Berkeley, Berkeley, CA, [2]UC San Diego, La Jolla, CA.

Force generation due to actin assembly is a fundamental aspect of membrane sculpting for many essential processes. We hypothesized that the spatial restriction of actin filament nucleators to the base of the pit and actin/membrane linkages in the clathrin coat might dictate how endocytic actin networks organize, produce force, and adapt to elevated membrane tension. Here, we use a multiscale computational model constrained by experimental measurements to show that a minimal branched actin network is sufficient to internalize endocytic pits against physiological membrane tension. A parameter sweep identified the number of Arp2/3 complexes as particularly important for robust internalization, which prompted the development of a molecule-counting method in live mammalian cells. From engineered GFP-tagged multimeric proteins of defined copy number, we constructed an intracellular calibration curve relating fluorescence of endogenously GFP-tagged proteins to numbers of molecules in live cells. A peak of ~200 molecules of Arp2/3 complex assemble at endocytic sites marked by the clathrin adaptor AP2-RFP in human induced pluripotent stem cells. Our simulations also revealed that actin networks self-organize in a radial branched array with barbed filament ends oriented to grow toward the base of the pit, and that the distribution of linker proteins around the endocytic coat is critical for this organization. Surprisingly, our model predicted that long actin filaments bend from their attachment sites in the coat to the base of the pit and store elastic energy that can be harnessed to drive endocytosis. This prediction was validated using cryo-electron tomography on cells, which revealed the presence of bent actin filaments along the endocytic site. Furthermore, we predict that under elevated membrane tension, the self-organized actin network directs more growing filaments toward the base of the pit, increasing actin nucleation and bending for increased force production. Thus, our study reveals that spatially constrained actin filament assembly utilizes an adaptive mechanism that enables endocytosis under varying physical constraints. We anticipate that the principles of self-organization and load-adaptation uncovered in this study will apply to other cellular membrane-deforming processes driven by the cytoskeleton.

M55

**Intrinsic Constraint of the Phenotypic Plasticity of the Actin Cytoskeleton Reveals Limited Attractor States**

**P. W. Gunning**[1], N. S. Bryce[1], T. W. Failes[1], J. R. Stehn[1], K. Baker[2], S. Zahler[3], I. Dedova[1], G. M. Arndt[1], B. T. Goult[2], E. C. Hardeman[1], J. G. Lock[1]; [1]University New South Wales, Sydney, AUSTRALIA, [2]University of Kent, Canterbury, UNITED KINGDOM, [3]Ludwig-Maximilians-University, Munich, GERMANY.

Numerous proteins and pathways regulate F-actin organisation. In combinatorial terms, this suggests that an almost unlimited number of regulatory states are conceivable. Consequently, the potential for plasticity in F-actin phenotypes appears virtually unbounded. To estimate the actual degree of F-actin phenotype plasticity quantified by 74 parameters, we used a library of 114,400 structurally diverse compounds to induce unbiased chemical perturbations. Remarkably, we estimated the number of recurrent F-actin phenotypes that emerged to be approximately 25. This predicts that compounds with distinct molecular mechanisms induced equivalent phenotypes, suggesting that these recurring

phenotypes reflect a low number of equilibrium or attractor states in actin organisation. Compounds which produce a Latrunculin A-like F-actin phenotype use at least three different pathways. Similarly both a talin activation phenotype and an anti-tropomyosin Tpm3.1 phenotype are produced by compounds using multiple pathways. This was further supported by dynamic analyses comparing phenotype trajectories over time, showing how initially divergent phenotypes ultimately converged into equivalent end-states. We propose that infrequent attractor states in the actin phenotypic landscape reflect a channelling of high perturbative diversity into low phenotypic variety. The existence of such attractor states may have provided a mechanism to suppress chaotic outcomes during the evolution of this complex, functionally integral system.

M56

**Dynamically Heterogeneous Plasma Membrane Is Poised for Initiation of Receptor-mediated Mast Cell Signaling**

N. Bag, D. Holowka, **B. Baird**; Cornell University, Ithaca, NY.

Cell surface receptors distinguish specific stimuli from biological noise, and underlying mechanisms depend on the resting steady-state organization of plasma membrane components. The nanoscale distribution of signaling proteins, including kinases and phosphatases, is coordinately rearranged within the poised membrane after the receptor is stimulated by extracellular ligand, such that changes in relatively weak interactions serve to transmit the signal across the membrane. Central to membrane organization are dynamically heterogeneous features such as cortical actin meshwork and asters, ordered proteo-lipid nanodomains, and protein clusters, any or all of which may slow the diffusion of membrane components. Correspondingly, microscopic diffusion of structurally and functionally distinct components provide the means for examining nanoscale organization in resting and stimulated cells. We evaluated three key components in the early stage of mast cell signaling by measuring their diffusion in live RBL cells: FcεRI receptor for immunoglobulin E (IgE), tyrosine kinase Lyn anchored to the membrane inner leaflet, and transmembrane phosphatase PTPα. We exploited the statistical robustness of multiplexed Imaging Fluorescence Correlation Spectroscopy (ImFCS), initially to evaluate subtle diffusion differences of these proteins in resting cells. We found that interactions between its saturated lipid anchor and Lo-like lipid nanodomains govern the diffusing distribution of Lyn, which also undergoes protein-based interactions. Diffusion of transmembrane proteins, IgE-FcεRI and PTPα appears limited primarily by protein-based interactions. Antigen-crosslinking of monomeric IgE-FcεRI causes their nanoclustering and consequent stabilization of a locally ordered membrane environment. ImFCS data show that this subtly reorganized region acts as a spatial filter to facilitate co-localization of Lyn through its favorable lipid-based interactions and, simultaneously, segregation of PTPα based on unfavorable interactions of its transmembrane domain with ordered lipids and protein-based steric exclusion. Supporting this view, a Lyn chimera with unsaturated lipid anchor does not couple with antigen-crosslinked IgE-FcεRI, whereas a PTPα anchored to the membrane by saturated lipids does couple. These observations underscore the pivotal importance of organized plasma membrane heterogeneity in the poised resting state, thereby enabling orchestration of critical protein-protein interactions during stimulated transmembrane signaling.This research is supported by NIH R01GM117552.

M57

**Structural organization of caveolin-1 8S oligomers determined by cryo-electron microscopy**

B. Han[1], J. Porta[2], E. Binshtein[3], E. Karakas[3], M. Ohi[2], **A. Kenworthy**[1]; [1]University of Virginia School of Medicine, Charlottesville, VA, [2]University of Michigan Life Sciences Institute, Ann Arbor, MI, [3]Vanderbilt University School of Medicine, Nashville, TN.

Cholesterol-rich membrane invaginations known as caveolae play a critical role in membrane buffering, mechanotransduction, and cellular signaling. The integral membrane protein caveolin-1 (Cav1) is essential for caveolae formation in eukaryotic cells and can even drive the formation of caveolae-like structures in bacteria. Cav1 contains an unusual hairpin shaped intramembrane domain predicted to bend caveolar membranes by a wedging mechanism and oligomerizes to form 8S complexes that function as the fundamental building blocks of caveolae. However, the molecular architecture of these complexes is currently unknown. To fill this gap in knowledge, we used negative staining and single particle cryo-electron microscopy to determine the structure of Cav1 8S oligomers. By negative staining we show that 8S complexes forms discs that are flat on one side, contain a central protrusion on the other, and vary in diameter. The diameters differ by a fixed amount, suggesting that they contain different numbers of Cav1 monomers. Using Venus as a fiduciary marker, we show the flat side of the disc corresponds to the membrane-facing side of the protein and the C-terminus of Cav1 is localized to the central protrusion. We also generated a ~10 Å resolution structure of the 8S Cav1 complexes using cryo-electron microscopy. At this resolution the discs are reminiscent of a wheel, consisting of an outer ring connected by spokes to an inner protruding ring. Interestingly, the intramembrane domains of Cav1 monomers are localized to the outermost portion of the wheel. Current work is focused on improving the resolution of 8S complex structure in order to build a more detailed molecular model of the complex and integrating a variety of biochemical, biophysical, and structural techniques to dissect how the 8S complexes form. Ultimately, these studies should provide a structural framework for understanding how caveolae assemble and control cellular functions.

M58

**Live-cell Imaging and Analysis of the Plasma Membrane Dynamics During Clathrin-mediated Endocytosis by High-speed Atomic Force Microscopy**

**A. Yoshida**[1], N. Sakai[2], N. Takahashi[1], S. H. Yoshimura[3], Y. Ohba[1]; [1]Department of Cell Physiology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, JAPAN, [2]R&D Group, OLYMPUS Corporation, Tokyo, JAPAN, [3]Laboratory of Plasma Membrane and Nuclear Signaling, Graduate School of Biostudies, Kyoto University, Kyoto, JAPAN.

Clathrin-mediated endocytosis (CME) is a process through which cells incorporate extracellular materials and membrane proteins into the inside of cells. Such a process is accompanied by a series of morphological changes of the plasma membrane, which is mediated by a successive assembly of many protein components. Although the protein assembly has been elucidated by fluorescence microscopy, dynamic changes in the plasma membrane morphology during CME have not been fully clarified in living cells. We have previously developed a combined system of a fast-scanning atomic force microscope and a confocal microscope and performed simultaneous imaging of morphological changes of the plasma membrane and protein dynamics in living cells. Through this observation, we have revealed that actin polymerization at a clathrin-coated pit (CCP) provides a major force to drive membrane protrusion and push vesicle downward for irreversible closing of CCPs in an Arp2/3 complex-dependent manner

(Yoshida et al., PLoS Biology, 2018). In this study, with the use of the combined system, the dynamic changes of plasma membrane morphology during CME in the presence of extracellular stimuli were studied. When serum-starved Cos-1 or A431 cells that stably express Venus-fused clathrin-light chain A were stimulated by EGF, a division and frequent appearance of CCP were observed. This resulted in the clustering of CCPs in the limited area of the membrane with a diameter of 800-1600 nm. This phenomenon was conserved among cell lines because it was observed in both Cos-1 and A431 cells. The clustering of CCP was suppressed by the treatment with EGF receptor inhibitors. Furthermore, upon the closure of CCPs in EGF-stimulated cells, it was frequently observed that membrane protrusion collectively covered and capped the clustered CCPs. Taken together, these results indicate that there exists a domain on the plasma membrane, where CME frequently emerges, and the formation of such the membrane domain is maintained by EGF receptor activity. In conclusion, contrary to the previous assumption that clathrin assembly is induced by ligand binding to the receptors, cells appear to have a “pre-opened gate” for coming extracellular materials to be incorporated.

M59

**Quantifying Cell Biology: Beyond Human Vision**

**A. E. Carpenter**; Broad Institute of Harvard and MIT, Cambridge, MA.

Microscopy images contain tremendous information about the state of cells, tissues, and organisms. Often, biomedical researchers use software to identify biological structures and extract metrics of interest from cell images; they quantify phenomena in conventional microscopy experiments as well as high-throughput screens to test drugs in disease model systems. We have developed computational approaches and open-source software, including CellProfiler, for this, bridging the gap between biologists’ needs and the latest computational science.Here, we describe going beyond human vision and biologists' existing hypotheses in a strategy called image-based profiling. Instead of measuring individual phenotypes that biologists already know are relevant to a particular disease, we stain many cellular components and extract thousands of morphological features from each cell’s image, often using an assay called Cell Painting and computational approaches such as deep learning. We then harvest similarities in these “profiles”, analogous to transcriptional profiling but less expensive and, surprisingly, more information-rich.Through this approach, we discover that unlabeled cells can be classified for their cell cycle and mitotic stage without any DNA labeling. We find that leukemia can be detected in unlabeled cells, eliminating the need for specific biomarker-based reagents. We find that degradation of red blood cells during blood bank storage can be detected, eliminating the need for tedious expert counting. We find that similarities in images of cells that overexpress genes contain sufficient information to determine unstudied genes' pathway identity and relationships. We identify cell biological impacts of rare disease alleles and mental illnesses. We find that cancer-associated alleles can be classified based on their functional impact without creating individual functional assays for each gene. Overall, cell images should now be considered a source of powerful data about cell state.

## Minisymposium 6: Cell Shape, Cytoskeletal Mechanics and Mechanosensing

M60

**Modular Lim Domains Are Direct Sensors of Actin Strain to Mediate Mechanotransduction**

**X. Sun**[1], D. Phua[1], L. Axiotakis, Jr.[2], M. Smith[3], A. Pasapera[2], R. Gong[1], R. Cail[2], S. Espinosa[1], C. Waterman[2], M. Beckerle[3], G. Alushin[1]; [1]Rockefeller University, New York, NY, [2]National Heart, Lung, and Blood Institute, NIH, Bethesda, MD, [3]Huntsman Cancer Institute, University of Utah, Salt Lake City, UT.

The LIM domain is a protein-interaction module that is implicated in cellular mechanosensation through the actomyosin-adhesion system. However, the molecular mechanism by which LIM proteins transduce mechanical signals remains elusive. Motivated by the hypothesis that mechanical deformations of actin filaments could serve as an upstream signal in mechanotransduction, we performed an imaging-based screen for LIM-domain proteins that accumulate on stress fibers in cells exposed to cyclic stretch ("mechanoaccumulate"). Three LIM-domain protein families, FHL, paxillin, and zyxin, emerged as mechanosensors. We identified a highly conserved phenylalanine present in mechanosensor LIM domains, and demonstrated that mutating this phenylalanine in each LIM domain abolishes the mechanoaccumulation of LIM proteins on stress fibers. We developed an in vitro actin force reconstitution assay wherein actin filaments are exposed to forces generated by surface-immobilized myosin motors, which reveals that strain in single actin filaments is necessary and sufficient for FHL, paxillin, and zyxin binding. FHL2 is a transcriptional coactivator which has been reported to translocate to the nucleus in soft environments to upregulate its transcriptional targets. We hypothesized that force-induced conformational changes in F-actin could generate binding sites for FHL2 in stiff microenvironments, serving as a platform for retaining FHL2 in the cytoplasm and preventing its nuclear shuttling. We found that our FHL2 mutant exhibits enhanced nuclear enrichment compared with wild-type in cells plated on stiff substrates. Furthermore, live cell imaging reveals nuclear translocation of wild-type FHL2 upon cytochalasin D treatment to disrupt F-actin and its return to the cytoplasm upon drug washout, consistent with our hypothesis. Our results demonstrate a novel mechanotransduction mechanism mediated by the direct binding of FHL proteins to strained actin.

M61

**Identification of Context-specific Force-sensitive Protein Complexes Within Focal Adhesions**

**A. Tao**, A. LaCroix, B. Hoffman; Duke University, Durham, NC.

Mechanical cues, such as applied loads or extracellular matrix (ECM) stiffness, activate specific cell signaling pathways to affect key cellular processes, such as migration and differentiation. Cellular detection of these physical cues is thought to be mediated by protein conformation changes and the formation of key force-induced protein complexes within focal adhesions (FAs), subcellular structures that mediate physical connections between the ECM and force-generating cytoskeleton. As the key constituents that comprise force-induced protein complexes are unknown, we developed a novel technique to identify them by probing for proteins, or phosphorylation states, that selectively localize to areas of high tension. We achieve this by combining a FRET-based molecular tension sensor that reports the loads across a key mechanical linker protein, vinculin, with standard immunofluorescence protocols to probe for protein localization and phosphorylation states. Using this technique, we identified ten FA proteins and six phosphorylation states that are preferentially and specifically recruited to areas of high vinculin tension, suggesting that they form different force-induced protein complexes. Furthermore, as FAs are diverse structures that contain vinculin in various biochemically-distinct and mechanically loaded states, we hypothesized that differential force-induced protein complexes form in various FA subclasses. To test this idea, we developed a machine learning algorithm to identify two FA subclasses, those associated with stress fibers or protrusions, and searched for distinct vinculin-based force-induced

protein complexes. Interestingly, nine proteins and two phosphorylation states exhibited differential force sensitive protein complex formation based on their associated FA class. In total, this work develops and uses a new imaging-based approach to demonstrate the existence of context-specific force-induced signaling capabilities amongst different FA proteins. Our observation that vinculin forms a variety of force-induced protein complexes in various FA subclasses suggests that vinculin load may activate different mechanosensitive signaling pathways in various adhesions. Indeed, preliminary experiments suggest that force-induced protein complexes that form in different FA subclasses are required for directed migration in response to aligned ECM.

M62

**Mechanical Force Promotes the Dissociation of Arp2/3 Complex Branches**

**N. G. Pandit**, W. Cao, J. Bibeau, E. Johnson-Chavarria, T. D. Pollard, E. M. De La Cruz; Yale University, New Haven, CT.

The branched actin network, primarily comprised of actin and the Arp2/3 complex, generates and sustains force to power cellular functions like motility, endocytosis, and vesicle trafficking. During assembly of the branched actin network, it experiences variable resistance from the cell membrane and responds by adapting it's growth speed, power, and architecture both in vitro and in cells. The network also experiences mechanical force during disassembly but much less is known about how forces affect the disassembly of actin filament networks. Here, we examine how mechanical forces affect disassembly of branched actin networks and focus on the dissociation rate of Arp2/3 complex branches. We used microfluidics to apply force to branches formed from purified actin and Arp2/3 complex and observed debranching events in real time with TIRF microscopy. We found that low pN forces on branches dramatically accelerated the dissociation of branches from the mother filament. We find that the Arp2/3 complex exists in distinct mechanical states and each mechanical state has different sensitivity to force. The mechanical states and sensitivity to force is regulated by the ATPase cycle of the Arp2/3 complex. Interestingly, we also show that the ATPase cycle of the Arp2/3 complex also regulates it's interactions with debranching proteins such as Glia maturation factor (GMF). These observations suggest that the ATPase cycle of the Arp2/3 complex tunes sensitivity to debranching by force and regulatory proteins. These findings have broad implications for cell biology because they reveal a mechanism were older portions of the branched actin network could be specifically targeted for disassembly by force and other debranching proteins.

M63

**Pi-3 Kinase Drives Adaptive 3D Migration by Polarizing Large Pressure-based Protrusions**

**E. Welf**, M. Driscoll, A. Weems, K. Dean, R. Fiolka, G. Danuser; UT Southwestern Medical Center, Dallas, TX.

A hallmark of highly metastatic cells is the ability to navigate complex and diverse microenvironments. Cells with this capability often switch between motility modes, utilizing both actin-driven and pressure-driven protrusions to find or make empty spaces in dense extracellular matrices. In contrast to the belief that actin and pressure-based protrusion operate independently, we have discovered in this study that actin polymerization plays a critical role in reinforcing pressure-based protrusions to generate large blebs that push and remodel a 3D collagen network to create space for invading melanoma cells. We found that this localized protrusion reinforcement occurs through spatially restricted PI-3 Kinase (PI3K)

signaling that drives actin polymerization through Rac1. By using a combination of high resolution light sheet microscopy along with 3D analytical tools, we show that regions of high PI3K create larger blebs. These blebs are reduced in size and redistributed in space under PI3K inhibition. By using photoactivatable forms of PI3K and Rac1, we found that bleb size can be increased by local activation of both PI3K and Rac1. Melanoma cells use these large pressure-based protrusions to dig tunnels through the soft but dense collagen network. Surprisingly, we found that this tunneling process does not require extracellular protease activity, but employs paxillin-containing adhesions along with repeated pressure-based "punches" to burrow through the network. Formation of adhesion structures at the front of these tunnels also localizes PI3K signaling to organize the large protrusions at the front of the cell where greatest contact occurs between adhesions and collagen. Classically, PI3K is thought to drive actin-based protrusion by recruiting Rac activators that generate a branched actin network. We also found actin inside growing blebs, suggesting that actin polymerization through Rac1 and PI3K drives bleb growth. This surprising result calls into question the very discrimination of actin-based and pressure-based protrusions and suggests that all protrusions may share common mechanisms. Furthermore, identification of PI3K as a common polarity cue explains how highly metastatic cells are able to switch protrusion mechanisms while maintaining directional polarity - cells use the same tools in different ways in order to switch protrusion mechanisms quickly when confronted with diverse microenvironments.

M64

**Coordination of Actomyosin Contractility and Mitochondrial Positioning During Interstitial Neutrophil Migration in Live Anesthetized Animal.**

**N. Melis**[1], B. Subramanian[1], D. Chen[1], W. Losert[2], C. Parent[3], R. Weigert[1]; [1]Laboratory of Cellular and Molecular Biology, Center for Cancer Research, National Cancer Institute – National Institutes of Health, Bethesda, MD, [2]Department of Physics, Physical Sciences Complex, University of Maryland, College Park, MD, [3]Department of Pharmacology, University of Michigan, Ann Arbor, MI.

Cell migration is a fundamental biological process in which membrane remodeling is a critical step occurring as a result of a constant re-arrangement of the actin cytoskeleton. Neutrophil migration has been particularly studied due to their role in the immune response, and because their immune function is directly associated to their ability to migrate. Neutrophil migration induces extensive plasma membrane remodeling through a combination of protrusion generated by actin polymerization at the leading edge of the cell and retraction generated by non-muscle myosin II A (NMIIA) contraction at the cell uropod. In addition, membrane remodeling is energetically unfavorable and has to be supported by an adjustment of the cellular metabolic activity.Most of our knowledge on membrane remodeling comes from *in-vitro* 2-D model systems and only very limited work has been performed in more complex models or *in-vivo*. To this end, here we use intravital subcellular microscopy coupled to a computational analysis in 3D to understand the underlying mechanisms of the coordination among plasma membrane remodeling, actomyosin cytoskeleton and cell metabolism during interstitial neutrophil migration in the mouse ear. In our model, migrating neutrophils exhibit a very active and dynamic membrane remodeling with a continuous formation of micron-scale membrane protrusions, which interact with the tissue microenvironment (i.e. extracellular matrix (ECM)). Differently from what has been previously described, we observe that NMIIA is not only present at the uropod of the cell but also at the leading edge and in large lateral protrusions. In these new locations, NMIIA is not actively retracting membrane, and its localization is correlated with the architecture of the ECM. Furthermore, NMIIA recruitment at the leading edge is RhoA/ROCK independent, possibly indicating a new role for NMIIA in a machinery

involved in sensing the ECM and a new mode of recruitment of NMIIA in neutrophil.Finally, we observe that mitochondria constantly reposition throughout the cells at the sites of NMIIA assembly, most likely in order to supply the ATP required to sustain the contractile activity, and change their polarization status during the various phases of migration. In summary, we have highlighted a novel correlation between mitochondria function and the dynamic rearrangement of the actomyosin complex *in-vivo*.

M65

**Mechanistic Insights Into Actin-based Force Generation During Clathrin-mediated Endocytosis Via *in Situ* Cryo-Electron Tomography**

**D. Serwas**[1], M. Akamatsu[1], K. M. Davies[2], D. G. Drubin[1]; [1]UC Berkeley, Berkeley, CA, [2]Lawrence Berkeley National Laboratory, Berkeley, CA.

Networks of interconnected actin filaments act in essential cellular processes, for example by providing force generated through polymerization. One of these processes is clathrin-mediated endocytosis (CME), a major pathway for down-regulation of signaling receptors and uptake of extracellular material, that involves a series of plasma membrane remodeling steps resulting in the formation of clathrin-coated vesicles (CCVs) containing cargo.Structural aspects of CME have been extensively studied by transmission electron microscopy of platinum replicas, and of thin sections of plastic embedded cells. However, both of these sample preparation methods have been shown to alter actin cytoskeleton organization. As a result, precise structural information on actin organization in CME, which is key to understanding actin's mode of function in this process, is lacking.Here, we made use of recent technological advances in cryo-electron tomography (cryo-ET) to discover the 3-dimensional actin cytoskeleton organization in mammalian CME at near-native state conditions. Combined with mathematical modeling, this analysis promises to reveal how actin functions in CME.We found growth conditions for SK-MEL-2 cells that allow cryo-ET of intact cells. Strikingly, individual actin filaments, actin branches, CME sites and CCVs can clearly be identified in our tomography data. The actin cytoskeleton associated with CME sites and CCVs consists of branched as well as unbranched filaments. Filaments at CME sites are not exclusively localized around the neck of membrane invaginations as was seen before by platinum replica EM, but rather form more complex 3-dimensional arrangements. We determined filament polarity based on branch point geometry and found an accumulation of plus-ends at positions that would allow for force production by polymerization to support plasma membrane deformation and CCV transport. Moreover, branched filaments do not arise from a common single filament, contradicting the theory that a single mother filament gives rise to the entire CME-associated network. Surprisingly, we also found varying amounts of very long, heavily bent actin filaments at CME sites. Based on mathematical modeling, these filaments are predicted to store elastic energy, which augments force generation by actin polymerization to support membrane deformation and thus represents a previously unrecognized mode of actin-based force production in CME.Taken together, our results reveal the complex actin filament organization at endocytic sites in unprecedented detail. We are in the process of in-depth quantitative analysis of our data, which will, in combination with mathematical modeling, ultimately result in a detailed understanding of actin function in CME.

M66

**Quantifying Non-muscle Myosin 2 Assembly Dynamics in 4D Super-resolution**

**M. A. Quintanilla**[1], H. Wu[1], M. Akamatsu[2], J. R. Beach[1]; [1]Loyola University Chicago, Chicago, IL, [2]University of California, Berkeley, Berkeley, CA.

How cells generate sufficient contractile forces to drive a diverse array of cellular processes is an outstanding question. Non-muscle myosin 2 is a motor protein that hydrolyzes ATP to contract actin filaments, generating the majority of contractile forces in non-muscle cells. To achieve the level of contractility needed for most processes, many myosin 2 filaments are needed. For decades, the only model to explain how a sufficient number of filaments are rapidly assembled was a model in which each filament was built individually. Recent work using high-resolution imaging demonstrated an alternative model in which nascent myosin 2 filaments are amplified by an actin-dependent partitioning process. We believe this partitioning process evolved as a dominant myosin 2 assembly mechanism to rapidly drive physiological levels of contraction - we observe the phenomenon in primary mouse fibroblasts, in 3D culture, on substrates of physiological stiffnesses, and in Drosophila haematopoietic cells. We do not understand the molecular mechanism for this phenomenon. Two mechanisms have been proposed (1) Single-filament partitioning - A single myosin 2 filament (~30 monomers) is ripped in two as dynamic actin fibers separate. (2) Multi-filament partitioning - Two or more myosin 2 filaments (>>30 monomers) are separated from one another as dynamic actin fibers separate. Differentiating these mechanisms is essential for understanding contractile force generation. To do this, we are using complementary methods of molecular counting and correlative light and electron microscopy (CLEM). Using protein nanocages with fixed numbers of subunits and fluorophores, we generated a standard curve of fluorescence with super-resolution imaging. By imaging endogenously-labeled EGFP-myosin 2 with identical conditions, we can interpolate the number of myosin 2 monomers present at the time of partitioning. Our in vitro, fixed-cell, and live-cell imaging suggest that 1) in vitro single filaments are 30 monomers, 2) live-cell bipolar structures can be upward of a single filament, and 3) partitioning events appear to occur at or above that of a single filament. Collectively, this work demonstrates that we can accurately quantify dynamic macromolecular complexes, and that we can use this technique to better understand the mechanism by which cells build contractile forces.

M67

**Actin Mechanics Across Scales, From Molecular Processes to the Control of Cortical Tension**

B. Truong Quang[1], **E. Paluch**[2], R. Peters[2], D. Cassani[1], G. Charras[1]; [1]University College London, London, UNITED KINGDOM, [2]University of Cambridge, Cambridge, UNITED KINGDOM.

A precise control of cell morphology is key for cell physiology, and cell shape deregulation is at the heart of many pathological disorders, including cancer. Cell morphology is intrinsically controlled by mechanical forces acting on the cell surface, to understand shape it is thus essential to investigate the regulation of cellular mechanics. In animal cells, shape is primarily determined by the cellular cortex, a thin network of actin filaments and myosin motors underlying the plasma membrane. We investigate how the mechanical properties of the cell surface arise from the microscopic organisation of the cortex, and how changes in these properties drive cell deformation. We have characterized the localization and orientation of cortical myosin motors using super-resolution microscopy and unveiled that myosin does not fully overlap with actin at the cortex. We propose that this incomplete overlap results from steric effects due to the large size of myosin mini-filaments compared to the meshsize of the cortical actin

network. We then uncovered a number of mechanisms that control the level of overlap between cortical actin and myosin, and thus regulate cortical tension. By combining cell biology experiments, quantitative imaging and physical modelling, we aim to understand the control of cell mechanics, and ultimately cell shape, across scales.

M68

**Short, Minimal Viral Fusogens Hijack the Actin Cytoskeleton to Drive Cell-cell Fusion**

**K. Chan**[1,2,3], S. Son[2,3], E. M. Schmid[2,3], A. L. Arthur[3], A. Bhat[3], J. Morstein[3], D. Schlesinger[3], D. A. Stevens[3], D. A. Fletcher[1,2,3]; [1]UC-Berkeley/UC-San Francisco Graduate Group in Bioengineering, Berkeley, CA, [2]Department of Bioengineering, University of California, Berkeley, Berkeley, CA, [3]Marine Biology Laboratory, Physiology Course, Woods Hole, MA.

Cell-cell fusion is essential for fertilization and tissue development in metazoans, and it is sometimes used by some viruses to form pathological syncytia. Cell-cell fusion is typically driven by fusogenic membrane proteins with tall (>10 nm) ectodomains that are similar to fusogens involved in enveloped viral entry. Their height likely enables them to bridge the gap between the densely-crowded plasma membranes and anchors into the opposing membrane. These fusogens then fold back to bring the two membranes into close contact to drive fusion. However, some fusogenic proteins, such as the non-structural fusion-associated small transmembrane (FAST) proteins from orthoreovirus and aquareovirus, have surprisingly short ectodomains (<2 nm) that reside within the repulsive hydration barrier that prevents membranes from coming in close contact. Yet, expression of FAST proteins in cells is sufficient to drive fusion with naïve neighboring cells, raising the question of how they bring plasma membranes in close contact for fusion. We found that p14, the reptilian reovirus FAST protein, hijacks the actin cytoskeleton to drive cell-cell fusion. Using bioinformatics, in vitro kinase and motility assays, splitYFP cell-cell fusion assay, fluorescence imaging and molecular biology techniques, we found that a tyrosine in the disordered cytoplasmic domain of p14 is important for fusion and is phosphorylated by c-src kinase. Upon phosphorylation, p14 binds to an adaptor protein, Grb2, and subsequently binds to N-WASP to nucleate branched actin assembly. This molecular pathway can be replaced by directly coupling p14 to actin assembly by using a 47-residue peptide to relieve the auto-inhibition of N-WASP. We propose that actin assembly coupled to the cytoplasmic tail of p14 provides localized force generation necessary to overcome the repulsive hydration barrier and to bring the plasma membranes in close contact so that fusion can occur. To investigate if other members of the FAST family also hijack the host actin cytoskeleton to drive cell-cell fusion, we used bioinformatics to screen all known FAST proteins for binding motifs to adaptor proteins. Interestingly, while none contained the same Grb2-binding motif found in p14, other actin-related adaptor proteins were predicted. Preliminary evidence suggests that other FAST proteins could couple to the actin cytoskeleton using these adaptor proteins to drive cell-cell fusion, suggesting a conserved mechanism. This work reveals that overcoming energetic barriers to cell-cell fusion does not require conformational changes of tall fusogens but can instead be driven by locally harnessing the actin cytoskeleton.

M69

**Signal Integration During Leukocyte Chemotaxis in Complex Microenvironments**

**P. J. Sáez**[1], M. Deygas[1], I. Lavi[1], M. Maurin[2], M. Piel[1], P. Vargas[1]; [1]UMR144 Cell biology and Cancer Unit, Institut Curie, Paris, FRANCE, [2]U932 Immunity and Cancer Unit, Institut Curie, Paris, FRANCE.

Migration of leukocytes, including dendritic cells (DCs), is a key process that determines the outcome of the immune response. To efficiently migrate, DCs quickly adapt to the properties of the microenvironment. This adaptation occurs due to high cellular plasticity, which allows fast integration of chemical and physical cues.Upon danger signal detection DCs undergo a maturation process and switch from slow/random migration to a fast/persistent mode, optimizing their locomotion through complex interstitial tissues to reach the lymph vessels. This phenomenon requires cytoskeleton re-organization and non-muscular MyosinIIA (MyoII) $Ca^{2+}$-dependent contractility. In addition, DC maturation increases the expression of CCR7, a chemokine receptor that enables DC chemotaxis. However, how cytoskeleton polarity contributes to couple directionality and speed during chemotaxis remains unclear.Here, we evaluated how increasing the geometrical complexity of the microenvironment affects DC chemotaxis, revealing new essential mechanisms required for 3D migration. By combining live cell imaging with new microfluidic devices, we evaluated how DCs resolve branching to advance in an irregular landscape. To do that, we screened the impact of cytoskeletal components in DC directionality and speed. In 3D gels, we found that disruption of acto-myosin contractility (i.e. low dose of Latrunculin A, MyoII inhibition, ROCK inhibition) specifically decreased DC speed, but did not affect directionality. On the other hand, disruption of microtubule dynamics (i.e. treatment with nocodazole or taxol) diminished the directionality of DCs undergoing chemotaxis. Importantly, the effect of nocodazole over directionality was independent of the effect of microtubule depletion on cell contractility, as nocodazole-treated MyoIIA knock-out DCs still presented a reduced directionality. Noticeably, the effect of nocodazole over directionality was lost in 2D confined environments, where DCs displayed no branching and faced no obstacles.In conclusion we showed that cell branching around physical obstacles introduces a specific constrain, which makes microtubules essential for directional migration. We also found that cell speed and directionality rely on distinct molecular mechanisms during guided migration through complex microenvironments.

## Minisymposium 7: From Stem Cells to Organoid Biology

M70

**Systematic Analysis of the Connectivity and Dynamics of the MAPK Signaling Network Reveals Critical Nodes of Cross-regulation**

**A. F. Peterson**, T. Aikin, S. Regot; Johns Hopkins University, Baltimore, MD.

The Mitogen Activated Protein Kinase (MAPK) family is a conserved signaling network that regulates a diverse array of cell fate decisions. There are three major MAPKs - ERK, JNK, and p38 - controlling different, and often opposing, cell fates. Here, we have used a live-cell imaging approach to measure the activity of each MAPK pathway simultaneously in single cells and in real time. We have probed the role of each mammalian MAP3K and MAP2K in human mammary epithelial cells (MCF10A) using a tet-inducible system and single-cell reporters for P38, JNK, and ERK activity. Using this technique, we have found that high P38 activity often functions cell autonomously to regulate both JNK and ERK activity and non-cell autonomously to promote ERK activity. Interestingly, when all three pathways are

simultaneously activated, lateral ERK signaling is accompanied by increased lateral NF-κB signaling. This research represents the first systematic analysis of the connectivity and dynamics of the MAPK signaling network and its role in regulating cell fate decisions at the single-cell and tissue level.

M71

**Epithelial Cells Act as a Regional Checkpoint for Immune Cell Organization**

**S. Park**, C. Martone, V. Greco; Yale University, New Haven, CT.

Skin protects our body against the outer environment, and its ability to repair upon injury is directly connected to both disease and survival. Despite recent advances in our understanding of skin homeostasis, it is still unclear how stem cells interact with their niche to sustain this process in a live mammal. The critical barrier to addressing these fundamental questions lies in the inability to simultaneously follow behaviors of different cell types and to define their functional interactions *in vivo*. Skin epidermis is an ideal system because of its accessibility and well-characterized epithelial stem cells and coexisting epidermal immune cells. In the epidermis, epithelial stem cells are closely intermingled with two resident immune cell populations: Dendritic epidermal T cells (DETCs) and Langerhans cells (LCs). Intravital imaging platform with multiphoton microscope allows us to dissect the coordination and functional significance of distinct cell activities, populations and interactions during homeostasis in live mice. In addition to immune surveillance, our data show that both immune populations can perceive and respond to the changes of their neighbors. Epidermal immune cells actively retain spatial organization within their own population while neighboring epithelial stem cells continuously divide and differentiate. Furthermore, skin epithelial stem cells act as regional checkpoints for the organization and number of epidermal immune populations, but not vice versa. This study reveals new principles of immune organization within the epidermis and elucidates dynamic epithelial-immune interactions that are in place to maintain homeostasis of the epidermis.

M72

**Stem Cell-Driven Lymphatic Remodeling Coordinates Tissue Regeneration**

**S. Gur-Cohen**[1], H. Yang[1], S. C. Baksh[1], Y. Miao[1], J. Levorse[1], R. P. Kataru[2], X. Liu[3], J. dela Cruz-Racelis[1], B. J. Mehrara[2], E. Fuchs[1]; [1]Rockefeller University, New York, NY, [2]Memorial Sloan Kettering Cancer Center, New York, NY, [3]Northwestern University, Chicago, IL.

Tissues rely on stem cells (SCs) for homeostasis and wound-repair. SCs reside in specialized microenvironments (niches) whose complexities and roles in orchestrating tissue growth are still unfolding. Here, using 3-dimensional deep imaging and molecular genetic approaches, we identify lymphatic capillaries as critical SC niche components. In skin, lymphatics form intimate networks around resting hair follicle (HF) SCs, and these capillaries remodel upon HF regeneration. Seeking understanding, we unravel a secretome switch within SCs that controls lymphatic behavior. Resting SCs express Angiopoietin-like 7 (*Angptl7)*, promoting lymphatic drainage. Upon activation, SCs trigger secretome dynamics, transiently sparking lymphatic disassociation and dampened drainage thereby enhancing tissue regeneration. In mammals, this dynamic duet between SCs and lymphatics regulates SC behavior and hair regeneration: Upon acute lymphatic collapse, *Angptl7* loss or super-activation of anagen associated SC-derived lymphatic factors, SCs spontaneously proliferate, and HF regeneration becomes asynchronous. In unearthing lymphatic capillaries as a hitherto under-appreciated SC-niche

element, we've learned how SCs can reshape the regenerative microenvironmentand coordinate their activity across a tissue.

M73

**Unraveling the Gene Regulatory Network of Early Lineage Commitment and Context-Dependent TGF-beta Response**

J. Valcourt[1], **R. E. Huang**[1], S. Kundu[2], R. E. Kingston[3], S. Ramanathan[1]; [1]Harvard University, Cambridge, MA, [2]Intellia Therapeutics, Cambridge, MA, [3]Massachusetts General Hospital, Boston, MA.

During mammalian development, progenitor cells differentiate and commit to ever-more-specific lineages. The cell's response to signaling molecules changes with commitment: selecting a given lineage entails reinterpreting signals that would have previously promoted an alternative lineage. This process of committing to a lineage by changing response to signaling molecules is of critical importance for proper development, but the dynamics and mechanism that govern the process are unclear. We investigate the binary response of human embryonic stem cells to BMP and Activin A during differentiation toward the bipotent ectoderm lineage by discovering a two-gene reaction coordinate that allows us to monitor the dynamics of differentiation and lineage commitment in real time. We show that knowledge of the expression levels of OCT4:RFP and SOX2:YFP in a double-tagged reporter line is sufficient to predict the cell's commitment to bipotent ectoderm and therefore its loss of mesendoderm-competent response to TGF-beta signals; in contrast, classical neural/ectodermal marker genes activate too late to be useful for this purpose. We predict candidate genes of the commitment gene regulatory network (GRN) using detailed RNA-seq and ATAC-seq analysis of cells that are pre- and post-commitment to bipotent ectoderm. We are able to extend or shorten the window of mesendoderm competency by overexpression of the putative GRN components, thereby validating their role in the network. This study elucidates the GRN governing commitment and provides high-time-resolution details of the commitment process.

M74

**Physical Mechanisms for Oncogene-induced Breakdown in Mammary Tissue Structure During Cancer Progression**

**V. Srivastava**[1], J. L. Hu[1], J. C. Garbe[2], M. R. Stampfer[2], M. A. LaBarge[3], Z. J. Gartner[1]; [1]University of California San Francisco, San Francisco, CA, [2]Lawrence Berkeley National Lanoratory, Berkeley, CA, [3]Beckman Research Institute, City of Hope, Duarte, CA.

Structural breakdown in the mammary epithelium is the hallmark of progression from ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC), and represents a major inflection point in the risk for patients. Normal epithelium is comprised of an inner luminal (LEP) compartment, also the site of origin for most breast cancers, that is surrounded by an outer myoepithelial (MEP) layer. Therefore, we propose that translocation of transformed LEP past the MEP layer is a key rate-limiting step preceding invasion, as most breast cancer drivers are already activated in DCIS lesions. We previously demonstrated that reconstituted organoids containing normal human LEP and MEP (isolated from reduction mammoplasty tissue) can self-organize in vitro, and that the capacity of MEP to exclude LEP from the basal compartment is determined by hard-wired and lineage-specific interfacial tensions at each cell-cell and cell-extracellular matrix (ECM) interface. Specifically, the LEP-ECM interface is highly unfavorable compared to the MEP-ECM interface. We developed a model for self-organization driven by

differences in interfacial energy, and predicted that altered interfacial tensions and dynamics (motility) promote basal LEP translocation, downstream of many cancer genes. Specifically, our model predicts that stabilization of the LEP-ECM interface and increased dynamics will increase occupancy of a high energy structural intermediate with a LEP in the basal compartment. To test this, we expressed twelve common breast cancer drivers in normal LEP, and observed that only PIK3CA-H1047R and PIK3CA-E545K expression disrupted their organization with normal MEP in 3D culture. Consistent with our predictions, PIK3CA activation increases the LEP-ECM contact angle (a measure of favorability of the LEP-ECM interface), promotes tissue dynamics and upregulates expression of several ECM molecules. Further, we used chemical inhibitors to show Akt activation downstream of PIK3CA is necessary for tissue disorganization. Collectively, these results show that signaling downstream of PIK3CA, a pathway dysregulated in >70% of breast cancers, drives the physical changes necessary for the structural transition that is a prerequisite for invasion. A more mechanistic understanding of the physical and molecular changes driving this transition will benefit the patients currently being overtreated due to lack of good markers for IDC progression.

M75

**Intracellular Ph Dynamics Regulates Intestinal Stem Cell Differentiation**

**Y. Liu**, T. Nystul, D. Barber; UCSF, San Francisco, CA.

Emerging evidence is revealing that intracellular pH (pHi) dynamics regulates different types of epithelial plasticity, including stem cell differentiation, epithelial-mesenchymal transition, and epithelial transformation. In testing the centrality of pHi dynamics in stem cell differentiation we found that increased pHi is necessary for intestinal stem cell (ISC) differentiation, using the well-established 3D *ex vivo* model of adult mouse small intestinal organoids. Using cells expressing the genetically encoded pHi biosensor pHluorin, we identified a pHi gradient along the crypt-villus axis, from lower pHi in crypt cells that include ISCs to higher pHi in differentiated villus cells. Dissipating this gradient and decreasing pHi by inhibiting H+ efflux by the plasma membrane Na-H exchanger NHE1 blocks two stages of organoid development: crypt budding and differentiation to the secretory cell linage. Inhibiting NHE1 activity completely blocks crypt budding over three days despite no effect on polarized expression of the crypt marker CD44. However, increased phosphorylation of myosin light chain 2 (pMLC) seen in controls is lost, which supports a role for actinomyosin contractility regulating budding. Additionally, inhibiting NHE1 and lowering pHi after crypt budding reduces the number of daughter cells expressing ATOH1, a master transcription factor for cells in the secretory lineage, as indicated by $ATOH1^{+}$cell-lineage tracing. Our data reveal a previously unrecognized critical role for pHi dynamics in intestinal epithelial development and further support an emerging view of pHi dynamics being a conserved regulator of stem cell differentiation.

M76

**Modeling Human Skeletal Muscle Development and Stem Cell Niche Formation in *Vivo***

**M. Hicks**, M. Yang, S. Younesi, H. Xi, A. Pyle; UCLA, Los Angeles, CA.

We are interested in using human pluripotent stem cells to generate skeletal muscle progenitor cells (hPSC-SMPCs) for development of cell therapies. My previous work showed that increased myogenic ability resides in the ERBB3+NGFR+ fraction of hPSC-SMPCs. We developed a single cell RNA-Sequencing atlas of human PAX7+ cells across fetal, juvenile and adult which identifies that hPSC-SMPCs align

between human fetal weeks 8-12 using diffusion map analysis.PAX7+ muscle cells fulfill different functional needs during myogenic development. Understanding how PAX7+ cells behave across different developmental states is critical to understanding their regenerative potential and to evaluating hPSC-SMPCs. We show that human fetal SMPCs and adult satellite cells (SCs) differ in their ability to make myofibers *in vitro* and *in vivo*; and fetal SMPCs have reduced ability to home to the SC niches of mdx-NSG mice compared to adult SCs upon engraftment.We have shown hPSC-SMPCs engraft to restore new myofibers similar to levels seen in uncultured fetal SMPCs. We also found hPSC-SMPCs fuse to form hundreds small human-specific only myofibers *in vivo*. PAX7+ hPSC-SMPCs were primarily associated with these regenerating human-only myofibers. We found that human-only myofibers continue to grow over 60 days *in vivo*, and PAX7+ hPSC-SMPC associate under the basal lamina of these emerging myofibers over time. This work demonstrates for the first time that fetal and hPSC-SMPCs can be used as a model to study human myofiber formation and niche occupancy *in vivo*.We further hypothesized that human SMPCs did not efficiently home to mouse SC niches due to competition with mouse Pax7+ SCs. Using mdx-NSG Pax7$^{cre/ERT2}$ RosaDTA mice, we evaluated whether population-specific ablation of mouse Pax7 SCs would improve retention of engrafted human PAX7 cells. We found an increase in human PAX7 cells in mdx-NSG PAX7-DTA mice in human niches but not mouse SC niches. Our work suggests that donor cell niche homing occurs in newly regenerating fibers, and not to empty SC niches of established myofibers.Evaluating human niche formation over time will improve our understanding of how human muscle SC niches develop. This could improve our ability to generate *de novo* human niches and better support human PAX7 cells *in vivo* for cell therapy.

M77

**Tension Suppresses Epithelial Stem Cell Differentiation**

M. King[1], R. Stewart[1], A. Zubek[2], E. Carley[1], A. Zieman[2], **V. Horsley**[2]; [1]Yale School of Medicine, New Haven, CT, [2]Yale University, New Haven, CT.

While several signaling pathways that control keratinocyte differentiation are known, how mechanical cues, and specifically the transmission of forces to the nucleus can impact these processes, is not well understood. The nucleus is mechanically integrated into the cytoskeleton by LINC (Linker of Nucleoskeleton and Cytoskeleton) complexes, which span the nuclear envelope and are composed of KASH proteins in the outer nuclear membrane that bind to the cytoskeleton and SUN proteins in the inner nuclear membrane that bind to lamins and chromatin. We recently showed that during differentiation of epidermal stem cells, nuclear position in mouse keratinocytes is driven towards the colony center by actomyosin contractility. Here, using a novel tension sensor, we show that tension on the LINC complex and lamin network is sensitive to extracellular matrix adhesion and decreases during epidermal stem cell differentiation. Furthermore, mouse skin in vivo and mouse keratinocytes in vitro lacking functional LINC complexes display precocious epidermal differentiation, which we tie to altered association of epidermal differentiation genes with the nuclear periphery. Together, these data provide a new function for nuclear tension in transcriptional changes that impact stem cell differentiation.

M78

**dek Modulates Global Intron Retention to Control Quiescence Exit in Muscle Stem Cells**

**T. H. Cheung**; Hong Kong University of Science and Technology, Hong Kong, HONG KONG.

Quiescent adult stem cells have the ability to respond rapidly to external stimuli, but mechanisms of such rapid activation remain elusive. Using quiescent skeletal muscle stem cells (QSCs), we showed that intron retention (IR) is prevalent. Genes possessing IR are essential for various fundamental cellular functions including RNA splicing, protein translation, cell cycle entry and lineage specification. Further analysis revealed that IR is a post-transcriptional mechanism that regulates QSC quiescence exit, which is dependent on the phosphorylated-Dek protein. While Dek is absent in QSCs, overexpression of Dek in QSC in vivo results in a global decrease of IR, quiescence exit, and consequently undermine muscle regeneration. Moreover, IR analysis on public RNA-seq data shows that other quiescent adult stem cells are enriched with retained introns, indicating IR as a feature of quiescent adult stem cells. Altogether, these findings suggest that intron retention plays an important role in stem cell quiescence exit.

M79

**Regenerative Landscape of Intestinal Organoids**

**P. Liberali**; Friedrich Miescher Institute, Basel, SWITZERLAND.

Development of intestinal organoids from single intestinal stem cells recapitulates the regenerativecapacity of the intestinal epithelium. To unravel molecular mechanisms orchestrating organoid formation and regeneration of intestinal tissue, we developed a high-content image-based screening assay for an annotated compound library. We generated multivariate feature profiles for hundreds of thousands of individual organoids to quantitatively describe the phenotypic landscape of organoid development. The resulting phenotypic fingerprints were then used to infer regulatory genetic interactions from a single screen, establishing a novel paradigm in genetic interaction screening applied to an emergent system. This allowed the identification of modules of genes that regulate cell identity transitions and maintain the balance between regeneration and homeostasis, unraveling novel roles for several pathways, among them retinoic acid signaling. We then characterized a crucial role for retinoic acid nuclear receptors in controlling the exit from the regenerative state and in driving enterocyte differentiation. By combining quantitative imaging with RNA sequencing we confirmed the role of endogenous retinoic acid signaling and metabolism for initiating transcriptional programs that guide intestinal epithelium cell fate transitions and identified a small molecule inhibitor of retinoid X receptor, RXR, that improved intestinal regeneration *in vivo*.

## Minisymposium 8: Lipid Regulation and Transport in Membrane Remodeling

M80

**The Nuclear Envelope Enriched Lipin Phosphatase Ctdnep1 Links Misregulation of Lipid Metabolism to Chromosome Instability in Dividing Cancer Cells**

H. Merta, J. Carrasquillo-Rodriguez, M. Deline, T. Vitale, **S. Bahmanyar**; Yale University, New Haven, CT.

Lipin1 is the key lipid metabolizing enzyme that controls ER lipid homeostasis. Lipin1 produces the precursors for triglycerides and membrane glycerophospholipids via its membrane binding domain while nuclear localized lipin1 inhibits the transcriptional activation of lipid synthesis genes. In the presence of

nutrients, lipin1 is phosphorylated by mTORC1 and excluded from the nucleus. Here, we show in dividing cancer cells that CTDNEP1, a highly conserved integral membrane protein phosphatase, dephosphorylates lipin1 to counteract mTOR and in turn limit the production of glycerophospholipids in the ER and prevents chromosome instability. We demonstrate that CTDNEP1-deleted U2OS cells contain constitutively phosphorylated and reduced levels of lipin1 leading to elevated levels of glycerophospholipids and triglycerides. In control cells, the majority of lipin1 is dephosphorylated upon inhibition of mTOR by Torin or in nutrient-poor conditions, yet lipin1 remains mostly phosphorylated in CTDNEP1-deleted cells under these conditions. Thus, CTDNEP1 dephosphorylates and stabilizes a pool of lipin by counteracting mTor in response to nutrient availability to maintain lipid homeostasis. Endogenous-GFP tagging showed that CTDNEP1 in U2 OS cells is enriched at the nuclear envelope (NE) suggesting that local regulation of lipin globally impacts ER lipid homeostasis. Increased glycerophospholipid synthesis in CTDNEP1-deleted cells causes expansion of ER membranes in mitosis leading to a faster rate of membrane wrapping around segregated chromosomes during nuclear formation and an increased incidence of micronuclei. Live imaging of CTDNEP1-deleted mitotic cells revealed ER membranes that invade the microtubule spindle region suggesting that excess ER membranes interfere with spindle assembly and chromosome segregation. Consistent with this, spindle morphology and nuclear reformation is severely disrupted after recovery from spindle microtubule destabilizing drugs. Together, these data demonstrate that NE-enriched CTDNEP1 activates a pool of lipin1 to maintain ER lipid homeostasis. The abundance of ER membranes, in turn, limit the rate of NE reformation to protect against chromosome missegregation and micronuclear formation. Thus, CTDNEP1 links mis-regulation of lipid metabolism to genome instability, two hallmarks of rapidly dividing cancer cells.

M81

**Defining the Subcellular Distribution and Metabolic Channeling of Phosphatidylinositol.**

**J. G. Pemberton**[1], Y. Kim[1], N. Sengupta[1], A. Eisenreichova[2], D. J. Toth[1], E. Boura[2], T. Balla[1]; [1]National Institute of Child Health and Human Development, NIH, Bethesda, MD, [2]Czech Academy of Sciences, Prague, CZECH REPUBLIC.

Among the structural phospholipids that form the bulk of eukaryotic cell membranes, phosphatidylinositol (PtdIns) is unique in that it also serves as the common precursor for low-abundance phosphorylated derivatives, called polyphosphoinositides (PPIn), which are central regulators of cellular physiology. In particular, the complex metabolic turnover of PPIn species has essential functions related to both intracellular signal transduction and membrane trafficking, however, there is still a limited understanding of how PtdIns synthesis and transport contributes to the turnover of the unique subcellular pools of PPIn lipids. To address these shortcomings, we established a molecular toolbox for investigations of PtdIns distribution and availability within intact cells that capitalizes on the substrate selectivity and high specific activity of the bacterial PtdIns-specific phospholipase C (bacPI-PLC) enzyme. Results using catalytically inactive mutants of the bacPI-PLC, which were designed to trap the bound PtdIns substrate, suggest that PtdIns is localized to the endoplasmic reticulum (ER), the biochemically-defined site of PtdIns synthesis, but is also enriched in the cytosolic leaflets of the Golgi complex, peroxisomes, and mitochondria. Strikingly, we did not observe significant localization of the bacPI-PLC variants within the plasma membrane (PM) or to endosomal compartments in any of the mammalian cell types examined. The membrane distribution of PtdIns was further investigated using recruitable versions of a modified bacPI-PLC scaffold to map PtdIns contents by monitoring the local production of

diacyglycerol (DAG), the direct cleavage product of bacPI-PLC-mediated PtdIns hydrolysis. A chemically-inducible protein heterodimerization system was used to target the bacPI-PLC to specific organelle membranes and revealed the rapid production of DAG within the cytosolic leaflets of the ER, mitochondria, peroxisomes, and Golgi complex. However, only minor increases in DAG production were observed following the targeted recruitment of the bacPI-PLC to the PM or to Rab5- and Rab7-positive endosomes. The recruitable bacPI-PLC construct was then used for kinetic studies on PPIn turnover that demonstrate the requirement for sustained PtdIns supply from the ER, rather than the absolute steady-state content of PtdIns, for the maintenance of monophosphorylated PPIn species within the PM, Golgi complex, and endosomal compartments. Overall, our findings provide a comprehensive map of the subcellular distribution of PtdIns within the membrane compartments of intact cells as well as support an important role for PtdIns transfer and substrate channeling in the spatial control of PPIn metabolism.

M82

**LetB Forms a Tunnel for Lipid Transport Across the Bacterial Periplasm**

**D. C. Ekiert**; New York University School of Medicine, New York, NY.

Gram-negative bacteria are surrounded by an outer membrane composed of phospholipids and lipopolysaccharide (LPS), which acts as a barrier to the environment and contributes to antibiotic resistance. While mechanisms of LPS transport have been well characterized, systems that translocate phospholipids across the periplasm, such as MCE transport systems, are less well understood. Here we show that *E. coli* LetB (formerly YebT), uses multiple MCE domains to form a ~0.6 megadalton complex in the periplasm. Our cryo EM structure reveals that LetB consists of a stack of 7 modular rings, creating a long hydrophobic tunnel through the center of the complex. LetB is sufficiently large to span the gap between the inner and outer membranes, and mutations that shorten the tunnel abolish function. Lipids bind inside the tunnel, suggesting that it functions as a pathway for lipid transport. Together, our results support a model in which LetB establishes a physical link between the bacterial inner and outer membranes, and creates a hydrophobic pathway for the translocation of lipids across the periplasm, supporting the integrity of the outer membrane permeability barrier.

M83

**Dual Role of Orp5 in Regulating Lipid Transport and Calcium Import to Mitochondria At Endoplasmic Reticulum (er)- Mitochondria Membrane Contact Sites**

L. Rochin[1], **F. Giordano**[1], C. Sauvanet[1], E. Jääskeläinen[2], A. Houcine[3], A. Kivela[2], X. MA[4], E. Marien[5], J. Dehairs[5], J. Neveu[1], R. Le Bars[1], J. Swinnen[5], D. Bernard[4], D. Tareste[6], V. Olkkonen[7]; [1]Institut de Biologie Integrative de la Cellule (I2BC)-UMR9198/CNRS, Gif-sur-Yvette, FRANCE, [2]Minerva Foundation Institute for Medical Research, Biomedicum 2U, Helsinki, FINLAND, [3]Institut Jacques Monod, CNRS, UMR7592, Paris, FRANCE, [4]Centre de Recherche en Cancérologie de Lyon, UMR INSERM U1052/CNRS 5286, Lyon, FRANCE, [5]Laboratory of lipid metabolism and cancer, Department of Oncology, KU Leuven, Leuven, BELGIUM, [6]Institute of Psychiatry and Neuroscience of Paris (IPNP), UMR-S1266 INSERM, Paris, FRANCE, [7]Department of Anatomy, Faculty of Medicine, FI-00014 University of Helsinki, Helsinki, FINLAND.

Mitochondria are dynamic organelles essential for cell survival whose structural and functional integrity rely on selective and regulated transport of lipids from/to the endoplasmic reticulum (ER) and across the two mitochondrial membranes. As they are not connected by vesicle transport, the exchange of lipids between ER and mitochondria occurs at sites of close organelle apposition called membrane contact

sites. However, the mechanisms and proteins involved in these processes are only beginning to emerge. We have found that ORP5/8 mediate non-vesicular transport of Phosphatidylserine (PS) from the ER to mitochondria in mammalian cells. We have also found that ER-mitochondria contacts where ORP5/8 reside are physically and functionally linked to the Mitochondrial Intermembrane space Bridging (MIB) complex, that anchors the outer and inner mitochondrial membranes, to facilitate PS transfer from the ER to the mitochondria. Finally, we show that ORP5 but not ORP8, additionally regulates import of calcium to mitochondria and consequently cell senescence.

M84

**The Regulation of Plasma Membrane Cholesterol States Via GRAMD1 Lipid Transfer Protein Complex**

T. Naito[1], B. Ercan[1], L. Krshnan[1], A. Triebl[2], D. Koh[1], F. Wei[3], K. Tomizawa[3], F. Torta[2], M. Wenk[2], **Y. Saheki**[1,4]; [1]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, SINGAPORE, [2]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, SINGAPORE, [3]Department of Molecular Physiology, Faculty of Life Sciences, Kumamoto University, Kumamoto, JAPAN, [4]Institute of Resource Development and Analysis, Kumamoto University, Kumamoto, JAPAN.

Lipid homeostasis plays a critical role in membrane dynamics and integrity, as well as in cell viability in all eukaryotic cells. The endoplasmic reticulum (ER), where the majority of membrane lipids are synthesized, extends throughout the cells and forms close appositions with virtually all other membranous organelles and with the plasma membrane (PM). Growing evidence suggests that these membrane contact sites play general roles in cell physiology, including lipid exchange between bilayers independent of membrane traffic via lipid transfer proteins. Such non-vesicular lipid transport plays critical roles in the intracellular traffic of lipids, including cholesterol. Cholesterol is important for structural integrity of cellular membranes and cell physiology, including neuronal function. Cells either take it up from dietary sources or synthesize it *de novo* in the ER. Close to 90% of cellular cholesterol is enriched in the PM, where it contributes to approximately half of the total lipids of this bilayer. Thus, the levels of PM cholesterol need to be constantly monitored by the cells to control its uptake or biosynthesis. This is thought to be in part mediated by the communication between the PM and the ER, the major source of membrane lipids that is responsible for the entire biosynthesis of cellular cholesterol. How the cells mediate such crosstalk has been unknown. We found that a family of evolutionarily conserved ER proteins [GRAMD1s (GRAMD1a/1b/1c)] transports cholesterol from the PM to the ER, facilitating such crosstalk at ER-PM contacts. GRAMD1s possess a GRAM domain and a StART-like cholesterol-harboring domain followed by a C-terminal transmembrane domain that anchors them to the ER. We found that they form homo- and hetero-meric complexes via their transmembrane domains and luminal helices. They accumulate at ER-PM contacts upon increase of the accessibility of PM cholesterol, which is limited by sphingomyelin at rest. PM recruitment of GRAMD1s is regulated by their GRAM domain, which acts as a coincidence detector of cholesterol and anionic lipids. Acute recruitment of GRAMD1b to ER-PM contacts by a drug-induced dimerization method mediates StART-like domain-dependent PM cholesterol extraction. Cells lacking all three GRAMD1s exhibit striking expansion of the accessible pool of PM cholesterol due to impaired cholesterol transport from the PM to the ER, which can be rescued by re-expression of any one of GRAMD1s but not by a version of GRAMD1s that is defective in cholesterol transport or complex formation. Collectively, our study shows that GRAMD1s regulate the homeostasis of PM cholesterol by counteracting its acute changes via sensing and extracting the accessible pool of PM cholesterol for its transport to the ER.

M85

**Loss of Lysosomal Npc1 Function Increases PtdIns4P to Sustain ER to Golgi Cholesterol Transfer**

O. Vivas, **C. Kutchukian**, M. Casas Prat, J. G. Jones, S. A. Tiscione, R. Dixon, E. J. Dickson; University of California, Davis, Davis, CA.

Cells utilize vesicular and non-vesicular transport mechanisms to transport lipids to target organelles. Non-vesicular lipid transport is mediated by transfer proteins that tether the gap between two closely apposed organelle membrane contact sites. At contact sites between Trans-Golgi network (TGN) and Endoplasmic reticulum (ER), oxysterol-binding protein (OSBP) mediates cholesterol/ PtdIns4P exchange. In health, TGN PtdIns4P is transported down its concentration gradient to ER, fueling the countertransport cycle of cholesterol to TGN against its steep concentration gradient. Maintaining the PtdIns4P gradient and thus perpetuating this exchange cycle, are PtdIns4P kinases on the TGN and a PtdIns4P phosphatase (Sac1) on the ER. Consequently, this cholesterol/PtdIns4P exchange contributes to the establishment of a cholesterol gradient between organelles of the secretory pathway, essential for protein trafficking to the plasma membrane (PM). Despite this knowledge, little is known about how this molecular choreography is disrupted during pathophysiological conditions. In Niemann-Pick Type C1 (NPC1) disease, a pathological condition that leads to hyperexcitability and neurodegeneration, there is an alteration of lysosomal cholesterol homeostasis. Here we show that in models of NPC1 disease, there is a rearrangement of molecular components of the cholesterol/PtdIns4P cycle. Using a combination of mass-spectrometry, super-resolution imaging, and electrophysiology, we have discovered that inhibition or disease-causing mutations in the lysosomal transporter Niemann-Pick Type C1 result in a significant elevation in Golgi PtdIns4P. The increase in TGN PtdIns4P is not due to a slower rate of OSBP-mediated PtdIns4P transfer, or increased protein levels of PI4K isoforms. Instead, the increase in TGN PtdIns4P levels is due to spatial redistribution of PI4KIIα and PI4KIIIβ isoforms. Concurrent with the enhanced recruitment of PI4KIIIβ to the TGN is a significant accumulation of endogenous ER Sac1 at the contact site between the ER and TGN. The parallel reorganization of PI4KIIIβ and Sac1 ensures the maintenance of a steep PtdIns4P gradient that strengthens cholesterol transport to the Golgi. We also find that a downstream consequence for the increased cholesterol/ PtdIns4P exchange in NPC1 disease, is a dysregulation of vesicular trafficking of the AMPA Receptor Subunit GluA1. Physiologically, altered GluA1 subunit density at the PM aberrantly modifies the influx of calcium into neurons and may contribute to neuronal cell death in NPC1 disease. We propose that lysosomal NPC1-mediated cholesterol efflux modulates the molecular organization of TGN-ER contact sites to influence the driving force for cholesterol transport in health and disease.

M86

**RASSF4 Functions At the Interface between Actin and Intracellular Vesicles to Regulate PI(4,5)$P_2$ Synthesis**

**Y. Chen**, D. Ryskamp, I. Bezprozvanny, J. Liou; UT southwestern at Dallas, Department of Physiology, TX.

The tumor suppressor RAS association domain family 4 (RASSF4) interacts with the small G protein ARF6 to regulate the level of phosphatidylinositol 4,5-bisphosphate (PI[4,5]$P_2$) important for a wide array of cellular functions. Nevertheless, the biological context of this RASSF4-ARF6-PI(4,5)$P_2$ pathway is not well understood. We found that actin dynamics controls RASSF4-ARF6 association at F-actin-associated intracellular vesicles, where RASSF4 activates ARF6 to stimulate PI(4,5)$P_2$ synthesis. Disruption of the

SARAH domain of RASSF4 resulted in an enhanced association with ARF6 at intracellular vesicles and an increased level PI(4,5)$P_2$ at the plasma membrane (PM). Additional data derived from acute manipulation and live-cell imaging experiments indicate that PI(4,5)$P_2$ produced at intracellular vesicles by the RASSF4-ARF6 pathway is delivered to the PM in an actin-dependent manner. Disruption of this intracellular PI(4,5)$P_2$ synthesis pathway in RASSF4 knockout cells resulted in reduced PM PI(4,5)$P_2$, enlarged cell size, disrupted actin cytoskeleton, and abnormal lysosomes. Consistently, knockdown of RASSF4 greatly suppressed neurite outgrowth in hippocampal neurons. Together, our study reveals a novel PI(4,5)$P_2$ synthesis pathway mediated by actin-regulated association of RASSF4 and ARF6 at intracellular vesicles. This RASSF4-mediated pathway is important for actin cytoskeletal integrity, lysosome homeostasis, and numerous PI(4,5)$P_2$-dependent physiological functions.

M87

**A PI3KC2 Noncatalytic Splice Variant Regulates Phosphoinositide Activities in Endolysosomal Trafficking**

**A. A. Kiger**, J. Groulx, S. Jean, C. Bao; University of California, San Diego, La Jolla, CA.

Membrane trafficking must adapt to changing cellular demands, in part through the coordinated activity of phosphoinositide lipid regulators. Class II PI3-kinase (PI3KC2) is implicated with roles in endocytosis, endosomal transit toward recycling and lysosomal identity. We discovered that the single Drosophila PI3KC2, Pi3K68D, encodes for two alternatively spliced protein variants: the full-length kinase and a short noncatalytic variant, PI3KC2-S. Strikingly, targeted genomic deletions revealed opposing functions for the two variants throughout the endolysosomal pathway in macrophages. PI3KC2-S was required to inhibit each of the PI3KC2 kinase functions, thus serving wildtype roles to both restrict PI3KC2-mediated integrin endocytic uptake and cortical recycling, and conversely, to de-repress PI3KC2-inhibited lysosomal maturation. PI3KC2-S interacted in a protein complex with PI3KC2 that restricted in vitro PI3-kinase activity. Consistent with this negative regulation of kinase activity, PI3KC2-S was required in vivo to restrict endosomal PI(3)P and compartment identities, as well as regulate PI3KC2 protein localization dynamics. Interestingly, PI3KC2-S also interacted with Mtm PI3-phosphatase protein, antagonized Mtm catalytic activity, and inhibited Mtm endosomal function and repression of lysosomal maturation. Altogether, we show that PI3KC2-S is a critical activator of endolysosomal progression through coordinate downregulation of PI3KC2-Mtm phosphoinositide functions in an endosomal pathway. Importantly, an imbalance in PI3KC2 and PI3KC2-S functions altered adult fly longevity and survival in response to stress, signifying cellular as well as organismal dependence on phosphoinositide regulation that balances levels of endosomal and lysosomal trafficking.

M88

**The Lipid Kinase, Vps34, Is a Driver of Selected Membrane Trafficking Pathways**

**N. Steinfeld**, V. Lahiri, A. Morrison, D. J. Klionsky, L. S. Weisman; University of Michigan, Ann Arbor, MI.

Phosphoinositide lipids are low-abundance signaling molecules that control signal transduction pathways and are necessary for cellular homeostasis. The phosphoinositide lipid, phosphatidylinositol 3-phosphate (PI3P), recruits downstream effector proteins to control a wide-range of membrane trafficking events. In the budding yeast, *Saccharomyces cerevisiae*, Vps34, a phosphatidylinositol 3-kinase, is the sole source of PI3P. PI3P is required for several cellular processes including synthesis of PI3,5$P_2$, retromer function, autophagy, and formation of multivesicular bodies via the ESCRT machinery.

Moreover, PI3P levels are acutely elevated in response to some stimuli, which suggests that dynamic changes in PI3P drive some downstream pathways. This possibility would not be revealed in most studies, which utilize knock-down or knock-out approaches. Here we seek to extend current knowledge by determining the consequences of increasing PI3P levels. We designed a hyperactive mutant based on a high-resolution structure (Rostislavleva et al., Science 2015) combined with a study that suggested that activation of Vps34 occurs in part via dissociation of the Vps34 kinase domain from its scaffold protein, Vps15 (Stjepanovic et al., Mol. Cell 2017). In addition, we identified Vps34 mutants that increase PI3P levels by screening for mutants that suppress an impaired growth phenotype of a catalytically damaged Vps34 allele. These hyperactive mutants increase cellular PI3P as well as $PI3,5P_2$ levels both at basal conditions and during hyperosmotic shock.Study of these hyperactive mutants has revealed that elevation of PI3P selectively drives certain trafficking pathways while not affecting, or even inhibiting others. Hyperactive Vps34 alleles were found to enhance Snx4-dependent retrograde traffic of Atg27 from the vacuole membrane. In contrast, ESCRT- and membrane fusion-dependent degradation of the plasma membrane methionine transporter, Mup1, following addition of methionine, is unchanged by elevated PI3P. Finally, our data suggests that while autophagy induction is unchanged by excess PI3P, increasing PI3P levels correlates with increasing defects in the degradation of autophagy cargoes. This phenotype may be due to defects in maturation of autophagosomes, their fusion with the vacuole, and/or the proteolytic capacity of the vacuole. Taken together, our data suggests that changes in phosphoinositide lipid levels may provide a way for cells to acutely respond to some stimuli. More broadly, the approach of using hyperactive mutants can be applied to other lipid kinases and may contribute insights into phosphoinositide lipid signaling.

M89

**Rab3 Is a Key Player in Microdomain-dependent Plasma Membrane Recycling**

**B. Diaz-Rohrer**[1], K. Levental[2], I. Levental[2]; [1]University of Texas Hlth Sci Ctr Houston, Houston, TX, [2]UT Health Science Center at Houston, Houston, TX.

The composition of the plasma membrane (PM) must be tightly controlled for the PM to maintain functionality. Because *de novo*synthesis of proteins and lipids is energetically costly, the cell depends on recycling of most endocytosed membrane components back to the PM. We observed that raft association is fully sufficient for PM recycling of certain proteins and that abrogation of raft partitioning for these proteins led to their degradation in lysosomes.These findings strongly support a model wherein ordered membrane domains mediate PM recycling of membrane components from the endosomal system. Furthermore, using a high-throughput knock-down screen using orthogonal transmembrane proteins as probes for raft and non-raft domains, we identified and validated a number of proteins that play a role on PM recycling, including known players of early endocytic traffic (Rab5 family and EEA1), as well as novel players that appear to represent a distinct class of trafficking mediators specific for raft-associated proteins. This raft mediated pathway is not dependent on the classical recycling pathways defined by Rab4A and Rab11A but instead represents a novel route for PM recycling of raft-preferring cargo from late endosomes. We implicate the Rab3 family as a central regulator of this pathway and show that the Rab3 family is essential for PM homeostasis, as abrogation of all four members of the Rab3 family disrupts PM lipidomes and proteomes. Altogether, our findings reveal a fundamental role for raft microdomains in endocytic sorting and recycling and support a novel role for Rab3 as a central regulator of this previously unrecognized mechanism for PM and endosome homeostasis.

## Minisymposium 9: Managing Gene Expression: DNA to RNA and Beyond

M90

**Origins and Implications of Transcriptional Bursts**

**J. Chubb**; University College London, London, UNITED KINGDOM.

We are interested in how cells make decisions. To understand how cells choose, it is necessary to monitor the gene expression decisions of individual cells, as they make their decisions. To achieve this, we use a combination of live cell imaging of transcriptional dynamics, combined with single cell transcriptomics and molecular genetics. I will present data showing that the changing transcript profiles of cells during development are discrete, rather than continuous, and that cell type specific changes in transcript content are coupled to large transcriptome-wide changes common to all fates. I will then describe how the dominant motive force underlying transcription dynamics arises from the promoter, rather than features specific to genome context.

M91

**Transcriptional Bursting At a Single Promoter Level Is Regulated by Transcription Factor Mobility and Hormone Release Pattern**

**D. Stavreva**[1], D. Garcia[2], G. Fettweis[1], A. McGowen[1], M. Ferguson[3], A. Upadhyaya[2], G. Hager[1]; [1]NCI, NIH, Bethesda, MD, [2]University of Maryland, College Park, MD, [3]Boise State University, Boise, ID.

Genes in eukaryotic cells are transcribed in a discontinuous pattern referred to as RNA bursting, but mechanisms regulating this process are unclear. Furthermore, many physiological signals, including glucocorticoid hormones, are pulsatile, the effects of transient stimulation on bursting are unknown. Here, we characterize RNA synthesis from single copy glucocorticoid receptor (GR)-regulated transcription sites (TSs) under pulsed (ultradian) and constant hormone stimulation. In contrast to constant stimulation, pulsed stimulation induces restricted bursting, centered around the hormonal pulse. Moreover, we demonstrate that TF nuclear mobility determines burst duration, while its bound fraction determines burst frequency. Using 3D tracking of TSs, we directly correlate TF binding and RNA synthesis at a specific promoter. Finally, we uncover a striking co-bursting pattern between TSs located at proximal and distal positions in the nucleus. Together, our data reveal a dynamic interplay between TF mobility and RNA bursting that is responsive to stimuli strength, type, modality, and duration.

M92

**RNA Splicing, Chromatin Modification, and the Coordinated Control of Gene Expression**

**T. Johnson**; UCLA, Los Angeles, CA.

RNA splicing is carried out by a large ribonucleoprotein machine, the spliceosome. Biochemical dissection of the splicing reaction has uncovered dynamic spliceosome rearrangements that are critical for proper recognition of splice sites and removal of introns. Recently, there has been growing evidence that assembly of the spliceosome onto pre-mRNA occurs co-transcriptionally, while the RNA polymerase is actively engaged with a chromatin template, which raises the question of how transcription, and more specifically, the state of the chromatin affects spliceosome assembly. Here we describe how a number of conserved chromatin modifications influence spliceosome assembly and disassembly to affect splicing outcomes.

M93

**Availability of Splicing Factors in the Nucleoplasm Can Regulate the Release of Mrna From the Gene After Transcription**

**Y. Shav-Tal**, H. Hochberg-Laufer, N. Neufeld, Y. Brody; Bar-Ilan University, Ramat Gan, ISRAEL.

Gene expression dynamics can be measured in single living cells. Using a detectable transcriptionally active gene in living cells, we previously found that an mRNA undergoing several splicing events was retained on this gene until completion of mRNA processing. We showed that this lag in the release of the retained mRNA was splicing dependent. Now, to determine the reason for this delay in release and whether mRNA retention on the gene might depend on splicing factor availability, we modulated the levels of splicing factors in the nucleus. We hypothesized that varying the abundance of splicing factors in the nucleoplasm by dismantling nuclear speckles might modify the lag between the end of transcription and the release of the mRNA from the gene. Using live-cell imaging and quantitative analysis of splicing factor dynamics in the cell nucleus we could address whether the levels of splicing factors in the nucleoplasm can influence the rates at which a spliced mRNA is released from a gene. Indeed, increasing the abundance of the diffusing fraction of splicing factors by their overexpression, or by Clk1 kinase overexpression, led to a reduction in splicing factor residence times on the active gene, and the retained mRNA was rapidly released from the gene. Other treatments such as overexpression of a mutant inactive Clk1, the downregulation of MALAT1 lncRNA or of the Son protein, or the overexpression of TNPO3 a splicing factor import factor, did not affect the dynamics of mRNA release from the gene. We found that the faster release of the mRNA from the gene mediated by increased availability of splicing factors, was dependent on the RS domain of the splicing factors and its phosphorylation state. We propose that the relative abundancies of splicing factors in the nucleoplasm can affect their availability for the splicing events taking place, and regulate the kinetics of mRNA release from the gene after processing.

M94

**The Midbody Is a Novel Translating Organelle Mediating Intercellular Communication**

R. Dahn[1], S. Park[1], E. Kurt[1], A. Presle[2], J. Gilbert[1], K. VanDenHeuvel[1], A. Jambhekar[3], J. Shivas[4], L. Qin[1], O. Olukoga[1], A. Echard[2], M. Blower[5], **A. R. Skop**[1]; [1]University Wisconsin-Madison, Madison, WI, [2]Instit Pasteur, Paris, FRANCE, [3]Harvard, Boston, WI, [4]Leica Microsystems, Buffalo Grove, IL, [5]Harvard, Boston, MA.

The midbody is a transient structure at the spindle midzone that is required for the terminal stage of cell division, cytokinesis. Long ignored as a vestigial remnant of cytokinesis, emerging data suggest midbodies are released from post-mitotic cells and can modulate cell fate decisions, proliferative state, tissue polarity, cilia formation, neuronal architecture and function, and oncogenesis. Our ‘structure reveals function’ approach first demonstrated that the midbody matrix- a mysterious electron-dense region of unknown composition- is the assembly site of a phase-separated biomolecular condensate, enriched for mRNAs encoding proteins involved in cytokinesis, oncogenesis and pluripotency regulation, including transcription factors. Hexanediol-sensitivity and FRAP analysis confirmed that the midbody matrix exhibits biophysical properties expected of an RNP condensate. SUnSET analysis of protein synthesis showed that both the mitotic and post-mitotic midbody are sites of active translation. Consistent with this, ribosomal subunits and elongation factors colocalize with puromycin labeling in a

ring structure surrounding the midbody RNA core. We discovered that translation of midbody-enriched mRNAs is under tight temporal regulation, beginning in G1 after cells have formally exited mitosis, and continues after the midbody is abscised and released extracellularly from the daughters that birthed it. Our data suggest a model in which the midbody functions as a novel organelle with a complex life cycle comprised of both membraneless and membrane-bound phases: 1) an RNP condensate is assembled at the spindle overlap; 2) spatiotemporally regulated translation is initiated as the daughter cells leave mitosis and reenter G1; 3) abscission occurs and the membrane-bound RNP granule is released; 4) the midbody is bound, internalized, and transfers information to recipient cells.

M95

**Cross-kingdom Recognition of Bacterial Small RNAs Induces Transgenerational Pathogenic Avoidance**

**C. T. Murphy**; Princeton University, Princeton, NJ.

We recently discovered that *C. elegans* can pass on a learned avoidance of pathogenic *Pseudomonas aeruginosa* (PA14) to four generations of its progeny. This transgenerational inheritance is bacterial species-specific, but how C*. elegans* recognizes and distinguishes different bacteria and transmits this information to future generations is not apparent. Here we show that small RNAs purified from pathogenic PA14 are sufficient not only to induce avoidance of pathogens in mothers, but also to confer transgenerational inheritance of this species-specific behavior for four generations, all without direct contact with pathogenic bacteria. This behavior requires the small RNA transporters SID-1 and SID-2, RNA interference pathway components, the piRNA Piwi/Argonaute pathway, a functioning germline, and TGF-β ligand *daf-7* expression in the ASI sensory neuron. Our results suggest that *C. elegans*"reads" small RNAs expressed by pathogenic bacteria, and uses this information to induce an escape behavior that lasts for four additional generations. *C. elegans* may have evolved this trans-kingdom signaling system to avoid pathogens in abundant classes of bacteria in its environment and its microbiome.

M96

**Transcription Factor Foxa1 Clusters Dna through Wetting-mediated Capillary Forces**

**T. Quail**, S. Golfier, D. Oriola, J. Brugues; Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, GERMANY.

Direct interactions between enhancers and promoters are necessary for the activation of gene regulatory programs in vivo. However, how transcription factors, co-factors, and general transcriptional machinery work in concert to physically bring DNA together in space and time remains unclear. Here, we used super-resolution TIRF to image the interactions of single-molecules of linearized lambda-phage DNA and Forkhead Box Protein A1 (FoxA1). We discovered that FoxA1 wets DNA, generating capillary forces that reel-in DNA into clusters on the order of 20 KB in a DNA tension-dependent manner. To understand the source of this reeling-in force, we imaged pairs of DNA molecules that were bound spatially proximal to each other, and we found that these DNA molecules exhibited "zipping", consistent with a mechanism where FoxA1 minimises its surface area due to surface tension effects, and, consequently, generates capillary forces. We proposed that the origin of these capillary forces is a combination of FoxA1 self-interactions and FoxA1-DNA adhesion. To test this theory, we generated a C-terminal truncation of FoxA1, a disordered region that we thought may play a role in FoxA1 self-interaction. FoxA1ΔCTD did not cluster DNA, suggesting that FoxA1-FoxA1 interactions are relevant for capillary force generation. This reveals that transcription factors could display a range of behaviors

depending on their structure. To investigate this, we used Tata-Box Binding protein (TBP), a general transcription factor, and linker histone H1, a chromosomal architecture protein structurally similar to FoxA1. We found that TBP wets DNA, forming droplets that translocate along the strand, though was unable to reel-in DNA. However, H1 reeled-in DNA with stronger capillary forces, consistent with its known compacting activity. We propose that capillary-force-induced DNA clustering is a novel mechanism that may play a fundamental role in DNA organization in the cell nucleus.

M97

**Confined Migration Induces Heterochromatin Formation in Cancer Cells**

**C. Hsia**[1,2], O. Hasan[2], C. Chang[2,3], J. Lammerding[1,2,3]; [1]Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, [2]Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY, [3]Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY.

During cancer metastasis, cancer cells migrate through confined interstitial spaces, requiring extensive deformation of the cell body and nucleus. The severe physical stress on the nucleus during this frequently results in rupture of the nuclear envelope (NE) and nuclear fragmentation. Previous studies found that mechanical force application on cells can induce mechanosensitive gene expression and chromatin rearrangement. However, it remains unclear whether physical stress on the nucleus during confined migration can induce histone modification and gene expression changes. Here, we performed live-cell and immunofluorescence imaging of HT1080 fibrosarcoma and MDA-MB-231 breast cancer cells migrating through collagen gel matrices and custom-made microfluidic migration devices that mimic interstitial spaces *in vivo*. Cell migration in confined 3D environments resulted in increased H3K9me3 and H3K27me3 heterochromatin marks and DNA methylation compared to unconfined conditions. The heterochromatin enrichment was particularly prominent in cells with visible nuclear deformation and in chromatin protrusions that formed inside nuclear blebs. In addition, we observed GFP-labeled HP1α enrichment within nuclear blebs in HT1080 and MDA-MB-231 cells during confined migration. By using a fluorescence recovery after photobleaching (FRAP) assay, we verified those GFP-HP1α enrichments to be heterochromatin. Treating cells with a pan histone methyltransferase inhibitor 3-Deazaneplanocin A (DZnep) significantly reduced the extent of heterochromatin formation after confined migration, suggesting an active enzymatic process underlying the increased heterochromatin formation. In particular, enriched phosphorylated (pSer424) HDAC3 nuclear staining suggested activation of HDAC3, potentially due to the mechanical force cells encountered during confined migration. Consistent with this idea, chemical inhibition of HDAC3 significantly reduced heterochromatin formation during confined migration. Interestingly, cells treated with DZnep showed significantly slower migration when compared to control treatment, suggesting the importance of chromatin condensation for efficient migration in 3D environment. Taken together, our research indicates that migration of cancer cells through confined spaces can induce heterochromatin formation, which is expected to both alter the physical properties of the nucleus and to modulate gene expression. These modifications could promote further metastatic cancer progression. Targeting the ability of chromatin condensation upon confined migration may potentially serve as a novel approach in treating metastatic cancers.

M98

**Identification of the Cellular Determinants of Stochastic Transcriptional Bursting**

**V. Sood**, Y. Wan, G. Pegoraro, P. Gudla, D. Larson, T. Misteli; National Cancer Institute, NIH, Bethesda, MD.

Stochastic gene bursting is a fundamental hallmark of transcription across species, yet the molecular basis for this property are unknown. To identify cellular determinants of stochastic transcriptional bursting, we performed high-throughput imaging screens of a chemical library containing 410 inhibitors of chromatin modifying enzymes and a library of 521 siRNAs that target chromatin factors. We assayed bursting changes using both RNA-FISH and a live cell transcription assay for 14 target genes that covered a wide range of expression level and burst frequencies, in human bronchial epithelial cells. The chemical screen revealed that genes with high burst frequencies showed only modest changes in response to most inhibitors, while genes with low burst frequencies displayed significant changes in bursting rates with several inhibitors. These differences suggest that bursting for highly expressed genes is less sensitive to chromatin modifiers, whereas chromatin has a significant influence on the bursting of genes with low expression levels. The most common molecular pathways to either increase or decrease bursting rates of a given gene involved histone deacetylases (HDAC) and bromo-domain containing proteins (BCPs). Live-cell measurements revealed that the increased bursting resulted from a specific decrease in off-time, whereas the decreased bursting was primarily caused by reduction in on-time. The complementary RNAi screen showed that long-term depletion of HDACs and BCP proteins exclusively downregulated bursting frequency whereas knockdown of two lysine demethylases increased bursting. The differences in bursting between short-term inhibition and long-term depletion of same chromatin factors in chemical and RNAi screen, respectively, suggest distinct mechanism in the establishment and maintenance of bursting frequencies. Our results provide the first insights into the molecular basis of transcriptional bursting and they suggest that regulation of gene-specific bursting is a multi-factorial process influenced by expression levels, chromatin context and the duration of the inhibition.

M99

**Structure and Function of Mammalian Swi/snf Chromatin Remodeling Complexes in Human Cancer**

**C. Kadoch**; Dana- Farber Cancer Institute and Harvard Medical School, Boston, MA.

Genome-wide sequencing studies in human cancer have unmasked a striking frequency of mutations in the genes encoding subunits of the mammalian SWI/SNF (BAF) family of ATP-dependent chromatin remodeling complexes. Our laboratory uses biochemical, structural, and functional genomics-based approaches to study rare, genetically well-defined pediatric cancers including synovial sarcoma, Ewing sarcoma, malignant rhabdoid tumor and others, all of which involve BAF complex perturbations as critical drivers of their oncogenic programs. These studies have informed the mechanistic basis underlying BAF complex targeting and function and have provided new foundations for therapeutic development.

## Minisymposium 10: Mitosis and Meiosis

M100

**Cryo-ET Analysis of the Yeast Synaptonemal Complex in *Situ***

**L. Gan**, O. X. Ma, S. Cai, J. Shi; National University of Singapore, Singapore, SINGAPORE.

The synaptonemal complex (SC) is the large proteinaceous scaffold that assembles between homologous chromosomes by the end of meiotic prophase. While its functions are numerous -- and mysterious, in yeast the SC is required for the phenomenon known as crossover interference. Knowledge of the native structure of this complex is needed to evaluate how the SC carries out its functions. Traditional electron microscopy and super-resolution light microscopy have revealed that in many organisms, the SC has a ladder-like structure: two rail-like lateral elements are bridged by a set of rung-like transverse filaments. The filaments are connected along their centers by a central element. To determine the 3-D architecture of the SC and nuclear macromolecular complexes in situ, we studied frozen-hydrated meiotic yeast cell cryosections by Volta electron cryotomography and subtomogram analysis. We find the predominant SC motif is a duster-like arrangement of densely packed triple-helical filaments, both of which are also abundant in the polycomplexes of pachytene-arrested cells. There was no evidence for a ladder-like organization. Partial dissolution by 1,6-hexanediol treatment suggests that these triple-helical filaments belong to the central region of the SCs and are most likely the abundant Zip1 transverse element protein. Subtomogram averaging revealed that the SC's triple helix is up to 12-nm thick and has a rise of 5 nm and a pitch of 130 nm. Polymers thinner than the triple helix, such as single or double strands, were not detected; this observation is consistent with the strong self-oligomerization properties of SC proteins. The dense packing of SC subunits supports the notion that the SC's mechanical properties help coordinate the rapid end-to-end communication across synapsed chromosomes. Finally, our study provides a 3-D framework for understanding the other macromolecule machines of meiosis *in situ*.

M101

**CDK-2 Mediates Crossover Designation through Phosphorylation of the Mutsγ Complex**

**J. Haversat**[1], A. Woglar[2], K. Klatt[1], V. Roberts[1], S. Arur[3], A. M. Villeneuve[2], Y. Kim[1]; [1]Johns Hopkins University, Baltimore, MD, [2]Stanford University, Stanford, CA, [3]M.D. Anderson Cancer Center, Houston, TX.

Meiotic recombination initiates with the generation of programmed DNA double-strand breaks (DSBs). Numerous early recombination intermediates accumulate until each homolog pair receives at least one potential crossover intermediate; a few of these early intermediates are selected to mature into crossovers, whereas the majority of DSBs are repaired as non-crossovers. Nevertheless, the mechanisms governing the designation of crossovers remains poorly understood. A genetic screen in *C. elegans* identified a cyclin-like protein, COSA-1, that is essential for processing meiotic DSBs into crossovers. This finding was subsequently followed by the identification of its mammalian ortholog CNTD1, which has conserved roles in crossover formation. However, no CDK has been identified as a kinase partner for COSA-1/CNTD1, and how these cyclin-like proteins designate crossovers is not known. Here we report that CDK-2, the *C. elegans* ortholog of CDK2, associates with COSA-1 to promote crossover formation. CDK-2 and COSA-1 form a complex *in* vitro, exibit colocalization in structured-illumination microscopy images both at early recombination intermediates and at crossover-designated sites in late pachytene, and are interdependent for localization at recombination sites While CDK-2 depleted germ cells exhibit normal homolog pairing and synapsis, they fail to designate meiotic DSBs into crossovers. In the absence of CDK-2, MutSγ component MSH-5 and the BLM helicase load onto early recombination intermediates at numerous foci during a prologed early pachytene stage; however, they fail to form the cruciform

structures observed at crossover-designated sites upon transition to late pachytene in wild-type. MSH-5 contains multiple putative CDK phosphorylation sites within its C-terminal tail, and it is phosphorylated *in vivo* in a CDK-2 and COSA-1 dependent manner. Further, truncation mutants of MSH-5 lacking C-terminal CDK phosphorylation sites fail to form crossover-designated intermediates and exhibit a prolonged early pachytene. Taken together, our work suggests that CDK-2 promotes designation and maturation of crossover sites through phosphorylation of MSH-5.

M102

**Mechanisms of Acentrosomal Spindle Assembly and Maintenance in *C. Elegans* Oocytes**

**I. D. Wolff**, S. M. Wignall; Northwestern University, Evanston, IL.

In female germ cells, or oocytes, the meiotic divisions are mediated by a microtubule-based spindle that is built and stabilized in the absence of centrosomes. While much is known about the proteins and forces underlying centrosome-based spindle assembly in mitotically-dividing cells, the molecular basis of force generation in acentrosomal spindle assembly and maintenance is far less understood. We are using *C. elegans* as an *in vivo* model to investigate these mechanisms. We previously characterized the stages of acentrosomal spindle assembly in *C. elegans* oocytes and found that KLP-18, a kinesin-12 family microtubule motor, and MESP-1, a rapidly evolving adaptor protein, are essential for outward force generation during this process. However, the biochemical mechanism of how these proteins generate force was unknown. We have now employed a combination of *in vitro* and *in vivo* approaches to gain insight into this important problem. First, we purified recombinant truncations of the KLP-18 coiled-coil stalk domain along with full length MESP-1 to use in microtubule binding experiments *in vitro.* We identified a novel non-motor microtubule binding site at the C-terminus of the KLP-18 stalk and found that this microtubule binding site is activated through MESP-1 interaction with an adjacent region of the stalk. Therefore, MESP-1 binding to KLP-18 may allow the motor to crosslink two microtubules using its motor and C-terminal microtubule binding domains, thereby generating force. To interrogate this model, we tested the importance of the KLP-18 C-terminal microtubule binding site *in vivo* using a temperature sensitive mutant strain containing two amino acid substitutions in the mapped domain. Prolonged incubation at the restrictive temperature caused spindle assembly defects that are identical to those observed following depletion of KLP-18 by RNAi; monopolar instead of bipolar spindles formed. In addition, we found that short incubation of this mutant at the restrictive temperature caused the collapse of already formed bipolar spindles into monopoles. In both cases, KLP-18 still localized to the aberrant spindles, indicating that the protein is present but non-functional. These results demonstrate that the C-terminal microtubule binding site that we identified *in vitro* is required for both spindle assembly and for the maintenance of spindle bipolarity *in vivo*. This work sets the basis for further investigation into how microtubule-associated proteins govern spindle assembly and maintenance, specifically in a system lacking centrosomes.

M103

**Non-overlapping Functions of Aurkb and Aurkc in Regulating the Spindle Assembly Checkpoint During Oocyte Meiosis**

**C. S. Blengini**, A. L. Nguyen, G. Jung, K. Schindler; Rutgers University, New Brunswick, Piscataway, NJ.

Aneuploidy is the leading genetic abnormality causing infertility and congenital birth defects in humans. Meiotic errors leading to aneuploidy are most frequently during meiosis I (MI) in females. The Aurora

Kinase (*Aurk*) gene family plays a major role in both mitotic and meiotic cell divisions. Mammalian mitotic cells require two Aurora Kinases, Aurora Kinase A (AURKA) and Aurora Kinase B (AURKB), while germ cells that undergo meiosis require a third, Aurora Kinase C (AURKC). AURKB has well-defined roles in triggering the destabilization of improperly attached kinetochore-microtubules (KT-MT) and recruiting components of the SAC to prevent aneuploidy in mitosis. However, little is known about how AURKB and AURKC regulate the SAC in female meiosis. In mouse oocytes AURKB localizes to spindle microtubules and AURKC localizes to chromatin. Given the distinct localizations of AURKB and AURKC, we hypothesized that the two kinases evolved to regulate the SAC in different pathways during female meiosis in mammals. To test this hypothesis, we used genetic and pharmacological approaches. First, we compared the SAC integrity and the ability to correct improper KT-MT attachments in oocytes between AURK knockout (KO) mice: AURKC KO (C KO) and *Gdf9-Cre*; *Aurkb*$^{fl/fl}$ KO (B KO) mice in which *Aurkb* is deleted specifically in oocytes. We observed that C KO oocytes had more abnormal KT-MT attachments, recruited MAD2, and arrested in MI when matured in the presence of nocodazole. On the other hand, although B KO oocytes had normal KT-MT attachments, there was a deficiency in MAD2 recruitment to kinetochores and they completed MI in the presence of nocodazole, suggesting that the SAC is weakened in absence of AURKB. Furthermore, we observed that B KO oocytes recruited MPS1, suggesting that the function of AURKB functions in an MPS1-independent manner to activate the SAC in mouse oocytes. Finally, we combined the genetic approach with a pharmacological approach to create oocytes with one active Aurora kinase, AURKB. To this end, we matured C KO oocytes in an AURKA-specific inhibitor, MLN8237. Our data showed that AURKB alone is sufficient to recruit and activate the SAC. These results highlight the presence of two independent pathways that separately involve AURKB and AURKC in the activation of the SAC to ensure correct chromosome segregation during female meiosis in mouse.

M104

**Tetraploidy Causes Chromosomal Instability in Acentriolar Mouse Embryos**

**L. Gomes Paim**[1], G. FitzHarris[1,2]; [1]Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montréal, QC, CANADA, [2]Département d'Obstétrique-Gynécologie, Université de Montréal, Montréal, QC, CANADA.

Tetraploidy can trigger chromosomal instability (CIN) and drive aneuploidy in somatic cells, and is likely a common steppingstone in tumorigenesis. The accepted mechanism by which tetraploidy is thought to drive CIN in somatic cells relates to the excess of centrioles generated by the tetraploid state, leading to hazardous multipolar spindles and chromosome segregation error. Here we set out to address the consequences of tetraploidy in the mouse preimplantation embryo, which is naturally devoid of centrioles throughout the first ~4 days of development. Our experiments show that, similarly to somatic cells, tetraploidy causes increased rates of anaphase chromosome segregation defects (55% in tetraploids vs. 16.6% in controls) and increased levels of aneuploidy (76% in tetraploids vs 32% in controls), confirming that tetraploidy indeed drives CIN in the mouse embryo. High resolution live imaging revealed that the first division following tetraploidization was characterized by the formation of two independent bipolar spindles that fused to form a single bipolar spindle prior to anaphase onset. This phenotype is morphologically distinct from previously described multipolar spindles as the two spindles remained indistinguishable throughout fusion. The subsequent cell division was characterized by the assembly of a single bipolar spindle, confirming that the CIN observed cannot be attributed to supernumerary centrioles or multipolar spindles. Fluorescence dissipation after photoactivation of

PAGFP-tubulin analyses revealed significantly increased kinetochore-microtubule half-life in tetraploids (3.38 ± 0.33 min) as compared to controls (2.28 ± 0.12 min), suggesting decreased microtubule turnover and error correction activity. Consistent with reduced error correction activity, a classic cold-shock approach revealed a far greater proportion of kinetochore-microtubule mis-attachments in tetraploid embryos (7%) as compared to controls (0.9%). Moreover, we found that the microtubule depolymerizing protein MCAK was substantially underrepresented at the kinetochore in tetraploid embryos (94.53 ± 8.85 a.u.) as compared to controls (120.86 ± 5.71 a.u.), and that overexpression of MCAK:GFP was able to rescue the chromosome segregation error phenotype. These results suggest that defective MT-kinetochore error-correction activity is responsible for the high rates of segregation error in tetraploid embryos. Together, our experiments demonstrate that altered microtubule dynamics can confer CIN after tetraploidization, and we thus propose that altered microtubule dynamics and supernumerary centrioles are two separate defects each with the capacity to drive CIN in the first cell cycles following tetraploidization.

M105

**A Molecular Mechanism for Assembly of the Pre-procentriole**

T. A. McLamarrah[1], **G. C. Rogers, 85724**[1], J. M. Ryniawec[1], S. Speed[2], D. W. Buster[1], C. J. Fagerstrom[2], B. J. Galletta[2], N. M. Rusan[2]; [1]University of Arizona, Tucson, AZ, [2]National Institutes of Health, Bethesda, MD.

Centriole duplication begins with the accumulation of several assembly factors at a single asymmetric spot on mother centrioles, which eventually gives rise to a procentriole. Specifically, this involves the hierarchical recruitment of a conserved set of centriole proteins, including Polo-like kinase 4 (Plk4)/ZYG-1 and Ana2/SAS-5/STIL, followed by the cartwheel protein Sas6. Recruitment of these proteins occurs during late mitosis in *Drosophila* cells, first forming a nebulous structure known as the 'pre-procentriole' and later, during the subsequent S-phase, assembles into a procentriole containing a central cartwheel and a cylinder of microtubule bundles. Because the pre-procentriole is the progenerative platform for nascent centriole growth, our study focuses on determining the molecular details of its composition, structure and assembly, which are largely unknown. Initially, Plk4 decorates the surface of mitotic centrioles due to its interaction with a centriole-targeting factor. Next, Ana2/STIL is recruited and stimulates Plk4 kinase activity. In turn, Ana2/STIL's C-terminal STAN domain is phosphorylated by Plk4, generating a high-affinity phospho-binding site for Sas6 loading. Although the assembly steps immediately preceding Sas6-loading appear clear, the mechanism underlying the upstream pre-procentriole recruitment of Ana2/STIL is not. In contrast to proposed models of Ana2/STIL recruitment, we recently showed that loading of *Drosophila* Ana2 onto procentrioles is actually independent of Plk4 binding, but does require Plk4's catalytic activity. We have discovered that the amyloid-like domain of Sas4, a centriole surface protein, binds Plk4 and Ana2. Sas4 binding facilitates phosphorylation of Ana2's N-terminus which increases Ana2's affinity for Sas4. Consequently, Ana2 accumulates at the

procentriole to induce daughter centriole assembly. We propose a new multi-step pathway for the initial assembly of the pre-procentriole and provide insight into how Sas4 may define the site of daughter centriole assembly.

M106

**Crispr-cas9-based Tiling Screens Reveal Protein Domain Structure and Novel Functional Regions in Human Mitotic Factors**

**J. Herman**, L. Carter, S. Biggins, P. Paddison; Fred Hutchinson Cancer Research Center, Seattle, WA.

A critical knowledge gap for the human genome has arisen from our inability to resolve important functional domains and motifs within protein coding genes at the large scale. Historically, large scale annotation of protein domains and motifs relied on homology based-inference by searching against the current 5494 conserved protein family (Pfam) domains documented in the human genome (e.g., methyltransferase-like domain). This approach is ineffective for the ~45% of the proteome that is devoid of Pfam domains, and still requires validation for the remaining genes. Closing this knowledge gap is critical for both basic and disease-focused biomedical research, where years, if not decades, can be spent dissecting gene functions. Here we leverage the mutagenic properties of CRISPR-Cas9 by saturating sgRNAs across the coding sequence of a gene to identify essential domains and motifs. A panel of diploid and aneuploid cells suggest critical, phenotypically constrained regions do not tolerate in-frame CRISPR-Cas9 indels. As a result, each gene produces a unique mutational signature, with constrained regions scoring as phenotypic "peaks". As a test case, we performed tiling mutagenesis to resolve the domain structure for 48 well characterized kinetochore-associated genes and identified approximately 160 functional regions, of which nearly 1/4 have not been previously described. Novel regions were found in a diverse set of mitotic factors including: SKA3, Rod, Spindly, Shugoshin, Mad1, and chTOG and preliminary evidence suggests many of them contribute to protein-protein interactions responsible for sub-cellular localization. This powerful genetic approach allows rapid and inexpensive dissection of essential protein activities expanding our understanding of genic structure for application to both basic science and disease-focused questions.

M107

**Aurora B Kinase Is Recruited to Multiple Discrete Kinetochore and Centromere Regions in Human Cells**

**J. G. DeLuca**, A. J. Broad, K. F. DeLuca; Colorado State University, Fort Collins, CO.

Precise regulation of interactions between kinetochores and spindle microtubules is required for proper chromosome bi-orientation prior to cell division. Aurora B kinase has a critical role in this regulation by phosphorylating kinetochore substrates. Early in mitosis, kinase activity at kinetochores is high to promote attachment turnover, and in later mitosis, activity decreases to ensure attachment stabilization. Aurora B localizes prominently to inner centromeres, and a population of the kinase is also detected at kinetochores. How Aurora B is recruited to and evicted from these regions to regulate kinetochore-microtubule attachments remains unclear. Here, we identified and investigated discrete populations of Aurora B at the centromere/kinetochore region. An inner centromere pool, recruited by Haspin phosphorylation of histone-H3, and a kinetochore-proximal outer centromere pool, recruited by Bub1 phosphorylation of histone-H2A, although spatially distinct, both contribute to accumulation of inner centromere Aurora B. A third pool, localized ~20 nm outside of the inner kinetochore protein CENP-C in early mitosis, does not require Bub1/pH2A/Sgo1 or Haspin/pH3 pathways for localization or

activity. Finally, we investigated the mechanisms for recruitment of Aurora B kinase to the outer kinetochore. Our results suggest that distinct molecular pathways are responsible for Aurora B recruitment to centromeres and kinetochores.

M108

**Cellular Limits on the Anaphase Spindle Elongation Rate Ensure that Chromosomes Are Dynamically Disentangled During Cell Division**

**S. Mukherjee**[1], D. Tank[1], E. Davidson[2], M. McClellan[1], T. Davis[2], M. K. Gardner[1]; [1]University of Minnesota, Minneapolis, MN, [2]University of Washington, Seattle, WA.

At anaphase, elongation of the mitotic spindle facilitates the segregation of duplicated sister chromatids into future daughter cells. This elongation is driven by forces derived from the combined action of motor proteins and microtubule polymerization at the spindle midzone. In all known eukaryotes, a regulatory switch linked to the metaphase to anaphase transition greatly reduces the magnitude of force that is produced at the spindle midzone, thereby limiting the maximum speed at which the spindle elongates in anaphase. While the molecular correlates of this regulatory switch have been described in detail, the cellular consequences of exceeding this speed limit is unknown. In this study, we dramatically increased the speed of anaphase spindle elongation in budding yeast by introducing four point mutations into *Cik1*, a binding partner of the kinesin 14 motor protein Kar3. These mutations abrogate the interaction of the Kar3-Cik1 complex with the plus end microtubule tracking protein Bim1. Loss of this interaction leads to a ~3-fold increase in anaphase spindle elongation rate, without causing spindle collapse. We found that cells with these rapid anaphase spindle elongation rates were unable to properly disentangle separating chromosomes, leading to increased persistence, and more frequent breakage, of ultrafine DNA bridges. As a result, cells with rapid anaphase spindle elongation rates had increased incidence of DNA damage at anaphase, as well as delays in cytokinesis, ultimately leading to a ~6-fold increase in cells that were stalled in G2 with improper chromosomal content. Taken together, these findings describe a novel and critical protective function of the speed limit that cells impose upon the anaphase spindle elongation rate: the timing that is set by a tightly regulated anaphase spindle elongation speed is required to ensure that chromosomes are dynamically disentangled during anaphase chromosome segregation.

M109

**Abscission Couples Cell Division to Exit From Pluripotency**

**A. Chaigne**[1], M. B. Smith[2], C. Labouesse[3], E. Hannezo[4], K. J. Chalut[3], E. K. Paluch[5]; [1]MRC/LMCB, University College London, London, UNITED KINGDOM, [2]The Francis Crick Institute, London, UNITED KINGDOM, [3]Stem Cell Institute, University of Cambridge, Cambridge, UNITED KINGDOM, [4]IST Austria, Klosterneuburg, AUSTRIA, [5]Department of Physiology, Development and Neuroscience, University Of Cambridge, Cambridge, UNITED KINGDOM.

Embryonic stem cells are pluripotent cells that can give rise to the three germ layers and the germline after exit from naïve pluripotency. In some adult stem cells, asymmetric division acts as fate switch where one daughter cell go towards differentiation while the other remains pluripotent, suggesting that cell division could be a universal fate switch. In embryonic stem cell, inhibiting cell division impairs exit from naïve pluripotency, yet the mechanism for this has remained elusive. We demonstrate that mouse embryonic stem cells exit from pluripotency at cell division. Strikingly, cells in 3D colonies, a geometry

resembling a 3D embryo, can divide asymmetrically, and we demonstrate that E-Cadherin dependent cell-cell junctions control the geometry of the division. Surprisingly, the timing of division itself, but not asymmetric division is responsible for heterogeneities in exit from pluripotency, as generating substantial asymmetries in size does not alter cell fate. Strikingly, we identify the last step of cell division, abscission, to be responsible for the coupling of cell division and exit from pluripotency. Indeed, pluripotent cells maintain tubulin bridges long after cell division; the maintenance of these bridges are detrimental to exit from pluripotency. Conversely, cells exiting pluripotency do not maintain tubulin bridges. Thus, we propose that cell division constitutes a universal fate changing switch, while the specific mechanisms of this switch are cell type dependent.

## Minisymposium 11: New Tools and Organisms in Quantitative Cell Biology

M110

**Applying Genetic Code Expansion and Bioorthogonal Labeling to Quantitative High-resolution Live Cell Imaging**

**N. Elia**, A. Koening, I. Segal; Ben Gurion University of the Negev, Beer Sheva, ISRAEL.

Genetic code expansion (GCE)-based bioorthogonal labeling is an emerging approach for fluorescence labeling of proteins in live cells. In this approach, proteins are directly labelled with fluorescent dyes at 1:1 ratio. The superb photophysical properties of fluorescent dyes together with the minimal size of the tag (an order of magnitude smaller than GFP) hold great potential for improving the performance of current methods for quantitative imaging and super resolution microscopy. However, in spite of its potential, this approach is not widely used in live cell imaging applications. Here, we set out to expand the use of this approach and test its performance in quantitative single molecule applications. To this aim, we: 1) optimized conditions for intracellular and extracellular labeling of proteins in live mammalian cells; 2) engineered a 14-residues long N-terminal peptide tag for straightforward bioorthogonal labeling of essentially any cellular protein and constructed a library of GCE-tagged organelle markers; and 3) calibrated conditions and performed Single Particle Tracking (SPT) and live-Single Molecule Localization Microscopy (SMLM) of bioorthogonally labeled proteins in mammalian cells. Conclusively, our results show that GCE and bioorthogonal chemistry is a suitable, flexible approach for protein labeling in quantitative high-resolution live cell microscopy that outperforms current live cell labeling approaches. This opens up new opportunities for quantitative analysis of cellular processes in the cellular milieu with improved spatiotemporal accuracy.

M111

**Towards “Autonomous” Microscopy: Artificial Intelligence Microscopy Screening (aims)**

**G. Kanfer**[1], Y. Maman[2], S. Sarraf[1], H. Baldwin[1], J. Lippincott-Schwartz[3], R. Youle[1]; [1]Biochemistry Section, Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, [2]Laboratory of Genome Integrity, National Cancer Institute, NIH, Bethesda, MD, [3]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA.

In cell biology, the identification of new pathways via genetic screens has been a central discovery strategy. Historically, this work was performed most readily in bacteria and yeast; however, it is important to advance this platform in mammalian cells. RNAi-based screens have been used to elucidate pathways in human cells. This approach has relied on two major strategies: arrayed screens, which have

high specificity but require the production of each RNAi separately creating a technical bottleneck, and pooled screens, in which production is easier, but specificity and reproducibility suffer. To overcome these limitations, we have developed a novel screening approach termed **A**rtificial-**I**ntelligence **M**icroscopy **S**creening (designated AIMS). The new platform "converts" single cells into “separate” wells by applying machine learning and deep learning algorithms to detect subcellular phenotypes. In brief, a genome-wide suppressor screen is performed on cells expressing dCas9 (CRISPR inhibition) by infecting a single well of cells with pooled guide-RNA (gRNA)-expressing lentivirus so that every cell will express a distinct gRNA. These cells also stably-express a photo-activatable red fluorescent protein (pa-mCherry). Before the screen, a deep learning neural network model is trained on several examples of the phenotype to be queried. Pooled gRNA-infected cells are screened by microscopy and individual cells exhibiting the phenotype that was screened are identified, photoactivated, and isolated via flow cytometry. To identify phenotype to genotype connections, the gRNA lentiviral integration site is sequenced in single cells from the selected sorted cells. We explored the regulation of the transcription factor TFEB. In response to the cellular metabolic state, TFEB initiates lysosomal and autophagy transcriptional programs. Using AIMS, we screened for factors involved in TFEB translocation in or out of the nucleus. In addition to several known hits, novel TFEB regulators were also detected. These results demonstrate the feasibility of AIMS. Our approach is not only a novel implementation demonstrating how machine learning can be used to to explore cell biology, but, this new platform also enables phenotypic-based screening at the subcellular level, an approach which has been largely unavailable.

M112

**Live Simultaneous Multi-receptor Tracking in Neurons with 5-D Single Molecule Localization Microscopy**

**C. Butler**[1,2], G. E. Saraceno[1,2], M. A. Kechkar[3], V. Studer[1], L. Groc[1,2], R. Galland[1,2], J. Sibarita[1]; [1]University of Bordeaux, Interdisciplinary Institute for Neuroscience, Bordeaux, FRANCE, [2]CNRS UMR5297, Bordeaux, FRANCE, [3]Ecole Nationale Supérieure de Biotechnologie, Constantine, ALGERIA.

Single particle tracking (SPT) techniques such as sptPALM, uPAINT, and quantum dot tracking have given unprecedented insight into molecular dynamics in living cells. They allow monitoring the behavior and molecular interaction of individual proteins at millisecond temporal resolution and high spatial resolution (<30 nm) by fitting the point spread function (PSF) of individual emitters and tracking their position over time. While these SPT methods have been extended to study the temporal dynamics and co-organization of multiple proteins, conventional experimental setups used to perform multicolor imaging are typically limited to two simultaneous wavelengths. Increasing the number of colors requires additional filters for specific fluorescent tags and is usually performed at the expense of spatial or temporal resolution and/or field of view. This limits the minimum diffusion coefficient that can be measured and reduces the statistics that can be gathered from a single experiment, thereby degrading the ability to differentiate between molecular diffusion regimes like immobilization and confined diffusion. Moreover, simultaneous multi-receptor tracking could also reveal specific interactions between different protein populations, which could previously only be inferred from the behavior of a single population without any knowledge of the presumed partner behavior.By employing a dual-objective imaging configuration compatible with routine live cell imaging, we will present a single molecule tracking technique that allows for simultaneous 3D single particle tracking of multiple distinct species without compromising spatio-temporal resolution. A dispersive element introduced into the second optical path induces a spectrally-dependent displacement, which is used to separate numerous

fluorescent species of single emitters based on their emission spectra. A proof of concept of the spectral separation abilities of the system will be shown via simultaneous 3D DNA-PAINT of fixed samples, where the acquisition time is significantly reduced compared to conventional sequential multicolor imaging. Lastly, we will demonstrate how the technique can be applied to track multiple receptors in live neuron cultures, and we will discuss possibilities of how advanced data analysis techniques can fully exploit the 5-dimensional data (x,y,z,t,λ) to extend the capabilities of conventional single particle tracking, such as the investigation of protein-protein interactions.

M113

**High Speed Single Molecule Dynamics in the Endoplasmic Reticulum**

**C. J. Obara**[1], J. Nixon-Abell[2], F. Riccio[2], C. Blackstone[2], J. Lippincott-Schwartz, 20147[1]; [1]HHMI Janelia Research Campus, Ashburn, VA, [2]NINDS\NIH, 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 also maintains elaborate and highly dynamic contacts with essentially all the other subcellular organelles. Despite this clear role as a central regulator of many important processes in cell biology, the way in which the many functions of the ER are spatially regulated has remained enigmatic. Diffraction-limited imaging experiments have failed to identify clear separation between components of ER biology, suggesting that functional microdomains within the membrane or lumen are either too small or too transient to be detected by conventional imaging techniques. Here, we correlate high speed single molecule trajectories of proteins in the endoplasmic reticulum to simultaneously collected imaging data of the context in which the molecules are moving. We show this approach works well with structured illumination microscopy, hyperspectral imaging, and even simple diffraction-limited imaging using a standard commercial widefield scope. By analyzing the trajectories of ER-localized proteins in the context of the ER itself, we show that functional microdomains do frequently exist in the ER, however they appear to be highly regulated in both space and time. For some classes of proteins, the existence of these domains is highly dependent on the underlying membrane structure, while others are transiently recruited to sites of interaction with specific cytoplasmic factors, often contact sites with other organelles or large protein complexes. Collectively, this data suggests correlating single molecule trajectories into cellular context may provide a valuable tool for connecting biological process regulation across diverse spatial scales.

M114

**Engineering Biologically-relevant Boundaries: Optochemical Patterning of a Synthetic Cell Boundary to Probe Spindle Positioning**

**J. G. Bermudez, 19104**, M. C. Good; University of Pennsylvania, Philadelphia, PA.

In all multicellular organisms, proper development and adult tissue renewal rely on precise cell divisions. Cell division defects are associated with developmental disorders, neurological defects, and cancer, and they are linked to mispositioning of the mitotic spindle during anaphase. Mutations have been identified in a suite of proteins, including tumor suppressors Adenomatous polyposis coli (APC) and p53, that are thought to function in spindle positioning. This suggests that disruption of spindle positioning can lead to cell division defects and subsequent disease. However, a major challenge has been characterizing the

functional roles of these proteins and how their mutation contributes to disease pathology.Studies in living cells are confounded by the presence of tens of thousands of distinct macromolecules and the complex nature of their interactions. An approach that overcomes these hurdles is to characterize minimal components in emulsion systems, which match the geometry of cells. I encapsulated recombinant optochemical proteins and microtubules inside water-in-oil emulsions. The optochemical system consists of FRB and FKBP fusion proteins and a photocaged dRap. Upon 405 nm laser illumination, dRap is uncaged and free rapamycin promotes dimerization of FKBP and FRB, thereby translating an optical input to a biochemical output. After 1 second of illumination, GFP-FRB protein is quickly relocalized to the boundary to generate an anisotropic boundary. I light-inducibly recruited microtubule adapter proteins (i.e., LGN) and GFP-FRB fusion proteins in a similar manner to examine their effects on spindle microtubule position in a minimal context. An additional advance is that we can incorporate cell-free systems that can carry out aspects of the cell cycle *in vitro*, including spindle assembly.By leveraging emulsion, cell-free extract, and optochemical approaches, we can pattern a synthetic boundary and build spindle interactions from the bottom-up to characterize the dynamics and spatial patterning of molecular factors required for proper cell division. This platform will provide new insights into the molecular mechanisms underlying spindle positioning, and ultimately generate paradigms to explain how defects arise in cell division and contribute to disease.

M115

**Blastoderm Formation in Crickets: a Crowding Mechanism Explains Nucleus Speed, Direction, and Division Timing**

**S. Donoughe**[1], J. Hoffmann[2], T. Nakamura[3], C. H. Rycroft[2,4], C. G. Extavour[2]; [1]University of Chicago, Chicago, IL, [2]Harvard University, Cambridge, MA, [3]National Institute for Basic Biology, Okazaki, JAPAN, [4]Lawrence Berkeley Laboratory, Berkeley, CA.

In most insects, development begins as a syncytium: that is, many nuclei divide and move within the single shared cytoplasm of the egg. It is essential for the nuclei to form a single layer—the blastoderm—which becomes the incipient embryo. A longstanding question is how these proliferating and migrating nuclei self-organize into a single layer with the proper number, timing, and spatial arrangement. Recent work has shed new light on this puzzle in *Drosophila melanogaster*, showing that flies employ cytoplasmic flows and a mechanism that synchronizes divisions. Here we show that the cricket *Gryllus bimaculatus* has an altogether different solution to the problem. By live-imaging yolk and 3D-tracking nuclei in transgenic cricket embryos, we find that: (1) Cytoplasmic flows are unimportant for nucleus movement. (2) Division cycle period varies widely among coexisting nuclei, correlating with local nuclear density. (3) Nucleus speeds and orientations throughout blastoderm formation can be explained by a local crowding mechanism. Based on these findings, we present a simple geometric model that describes embryo-wide patterns of nucleus behavior. We use the model to predict what would happen to nucleus movements if an egg were to be physically constricted. Then we empirically test this prediction by constricting eggs, finding that the resulting nuclear behaviors are consistent with simulated embryos. Finally, we put the cricket and fruit fly data in the greater context of insect diversity. Across insects, syncytial eggs come in an enormous range of shapes and sizes. We hypothesize that over a macroevolutionary timescale, egg size and shape co-vary predictably with aspects of nucleus dynamics in early insect embryos.

M116

**Location-dependent Noncanonical Elemental Composition and Cellular Architecture of the Statolith and Underlying Cells in the Early-diverging Metazoans, *Beroe Ovata* and *Mnemiopsis Leidyi*.**

**A. G. Moss**, W. Hames; Auburn University, Auburn, AL.

Ctenophores, the largest animals that move primarily by means of beating cilia (the comb plates), are thought to have diverged from all other multicellular animals at least 350 mya, and the ultimate origin of the entire group is probably set much deeper in time. In this context, the remarkable observation by Tamm's group recently of the unusual organization, mechanisms of construction and cellular origin of the statolith has considerable import, because it describes for the first time an alternative, extremely ancient solution to a common problem: the organization and structure of a gravity-sensing organ. Here, we explore further the exceptional characteristics of these cells. A common characteristic of bio-deposited gravity sensing structures across animals is that they are calcareous: containing calcium. We sought to determine the composition and organization of the lithocyte concretions in order to better appreciate how this ancient group would respond to changing pH in the world's oceans. We confirmed, using DNA staining (Hoechst 33342, 1 µg/mL), that the lithocytes are MOSTLY nucleated; not all labeled with the stain. Newly forming lithocytes were not associated with Hoechst-positive staining. We used energy dispersive spectroscopy (EDS) and electron microprobe analysis (EMPA) to compare and contrast the composition of ctenophore lithocytes from the Woods Hole (WH) region and the northern Gulf of Mexico (nGOM). We found several distinct features common to all of the ctenophores, with distinct differences by location. 1) lithocytes from both species, whether examined by EDS or EMPA, lacked Ca or Mg and did not display any divalent cations. 2) The primary composition of the positive ion was that of Na, and in some cases, potassium, although sodium was the most common positive univalent ion present. 3) Oxygen, which was detectable by EDS but no by our EMPA system, was always present. 4) Sulfur was always present. 5) Cl was much more evident in the nGOM samples. 6) Crystal structure was very uniform in the WH samples. There was variable structure in the nGOM crystal arrays in dried down samples, but 7) intact lithocytes, while very refractile, were distinctly non-birefringent. The relative contribution of the different elements varied somewhat by location, probably indicative of the local nutrient available to the animals, which could reflect a very adaptive and opportunistic metabolism. We interpret these unexpected results to be indicative of an ancient metabolism carried down to the present day. We acknowledge the generous help of Dr. Oldenbourg, and Mr. L. Kerr, Marine Biological Laboratory. Supported by Auburn University, Office of the Vice President for Research.

M117

**A New Window Into the Evolution of Fungal and Animal Cell Biology: Genetic Transformation of the Chytrid *Spizellomyces*.**

**E. M. Medina**[1], K. A. Robinson[1], L. K. Fritz-Laylin[1], N. E. Buchler[2]; [1]University of Massachusetts, Amherst, MA, [2]North Carolina State University, Raleigh, NC.

Chytrids are deep fungal lineages that display traits associated with both animals and fungi, making them evolutionary transitional lineages. Chytrid spores and gametes, called zoospores, lack a cell wall, move with amoeboid movement and swim with a motile cilium nucleated from a centriole, all characters found in animal cells but lost in other fungi. The zoosporic stage then transitions into a more typical fungal developmental program, by building a cell wall and undergoing synchronous nuclear

divisions without cytokinesis before cellularizing and releasing new zoospores. The later stages of this developmental program are reminiscent of the cellularization of the *Drosophila* embryo and *Dictyostellium* polarized epithelial development, suggesting that the toolkit for multicellularity may have predated the divergence of the animal and fungal lineage. This unusual cell biology makes chytrids important research organisms to explore the emergence of multicellularity. Moreover, chytrids can provide an evolutionary framework for integrating animal and yeast cell biology: this single research organism developmentally switches from a ciliated amoeba with an actin cortex and protrusions into a "yeast" that undergoes a multicellular-like developmental program. To capitalize on chytrid biology, we have developed a method for stable and reliable genetic transformation of the soil chytrid *Spizellomyces*. By combining expression of fluorescently tagged proteins and live-cell imaging we have taken the first steps towards developing *Spizellomyces* as a model for animal-fungi evolutionary cell biology. We show how polymerized actin drives zoospore motility, and forms transient perinuclear actin shells that coincide with nuclear division. Additionally, we describe how the formation of membrane cleavage planes during cellularization may depend on the formation of three-dimensional polygonal territories of polymerized actin. We are currently extending these tools to use *Spizellomyces* as a model system to study the evolution of key animal and fungal traits, particularly cell cycle regulation, the evolution of cellular motility and the fungal developmental program.

M118

**Dissecting the Role of Innate Immunity for Symbiont Tolerance in Coral Endosymbiosis**

**A. Guse**, M. R. Jacobovitz, S. Rupp; COS, Heidelberg University, Heidelberg, GERMANY.

Phagocytosis is ancient and evolved as a predation mechanism for feeding (phagotrophy) used in single-celled organisms. Multicellular animals co-opted it to engulf and destroy invading microbes. Reef-building corals phagocytose dinoflagellate symbionts into their endodermal cells which transfer essential nutrients to their hosts. In fact coral-algal endosymbiosis is the foundation of the productivity of coral-reefs, ecosystems of great ecological importance. Interestingly, the innate immune repertoire of corals is nearly as complex as that of vertebrates. A long-standing puzzle is how symbionts avoid recognition and phagolysosomal digestion by the host, partly because corals are unsuitable as model systems. Here, we will introduce *Exaiptasia pallida* (commonly *Aiptasia*), an emerging anemone model for coral endosymbiosis. We exploit *Aiptasia* larvae, which acquire symbionts from the environment similar to their coral relatives to dissect the underlying mechanisms of intracellular symbiont persistence. Specifically, we established a comparative system to analyze the host response for dinoflagellate symbionts and evolutionary distinct microalgae ('a pathogen-like one' and a 'food-like' one). Using cell biology, live imaging, cell-type specific transcriptomics and chemical perturbation, we find that initial phagocytosis of microalgae is largely indiscriminatory. However, all algae but true symbionts are post-phagocytotically cleared from the host cells. Unexpectedly, cellular clearance of unwanted particles did not occur via digestion but expulsion of seemingly healthy microalgae. We find that within the first 24h of infection, dinoflagellate symbionts massively suppress expression of innate immunity-related genes in their individual host cells but not in neighboring cells. Further, we provide evidence that this immune suppression is an important prerequisite to escape expulsion: unspecific stimulation of innate immunity by LPS reduces the efficiency of symbiosis establishment by increasing expulsion rates but not symbiont persistence. Specifically, using small molecule inhibitors we find that suppression of MyD88 and

activation of ERK5 play a role during symbiosis establishment. Finally, we find that symbionts quickly recruit LAMP1 indicating phagolysosomal maturation but other microalgae do not. Taken together, we propose a novel model in which symbionts modulate innate immunity and establish a LAMP1-positive niche to escape cellular clearance by expulsion. Our findings have important implications for understanding the evolution of innate immunity, the relation of symbiosis to parasitism and understanding coral-reef ecosystems which are greatly threatened by environmental change.

M119

**Scale-free Tracking Microscopy of Freely Suspended Cells: Towards Connecting Cell Biology and Ocean Ecology**

**D. Krishnamurthy**[1], H. Li[1], A. Larson[1], D. Mion[2], E. Li[1], F. B. D. Rey[2], M. Prakash[1]; [1]Stanford University, Stanford, CA, [2]Ecole Polytechnique, Paris, FRANCE.

Many cells spend their entire lives freely-suspended in a fluid, rarely encountering any substrate. Uni-cellular marine plankton epitomize this lifestyle, where single cells can travel several tens to hundreds of meters vertically in the water column, as part of the largest daily bio-mass migration on our planet. Such migrations are key components of vertical material fluxes in the ocean and drive planetary-scale biogeochemical cycles, and form a major component of oceanic carbon sequestration. Measuring sub-cellular-scale processes in single cells while allowing free movement over ecological-scale distances is, therefore, crucial for mechanistically understanding the cell biology and ecology of the living ocean. However imaging over large distances along the axis of gravity is a considerable challenge for conventional microscopy: Given the tradeoff between optical resolution and field-of-view, how can one measure single organisms or cells, at microscale resolution, while allowing them to freely move hundreds of meters in the vertical direction? Here we present a solution in the form of a scale-free, vertical tracking microscope, based on a circular “hydrodynamic-treadmill” for single cells, with no bounds for motion along the axis of gravity. Our method demonstrates a new paradigm for simultaneous multi-scale measurement where microscale characteristics like cell-state and organismal behavior can be directly connected to macroscale outcomes like depth in the water column. Using our tool, we demonstrate novel biophysical measurements in two classes of ecologically relevant plankton. In single-celled diatoms spanning four species, we discovered rapid density fluctuations over millisecond time-scales by concurrently measuring cell behavior (vertical sinking speed) and cell’s molecular state by synthesizing our tracking microscopy method with fluorescence imaging of signaling reporters. In the dinoflagellate cells *P. noctiluca*, we observed, for the first time, cell-division in suspension, far from substrates and resolve microscale fluctuations in cell density during division and rapid changes in cell volume post-division. Finally, we combined our tracking methodology with environmental patterning of light, chemical species and ambient pressure to bring the virtual-reality paradigm, which is well-established in neuroscience, to cell biology in the ocean. We anticipate that our method will open up new avenues for understanding key biophysical processes in our oceans at sub-cellular resolution, and allow connections between cell biology and ecology. Beyond the oceans, we foresee that our method will allow novel cell-biological measurements by freeing cells from the confines of the coverslip.

## Minisymposium 12: Autophagy, Protein Turnover and Quality Control

M120

**Molecular Mechanisms of the Mitochondrial Motors of Mass Destruction**

**G. Lander**[1], C. Puchades[1], M. Shin[1], S. Glynn[2], W. Karzai[2]; [1]Scripps Research, La Jolla, CA, [2]Stony Brook University, Stony Brook, NY.

Mitochondrial AAA+ quality control proteases regulate diverse aspects of mitochondrial biology through specialized protein degradation, but the underlying molecular mechanisms that define the diverse activities of these enzymes remain mysterious. Two different mitochondrial AAA+ proteases reside in the inner mitochondrial membrane but expose enzymatic domains to the intermembrane space and matrix. Using cryo-EM, we show that these hexameric complexes use a hand-over-hand mechanism of substrate translocation through a sequential ATP hydrolysis cycle. The basic translocation mechanism we describe is likely to be evolutionarily conserved from bacteria to humans. Our results provide a molecular basis for neurological phenotypes associated with different mutations and establish a structural framework to understand how different members of the AAA+ superfamily achieve specialized, diverse biological functions. While a hand-over-hand translocation is emerging as the conserved mechanism by which ATP hydrolysis drives substrate translocation within the classical clade of AAA+ proteins, the operating principles of the distantly related HCLR clade, which includes the important quality control protease Lon, is also of great interest. We determined a cryo-electron microscopy structure of Y. pestis Lon, revealing that although sequential ATP hydrolysis and hand-over-hand substrate translocation are conserved in this AAA+ protease, Lon processes substrates through a distinct molecular mechanism involving structural features unique to the HCLR clade. We define a previously unobserved translocation mechanism that is likely conserved across HCLR proteins and reveal how distinct structural configurations of distantly-related AAA+ enzymes can power hand-over-hand substrate translocation.

M121

**Novel Translation Repression Complex Prevents Ribosome Initiation on Faulty Messenger RNAs**

K. Hickey[1], **K. Kostova**[2], J. Replogle[1], K. Disckson[3], K. D'Orazio[4], N. Sinha[4], R. Green[4], J. Weissman[1]; [1]University of California, San Francisco, San Francisco, CA, [2]Carnegie Inst Washington, Baltimore, MD, [3]Lawrence University, Appletown, WI, [4]Johns Hopkins School of Medicine, Baltimore, MD.

Protein biosynthesis is the most energy-consuming process during cellular proliferation. Therefore, any event that interferes with protein production jeopardizes cell viability. A particular challenge is ribosome stalling where a ribosome gets trapped on an mRNA and can neither proceed with translation nor be released. To counter the threats posed by ribosome stalling, cells have evolved quality control pathways that detect and release the stalled ribosome, and degrade both the mRNA and the nascent polypeptide. Ubiquitination and subsequent degradation of the emerging nascent peptide is mediated by the Ribosome Quality control Complex (RQC). Although the RQC has been extensively studied in yeast, far less is known about the mammalian pathway.We have engineered a non-stop decay reporter and used it to perform a whole-genome CRISPR interference screen for factors that allow mammalian cells to cope with ribosome stalling. This unbiased approach allowed us to gain a comprehensive view of the mammalian RQC pathway, as well as to identify novel RQC components. We have discovered a three-component complex (ZNF598, GIGYF2 and 4EHP) that translationally silences faulty mRNAs by blocking

ribosome initiation. This novel quality control step prevents the formation of ribosome “traffic jams” on defective messages, alleviating the stalling burden in mammalian cells.

M122

**Activated Ire1 Oligomerizes Into Filaments Contained in Anastomosing 30 Nm Endoplasmic Reticulum Membrane Tubes**

**N. Tran**[1,2], S. Carter[3,2], V. Belyy[1,2], D. Acosta-Alvear[4], G. Jensen[3,2], P. Walter[1,2]; [1]University of California San Francisco, San Francisco, CA, [2]Howard Hughes Medical Institute, Chevy Chase, MD, [3]California Institute of Technology, Pasadena, CA, [4]University of California Santa Barbara, Santa Barbara, CA.

The unfolded protein response (UPR) is an intracellular signaling network that adjusts the abundance and protein folding capacity of the endoplasmic reticulum (ER) according to need. The most conversed branch of the UPR is mediated by the ER-resident transmembrane kinase/endoribonuclease IRE1. It senses unfolded protein accumulation within the ER and transduces the signal via a non-conventional mRNA splicing mechanism. In response to binding of unfolded proteins in the ER lumen, IRE1 activates by oligomerization and accumulates in dynamic foci. Fluorescence recovery after photobleaching (FRAP) and photoconversion experiments show that IRE1 molecules in the foci remain in equilibrium with IRE1 molecules in the surrounding ER network. We determined the structure of human IRE1 foci in whole cells by cryogenic correlative fluorescent light microscopy and electron tomography. Our results show that activated IRE1 oligomers induce membrane deformations and local ER membrane restructuring into extremely narrow, ribosome-free, anastomosing tubes (diameter ~30nm) with complex branching topology. Most excitingly, we found that the lumens of these ER tubes contain well-ordered filaments. We determined the lumenal filament structure *in situ* by subtomogram averaging to a resolution of 13Å. The electron density map reveals two intertwined helices formed by linear filaments that are likely composed of contacting dimers of IRE1’s lumenal domains. Our findings define a previously unrecognized subdomain of the ER membrane and shed new light on the structure and organization of activated IRE1 in mammalian cells.

M123

**ER Stress Response in a Premature Aging Disease**

**S. Vidak**, L. Serebryannyy, T. Misteli; NCI/NIH, Bethesda, MD.

Human aging is the biggest risk factor for many diseases including cancer, cardiovascular events and neurodegenerative diseases. Several human premature aging syndromes are characterized by features resembling normal aging, providing important insights into the molecular mechanisms underlying human aging. One such disease is Hutchinson-Gilford Progeria Syndrome (HGPS), an extremely rare premature aging disorder reflecting several aspects of normal aging. Classical HGPS is caused by a *de novo* heterozygous mutation in the *LMNA* gene encoding A-type lamins, major structural components of the cell nucleus. The HGPS-linked *LMNA* mutation leads to the expression of progerin, a mutant lamin A isoform, which causes numerous nuclear and cellular defects, including post-transcriptional reduction of select cellular proteins, pointing to an effect of the disease-causing progerin protein on protein homeostasis. To test this hypothesis, we have analyzed the levels and localization of several major cellular chaperones by high-throughput imaging. In inducible GFP-progerin and in patient-derived HGPS fibroblasts several cytosolic chaperones accumulate at the endoplasmic reticulum (ER). This re-localization is accompanied by the activation of an adaptive response to ER stress *in vitro* and *in vivo*, including an increase in various ER chaperones and transcriptional activation of Unfolded Protein

Response (UPR). In addition, several ER chaperones localize at the nuclear periphery where they co-localize with progerin. Interestingly, super resolution microscopy shows progerin-induced aggregation and clustering of inner nuclear membrane proteins SUN1 and SUN2 at the nuclear periphery, which may account for peripheral localization of ER chaperones. Taken together, our results suggest a novel role of progerin in activation of ER stress and provide new insights into the pathology of HGPS and aging.

M124

**In *Cellulo* Structure of the Nuclear Pore Complex and Its Implications in Mrna Export and Nuclear Pore Turnover by Autophagy**

**M. Allegretti**[1], C. E. Zimmerli[1], V. Rantos[2], P. Ronchi[1], F. Wilfling[3], K. H. Fung[1], C. Lee[3], Y. Schwab[1], J. Mahamid[1], B. Pfander[3], J. Kosinski[2], M. Beck[1]; [1]EMBL Heidelberg, Heidelberg, GERMANY, [2]EMBL Hamburg, Hamburg, GERMANY, [3]Max Planck Institute of Biochemistry, Munich, GERMANY.

The nuclear pore complex (NPC) is an essential membrane protein complex in eukaryotes shaping a hole in the nuclear envelope. Due to its dimensions (~100 MDa in *H. sapiens*) the elected method to determine the structure of the intact complex scaffold has been cryo electron tomography and subtomogram averaging (vonAppen, 2016) in combination with crystal structures from purified components (Lin, 2019). In addition, efforts to investigate the full NPC structure extracting it from its double membrane context have produced two structures in *S. cerevisiae* (Yang, 1988; Kim, 2018). In our study we perform extensive cryo-FIB-milling coupled to cryo-electron tomography and subtomogram averaging to get structures of the entire NPC scaffold in exponentially-growing cells with and without protein knock-outs. The new structures not only largely overcomes in resolution previously determined structures with the same method (Mahamid, 2016; Mosalaganti, 2018), but also shows significant differences in sub-complexes architecture to previous *in vitro* work (Kim, 2018), emphasizing the importance of determining structures of membrane proteins in their native membrane context. We combine the *in cellulo* structures with integrative modeling and crosslinks analysis: Y-complexes show a very extended conformation in comparison to previous studies and we propose a new model that takes into account our *in cell* conformation. In addition, the main mRNA export platform architecture reveals a new configuration that accommodates spatially and temporally the existent biochemical data with the release of transport factors occurring in the cytoplasm. Shifting a Nup116 knock-out strain to not-permissive temperature, we activate the formation of *herniae* (Wente, 1993), protrusions of the outer and inner nuclear membrane forming evaginations conserved in human cells and linked to Dystonia (Laudermilch, 2016). Milling this knock-out strain let us discover that at the basis of the *herniae* there is a partially assembled NPC, solving an outstanding conundrum in literature. The new mRNA export architecture discloses also the cytoplasmic exposure of an NPC-intrinsic autophagic receptor (Lee et al, submitted). We validate the NPC-Atg interaction obtaining high-resolution snapshots using correlative light and electron microscopy on plastic sections (Kukulski, 2011), conventional electron microscopy and 3D-cryo CLEM (Arnold, 2016) demonstrating that NPC degradation occurs through complex membrane remodeling events at the nuclear envelope and passes through an autophagosome intermediate owning NPC-containing nuclear vesicles in the cytoplasm. Our results highlight the power of *in cell* structural biology to provide novel insights into two fundamental processes of eukaryotic life.

M125

**Uncovering Cellular Mechanisms Controlling Tau Aggregation and Toxicity Using CRISPRi-based Genome-wide Screens in Human Neurons**

**M. Kampmann**, A. Samelson, G. Mohl, R. Tian; University of California, San Francisco, San Francisco, CA.

Protein aggregation is a hallmark of age-associated neurodegenerative diseases, but we lack an understanding of the cellular mechanisms underlying the formation and toxicity of aggregates. Aggregates of the protein tau are associated with Alzheimer's Disease and other tauopathies. Intriguingly, distinct types of neurons show selective vulnerability to tau aggregation, suggesting that cellular pathways play a key role in controlling aggregation and resulting toxicity. A systematic understanding of these pathways would enable a mechanistic understanding of the disease processes and point to potential therapeutic targets. To uncover such pathways, we used a CRISPR-based platform for genetic screening that we recently developed in human iPSC-derived neurons and glia, enabling us to study mechanisms of neurodegenerative diseases in the relevant human cell types. Here, we have applied this approach to uncover genes that control tau aggregation and toxicity in human. Among the hit genes were protein homeostasis factors, including components of the autophagy and ubiquitin-proteasome systems, and specific chaperones, some of which we found to interact directly with tau. But we also identified unexpected categories of hits, such as mitochondrial proteins, RNA-binding proteins and neuron-specific proteins. We are using biochemistry and cell biology to understand the mechanisms by which these factors act.

M126

**System-wide Profiling of Mitotic Ageing Reveals Pro-ageing Functions of the Autophagy Machinery.**

**M. Graef**; Max Planck Institute for Biology of Ageing, Cologne, Cologne, GERMANY.

We have successfully developed a high-throughput system for mitotic ageing in budding yeast using synthetic biology, which selectively and inducibly prevents newborn daughter cells from entering the cell cycle after asymmetric separation from dividing mother cells. As a consequence, cell growth in induced liquid cultures solely depends on the number of mother cell divisions over time and, thus, directly reflects mitotic ageing. We have established fully automated high-throughput mitotic ageing measurements using a newly developed bioinformatics pipeline. Using this genetic platform, we have determined the quantitative effects of each gene in the yeast genome on mitotic ageing under standard conditions at unprecedented resolution revealing a complex and unexpected picture of cellular ageing. Challenging current views, we found that defects in a subset of core autophagy components, generally involved in maintenance of cell homeostasis in response to stress, extend lifespan by improving mitochondria function during mitotic ageing. We are presenting mechanistic insights into this novel longevity pathway as a new paradigm for the multifaceted nature of ageing.

M127

**Selective Autophagic Clearance of Neurodegeneration-associated Protein Aggregates Is Mediated by the Autophagy Receptor, TAX1BP1**

**S. A. Sarraf**[1], H. V. Shah[1,2], G. Kanfer[1], A. M. Pickrell[3], L. A. Holtzclaw[4], M. E. Ward[1], R. J. Youle[1]; [1]Biochemistry Section, Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, [2]University of Maryland, College Park, MD, [3]School of Neuroscience Science, College of Science, Virginia Tech, Blacksburg, VA, [4]Microscopy and Imaging

Core, Office of the Scientific Director, Intramural Research Program, Eunice Kennedy Shriver National Institute of Child Health and Human Development,National Institutes of Health, Bethesda, MD.

Misfolded protein aggregates can disrupt cellular homeostasis and cause toxicity, a hallmark of numerous neurodegenerative diseases. Protein quality control by the ubiquitin proteasome system and autophagy is vital for clearance of aggregates and maintenance of cellular homeostasis. Autophagy receptor proteins bridge the interaction between ubiquitinated proteins and the autophagy machinery, allowing selective elimination of cargo. Aggrephagy, the selective elimination of protein aggregates, is critical to protein quality control, but how aggregates are recognized and targeted for degradation is not well understood. Using CRISPR-mediated gene editing, we examined the requirements for 5 autophagy receptor proteins: OPTN, NBR1, p62, NDP52, and TAX1BP1 in proteotoxic stress-induced aggregate clearance. Endogenous TAX1BP1 is both recruited to and required for the clearance of stress-induced aggregates while overexpression of TAX1BP1 increases aggregate clearance through autophagy. Furthermore, we found that TAX1BP1 is highly and specifically expressed in the brain compared to other autophagy receptor proteins, suggesting a potential protective role in neurodegenerative disease. In addition, TAX1BP1 depletion sensitized IPSC-derived neurons to proteotoxic stress and toxicity due to expression of Huntington's disease-linked polyQ proteins, whereas TAX1BP1 overexpression clears cells of polyQ protein aggregates by autophagy. TAX1BP1 knockout mice exhibited increased accumulation of ubiquitin conjugates in various brain regions compared to wildtype mice, further supporting a role for TAX1BP1 in prevention of aggregate accumulation. We propose a broad role for TAX1BP1 in the clearance of cytotoxic proteins, thus identifying a new factor with therapeutic potential in clearance of protein inclusions.

M128

**Synaptic Activity Regulates Local Autophagy in Dendrites of Primary Neurons**

**S. Maday**, V. V. Kulkarni, A. Anand, J. Brandt; Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA.

Neurons face the extraordinary challenge of firing action potentials at very high frequencies (up to ~50-100 impulses per second) for nearly a century of time. Consequently, the neuronal proteome is vulnerable to damage and aging. This stress necessitates robust quality control pathways to maintain the integrity and composition of the neuronal proteome in response to synaptic activity. Indeed, neurons are particularly dependent on autophagy, a lysosomal degradation pathway that recycles damaged and aged proteins and organelles. Neuron-specific knockout of key autophagy genes can lead to neuronal dysfunction and death. Further, loss of autophagy causes deficits in learning and memory in mice, indicating a critical role for autophagy at synapses. Surprisingly, however, little is known about the molecular details of autophagy in synaptic compartments. To address this gap in knowledge, we use live-cell confocal microscopy to define the autophagy pathway in primary hippocampal neurons under various paradigms of synaptic activity. We find that synaptic activity controls the dynamics of autophagic vacuoles (AVs) specifically in dendrites. Stimulating synaptic activity dampens AV motility in dendrites while silencing synaptic activity induces AV motility. Silencing synaptic activity also reverses activity-dependent decreases in AV dynamics. Interestingly, these effects are specific to dendrites and are not observed in axons, indicating compartment-specific regulation of neuronal autophagy with synaptic activity. Most strikingly, synaptic activity increases the number of AVs that colocalize with markers of proteolytic activity, suggesting that synaptic activity impacts the function of dendritic AVs by stimulating

their degradative capacity. By contrast, synaptic activity did not alter the degradative capacity of AVs in axons where degradative AVs were detected less frequently as compared with dendrites. Interestingly, across all paradigms of synaptic activity, non-degradative AVs were more motile and degradative AVs were less motile, suggesting that AV motility and function are correlated and AVs may need to stop to fully mature into proteolytically active compartments. Given that dendritic AVs exhibit bidirectional or oscillatory movement, these results suggest a more local pathway for autophagy in dendrites, in contrast to the long-range pathway for autophagy that we previously defined in axons. Combined, we propose a model where neuronal activity controls AV transport and function specifically in dendritic compartments for local regulation of the synaptic proteome.

M129

**Regulation of Cholesterol-dependent Mtorc1 Signaling by Inter-organelle Contacts**

**C. Lim**[1,2], O. Davis[1,2], H. Shin[1,2], J. Zhang[1,2], C. Berdan[1,3], X. Jiang[4], J. Counihan[1,3], D. Ory[4], D. Nomura[1,3], R. Zoncu[1,2]; [1]Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA, [2]The Paul F. Glenn Center for Aging Research at the University of California, Berkeley, Berkeley, CA, [3]Department of Nutritional Sciences and Toxicology, University of California at Berkeley, Berkeley, CA, [4]Diabetic Cardiovascular Disease Center, Washington University School of Medicine, St Louis, MO.

Organelle communication is key to cellular homeostasis, and its failure is an emerging driver of neurodegenerative and metabolic disease. Cholesterol, an essential building block for membranes and precursor for growth hormones, is exchanged by specialized sterol carriers located at physical contacts between the endoplasmic reticulum (ER) and other organelles. Recently, cholesterol was identified as an essential input that activates the master growth regulator, mTORC1 kinase, by promoting its recruitment to the lysosomal limiting membrane via the Rag guanosine triphosphatases (GTPases). Cholesterol triggers major programs downstream of mTORC1, including upregulation of anabolism and suppression of autophagy, but the mechanisms that regulate lysosomal cholesterol content to enable mTORC1 signaling are unclear. More generally, whether and how inter-organelle contacts govern cell-wide programs for growth and quality control is not understood. Here we show that Oxysterol Binding Protein (OSBP) and its anchors at the ER, VAPA/B, deliver cholesterol across ER-lysosome contacts to activate mTORC1. In cells depleted for OSBP, but not other VAP-interacting cholesterol carriers, mTORC1 recruitment by the Rag GTPases is inhibited due to impaired cholesterol transport to lysosomes. Moreover, in cells lacking the lysosomal cholesterol exporter, Niemann-Pick C1 (NPC1), unopposed ER-to-lysosome transport by OSBP drives aberrant buildup of cholesterol on the lysosomal limiting membrane. Consequently, genetic and small molecule-mediated inhibition of OSBP selectively reverses cholesterol accumulation on the limiting membrane but not in the lumen of NPC1-null lysosomes, suppresses aberrant mTORC1 signaling and restores defective autophagy, a major driver in the pathogenesis of Niemann-Pick type C. Thus, ER-lysosome contacts emerge as signaling hubs that enable cholesterol sensing by mTORC1, and their manipulation via OSBP inhibitors could be beneficial in Niemann-Pick type C and mTORC1-driven diseases.

## Minisymposium 13: Biological Phase Separation: From Phenomenon to Function

M130

**Er Membranes Exhibit Phase Behavior At Sites of Organelle Contact**

**C. King**, P. Sengupta, A. Seo, J. Lippincott-Schwartz; HHMI Janelia Research Campus, Ashburn, VA.

The plasma membrane of cells exhibits phase behavior that allows transient concentration of specific proteins and lipids, giving rise to functionally dynamic and diverse nanoscopic domains. This phase behavior is observable in giant plasma membrane-derived vesicles, in which microscopically visible, liquid-ordered ($L_o$) and liquid-disordered ($L_d$) lipid domains form upon a shift to low temperatures. The extent such phase behavior exists in the membrane of the endoplasmic reticulum (ER) of cells remains unclear. To explore the phase behavior of the ER membrane in cells, we used hypotonic cell swelling to generate Large Intra-Cellular Vesicles (LICVs) from the ER in cells. ER LICVs retained their lumenal protein content, could be retubulated into an ER network, and maintained stable inter-organelle contacts, where protein tethers are concentrated at these contacts. Notably, upon temperature reduction, ER LICVs underwent reversible phase separation into microscopically-visible domains. The ER-ordered domains marked ER contact sites with other organelles. These findings demonstrate that LICVs provide an important model system for studying the biophysical properties of intracellular organelles in cells.

M131

**Protein Phase Separation as a Membrane Curvature Sensing Switch**

**G. Kago**, F. Yuan, W. F. Zeno, J. C. Stachowiak; University of Texas at Austin, Austin, TX.

The ability of proteins to sense membrane curvature is essential to many cellular functions including endocytosis, cell motility, and the biogenesis of organelles. Previously characterized curvature sensing mechanisms rely on protein domains with specific structural features like amphipathic helices and curved BAR (Bin/amphiphysin/RVS) domains. However, more recent work has shown that intrinsically disordered proteins can sense convex membrane curvature through entropic mechanisms. Interestingly, in the cell, intrinsically disordered proteins are frequently associated with liquid-liquid protein phase separation. Further, many proteins involved in these liquid assemblies interact with membranes. How might protein phase separation impact membrane curvature sensing? Here we use a model phase separating protein, the Low Complexity (LC) domain of Fused-In-Sarcoma (FUS) to assess curvature sensing under both phase separating and non-phase separating conditions. We show that protein phase separation results in a curvature sensing switch. Specifically, when FUS bound to membranes under non phase-separating conditions, the amphipathic protein partially inserted into membrane defects, leading to a preference for convex membrane curvature. The behavior is similar to other well-characterized amphipathic sequences. However, upon protein phase separation, we observed a pronounced switch toward concave curvature sensing in which proteins strongly avoided areas of high convex curvature. Concave curvature sensing was correlated with high protein coverage. We verified protein phase separation by visualizing the emergence of protein dense domains on Giant Unilamellar Vesicles (GUVs). Ongoing experiments in live cells are evaluating whether plasma membrane-anchored FUS will partition preferentially to the concave curvature of filopodia. To explain this phenomenon, we propose a novel mechanism in which membrane-bound protein droplets prefer flat or concave surfaces, which maximize protein contact, amplifying protein assembly. This finding implies that the many phase separating

proteins that are being discovered may be able to sense and selectively assemble at sites of concave membrane curvature, such as filopodia, viral buds and dendritic spines, while avoiding convex curvatures associated with endocytic buds and trafficking vesicles.

M132

**Ph-triggered Coacervate Formation Activates Enzyme Reactions**

**C. Love**[1], J. Steinkühler[2], R. Dimova[2], D. Tang[1]; [1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, GERMANY, [2]Max Plank Institute of Colloids and Interfaces, Potsdam, GERMANY.

The field of liquid-liquid phase separation (LLPS) in biology has attracted much attention over the last decade and bimolecular condensates have now been identified in a wide range of eukaryotic organisms. Despite this, the precise function of membrane-free compartments within biological systems has not been entirely elucidated. As biology is innately complex, it is often challenging to determine the function of condensates within the myriad of biochemical processes. On the other hand, *in vitro* assays provide a simplified bottom-up approach that allow for the controllable testing of condensate function. We have therefore developed a method for the *in-situ* formation of coacervates (a form of condensates) within giant unilamellar vesicles (GUVs) to enable the controllable testing of the role of phase separation in regulating biochemical reactions.Coacervation, a specific type of LLPS driven by the electrostatic attraction of oppositely charged polymers, has been implicated as one of the driving forces for condensate formation. Here, we exploit the intrinsic pKa of coacervate-forming polymers polylysine and either ATP or carboxymethyl dextran to reversibly drive coacervate formation within lipid vesicles with pH changes. We show that the *in-situ* coacervate formation activates dormant reactions in solution, by the up-concentration of the reactants. Our results, demonstrate a clear functional role of condensates and show that minimal and dynamic *in vitro* models can offer fundamental insights into our understanding of membrane-free condensates in biology.

M133

**Proteome-wide Analysis of Cytoplasmic Meso-scale Organization**

F. Keber, **M. Wühr**, C. Brangwynne; Princeton University, Princeton, NJ.

Key organization principles for the eukaryotic cytoplasm are the formation of stable protein complexes, membrane-bound organelles, and membrane-less condensates that utilize liquid-liquid phase separation (LLPS). Despite the importance of LLPS in cytoplasmic organization for many biological processes, it is still unclear what fraction of proteins organize themselves through this mechanism. Here, we assay the organization of proteins into meso-scale assemblies on a proteome-wide scale and reveal the underlying organization principles with molecular resolution. We filtered undiluted cytoplasm from frog eggs through porous membranes under different pressures. We quantified the relative permeation of each protein with multiplexed proteomics. Protein complexes are retained by small pores regardless of applied pressure. In contrast, liquid assemblies are retained by small pores at low pressure but squeeze through the same pores under high pressure. We find that well-known LLPS proteins showed the predicted differential behavior and can be clearly separated from stable protein complexes. We identified hundreds of proteins with similar differential characteristics suggesting that LLPS plays a wide and still mostly undiscovered role in the self-organization of the cytoplasm. This new methods to assay the meso-scale organization of cytoplasm suggests that many condensate organelles still remain to be discovered.

M134

**The Transition Into and Out of Glucose Starvation Induced Cytoplasmic Freezing in Fission Yeast**

**A. Foote**, M. Williamson, E. Florin; University of Texas At Austin, Austin, TX.

The fluidity of the eukaryotic cytoplasm is essential for both active and passive forms of intracellular transport. It has been shown recently that the cytoplasm of fission yeast and budding yeast solidifies into a rigid state in minutes in response to drug induced energy depletion. Here, in contrast, we study a solidification of fission yeast cytoplasm in response to six day long glucose starvation. Because this state is characterized by the nearly complete immobilization of endogenous lipid droplets and other cytoplasmic structures, we named it “cytoplasmic freezing”. The origin of cytoplasmic freezing and how cells transition into and out of this state remains unclear. Here, we investigate how fission yeast cells enter into and recover from this solid-like state by tracking the motion of endogenous lipid droplets. In addition, we use a novel microscopy method that can detect motion of objects as small as 30 nm in featureless parts of the cytoplasm. We find that cells enter and recover from the frozen state in a multistage process. When deprived of glucose, the cytoplasm progresses through three distinct states of fluidity after two and six days of glucose starvation. When cells in the frozen state are exposed to glucose, the cytoplasm softens within seconds, remains in an intermediate state for ~ 2 minutes, and then gradually returns to a level of fluidity that is typical for exponentially growing cells. The transitions between states were observed with both techniques which demonstrates that cytoplasmic freezing is a multistage and cell-wide process.

M135

**The Properties of Membraneless Organelles Are Tuned to Environmental Conditions.**

**B. Stormo**[1], F. Dietrich[2], C. Roden[1], A. Gladfelter[1]; [1]University of North Carolina-Chapel Hill, Chapel Hill, NC, [2]Duke University, Durham, NC.

How cells pattern and compartmentalize their cytoplasm is a critical question in biology. Many cells rely on membraneless organelles for organizing biochemistry. These organelles are not separated from the rest of the cell by a lipid bilayer, instead they are formed through Liquid-Liquid Phase Separation (LLPS) in which a well-mixed solution demixes into a concentrated droplet phase and a dilute bulk phase. When the material properties, such as viscosity, of these droplets are measured they are found to vary by a thousand fold. Despite these differences we have very little understanding about whether specific properties are important for the function of droplets, although it has been suggested that dysregulation of droplet properties can result in diseases.In the free-living filamentous fungus *Ashbya gossypii* asynchronous nuclear division in the syncytial hyphae requires the phase separation of the protein Whi3 and the cyclin RNA *CLN3*. Whi3 also undergoes phase separation with the polarity RNAs *BNI1* and *SPA2*, which is required for hyphal branching. Like other phase-separating proteins Whi3 contains an RNA recognition motif (RRM) that allows for multivalent interactions, and it contains a disordered domain rich in glutamine (polyQ). Loss of either of these domains results in failure to phase separate. Because LLPS is a thermodynamically-driven process, temperature plays a role in determining whether phase separation happens and influences the properties of the resulting droplets. Free living fungi are exposed to the ambient temperature and therefore are under selective pressure to adapt to their local climate. We hypothesized that if the material properties of droplets are important then they should be optimized for a given climate.Using a collection of fifty wild-isolated *Ashbya* from different climates in the United States we sought to test the effects of temperature on the properties of membraneless organelles. We

found that the interbranch distance, which is controlled by membraneless organelles, varied between isolates when grown at high or low temperature. We then performed whole genome sequencing on the wild isolates. We found that the sequence of the Whi3 polyQ domain, which is critical for phase separation, differs between isolates and that purified proteins from these isolates behaves differently in *in vitro* phase separation experiments. We further found that the sequence of the RNA of *CLN3* and *BNI1* differs between isolates and that these changes affect the structure of those RNAs without changing the protein coding sequence. These results suggest that the material properties of phase separated droplets are tuned to the climate at which the cells live indicating that specific properties are important for function.

M136

**Adaptive Control of Cell Division Programs by Pervasive Protein Self-assembly**

**D. F. Jarosz**; Stanford University, Stanford, CA.

Spatiotemporal gene regulation is often driven by nucleic acid binding proteins. Many such proteins harbor long, intrinsically disordered sequences of amino acids. We report that the disordered region of the ancient developmental regulator Smaug drives its self-assembly into gel-like condensates. These proteinaceous particles are not composed of amyloid. Yet they are infectious, allowing them to act as a protein-based epigenetic element: a prion. In contrast to many amyloid prions, condensation of Smaug hyperactivates its function in mRNA decay. Its inheritance requires Hsp70 activity, and its self-assembly properties are conserved over large evolutionary distances. Yeast cells harboring [*SMAUG*$^{+}$] downregulate a coherent network of mRNAs, altering decisions between two broad growth and survival strategies: mitotic proliferation or meiotic differentiation into a stress-resistant state. This allows cells to anticipate nutrient repletion after periods of starvation, providing a strong selective advantage. [*SMAUG*$^{+}$] is common in laboratory yeast strains, where standard propagation practice produces regular cycles of nutrient scarcity followed by repletion. Distinct [*SMAUG*$^{+}$] variants are also widespread in wild yeast isolates from multiple niches, establishing that prion polymorphs can be utilized in nature. Our data provide a striking example of a protein-based epigenetic switch hidden in plain sight, establishing a transgenerational memory that integrates adaptive prediction into developmental decisions.

M137

**Mitochondrial Nucleoids Self-assemble Via Phase Separation**

**M. Feric**[1,2], T. G. Demarest[3], J. Tian[3], D. L. Croteau[3], V. A. Bohr[3], T. Misteli[2]; [1]NIGMS, Bethesda, MD, [2]NCI, Bethesda, MD, [3]NIA, Baltimore, MD.

Mitochondria contain an autonomous and spatially segregated genome. The mitochondrial DNA (mtDNA) is coated by architectural proteins to form physically discrete mitochondrial nucleoids, which are non-membrane bound nucleoprotein complexes of uniform size. Beyond the direct binding of protein to mtDNA, the physical mechanisms that govern how mt-nucleoids assemble, how they are maintained, and how they contribute to function remain unclear. Here, we provide direct evidence that mt-nucleoids arise from phase separation of nucleic acids and proteins. We show that the main mtDNA packaging protein mitochondrial transcription factor A (TFAM) readily phase separates into viscoelastic droplets in vitro. Mutation analysis of TFAM reveals that multivalent interactions, intrinsically disordered domains, and charged residues contribute to the observed phase behavior. Addition of mtDNA promotes the formation of multiphase structures with gel-like properties. With regards to in vivo

properties of nucleoids, pharmacological disruption with known mtDNA intercalators induces liquid-like fusion events between individual mt-nucleoids and global coarsening within the mitochondrial network. In support of a link to aging, which is associated with increased reactive oxygen species production and mitochondrial dysfunction, we find that cells derived from patients with the premature aging disease Hutchinson-Gilford Progeria Syndrome (HGPS) have an increased number of damaged mitochondria with accumulation of mt-nucleoid markers. The atypical organization and enlarged structures of mt-nucleoids in the damaged mitochondria in HGPS cells resemble those generated in vitro and are associated with impaired mitochondrial function. Together, these results suggest that mt-nucleoids represent phase separated droplets of protein and mtDNA in vivo. The phase behavior of mt-nucleoids represents a novel framework for understanding their assembly, maintenance, and organization, with potential implications for normal aging.

M138

**Robust Modulation of Kinase Activity within a Bacterial Biomolecular Condensate**

**S. Saurabh**, L. Shapiro; Stanford University, Stanford, CA.

The diversity of function and material properties found in cytoplasmic biomolecular condensates contributes to the robustness of enzymatic interactions in a crowded cellular environment. However, the molecular principles that organize chemical reactions in these condensates are poorly understood. To understand how molecular properties influence biochemical reactions in this constrained environment, we have used a multi-scale approach combining biochemical reconstitution with single molecule imaging to study polar microdomains in the Gram-negative bacterium *Caulobacter crescentus*. *Caulobacter* divides asymmetrically to yield a sessile stalked cell and a flagellated swarmer cell. Much of this asymmetric division is regulated through different signaling pathways that reside in phase separated microdomains at the two cell poles. Specifically, at the stalked pole, two intrinsically disordered proteins, PopZ and SpmX, form a stable complex and localize the Histidine kinase DivJ to establish stalked cell identity. Biochemical reconstitution experiments revealed that SpmX and PopZ can form phase-separated condensates that sequester DivJ and control its kinase activity. We further identified molecular determinants of SpmX phase separation that control material properties and enzyme kinetics in this condensate. Perturbing the interactions between SpmX and DivJ was found to regulate DivJ diffusion and its subsequent phosphorylation in the stalk bearing polar microdomain. Our results underscore the relationship between enzyme activity and protein phase separation that governs the physical environment in signaling hubs. They also demonstrate the utility of disordered scaffolds in robust modulation of biochemical reactions in a crowded cytoplasm that is devoid of membrane-bound organelles.

M139

**Material Aging Underlies Centrosome Weakening and Disassembly During Mitotic Exit**

**J. Woodruff**; UT Southwestern Medical Center, Dallas, TX.

Centrosomes are membrane-less organelles that must resist microtubule-mediated forces for mitotic chromosome segregation. During mitotic exit, however, centrosomes are deformed and fractured by those same forces, which a key step in centrosome disassembly. How the mechanical properties of centrosomes are tuned during the cell cycle is not known. Here, we used optically-induced flow perturbations to determine the molecular basis of centrosome deformation resistance and fracture

resistance in *C. elegans* embryos. Induced flows caused time-dependent deformation and eventual fracture of the centrosome scaffold. Centrosome resistance to these flows peaked in metaphase, then declined sharply in anaphase, ~150s prior to natural disassembly. This mechanical transition depended on PP2A phosphatase and correlated with the departure of PLK-1 (Polo Kinase homolog) and SPD-2 (Cep192 homolog) from centrosomes. Acute inactivation of PLK-1 or SPD-2 made the centrosome scaffold weak and brittle in metaphase and led to premature disassembly. *In vitro*, PLK-1 and SPD-2 protected centrosome scaffolds from force-induced disassembly, suggesting that they directly reinforce the centrosome scaffold *in vivo*. Thus, centrosome mechanical properties are tuned via compositional balance of PLK-1 and SPD-2 versus PP2A. In anaphase, centrosomes undergo material aging, whereby they lose PLK-1 and SPD-2 and transition from a strong, ductile state to a weak, brittle state that enables centrosome disassembly.

## Minisymposium 14: Cell Polarity

M140

**An Interphase Contractile Ring Reshapes Primordial Germ Cells to Allow Bulk Cytoplasmic Remodeling**

**J. Nance**; New York University School of Medicine, New York, NY.

Some cells discard undesired inherited components in bulk by forming large compartments that are subsequently eliminated. *C. elegans* primordial germ cells (PGCs) jettison mitochondria and cytoplasm by forming a large lobe that is cannibalized by adjacent intestinal cells. Although PGCs are non-mitotic, we find that lobe formation is preceded by formation of a contractile ring, and that conditional mutations in proteins required for contractile ring formation during cytokinesis (CYK-1/formin, NMY-2/myosin, ECT-2/Ect2) block PGC lobe formation. By contrast, disruption of centralspindlin, which positions the cytokinetic contractile ring, does not block PGC lobe formation. We propose that lobe contractile ring formation is locally inhibited by the PGC nucleus, which migrates to one side of the cell before the cytokinetic ring assembles on the opposite cortex. Our findings reveal how components of the cytokinetic contractile ring are reemployed during interphase to create compartments used for cellular remodeling, and they reveal differences in the spatial cues that dictate where the contractile ring will form.

M141

**Leading Edge Maintenance in Migrating Neutrophil-like HL-60 Cells Is An Emergent Property of Branched Actin Growth**

**R. M. Garner**[1], E. F. Koslover[2], A. J. Spakowitz[1], J. A. Theriot[3]; [1]Stanford University, Stanford, CA, [2]University of California San Diego, San Diego, CA, [3]Howard Hughes Medical Institute, University of Washington, Seattle, WA.

Actin-driven, directional cell motility is one of the most fundamental behaviors in animal cell biology. To maintain polarized migration, cells must coordinate the stochastic growth of tens of thousands of nanometer-sized actin filaments over micron-scale distances along their leading edge. We employed high speed, high resolution microscopy of migrating neutrophil-like HL-60 cells to directly observe leading edge maintenance evolving over time. To our surprise, we discovered that cells' leading edge shape continuously undergoes dynamic sub-micron, sub-second undulations, all while maintaining nearly constant overall cell shape. Under the assumption that these shape fluctuations reflect the

underlying actin dynamics driving migration, we set out to quantitatively characterize and then perturb shape dynamics in order to develop a deeper understanding of the mechanisms cells use to maintain leading edge shape, and thus polarized migration. Given that the overall leading edge shape is quite stable, we reasoned that any perturbation from the average shape would have a characteristic amplitude and relaxation timescale, and that this relaxation might have some wavelength dependence. Adapting well-established methods from polymer physics, we used Fourier mode time-autocorrelation analysis to investigate the relaxation of shape fluctuations over time. Surprisingly, we found that shape fluctuations at all wavelengths undergo oscillations as they decay, implying some memory exists in the system that causes fluctuations to "overshoot" as they relax, much like a Hookean spring. Furthermore, live-cell fluorescence imaging of HL-60 cells endogenously expressing GFP-β-actin revealed that actin density fluctuations also undergo oscillatory decay that is perfectly anticorrelated with leading edge shape fluctuations. The rich behavior and quantitative nature of this dataset made it primed for physical modeling. We thus employed a swath of simple "toy" models to explore potential molecular mechanisms that could explain leading edge oscillations and their relationship to actin density. Remarkably, we found that a simple evolutionary filament orientation model (Maly and Borisy 2001) is sufficient to explain the oscillations. In this model, the network oscillates between a fast-growing, low density state in which filaments grow perpendicular to the leading edge, and a slow-growing, high density state in which filaments grow towards the membrane at approximately one half of the branching angle. Excitingly, these results suggest that branched actin growth against a membrane is inherently self-correcting and thus sufficient for maintaining leading edge shape without requiring higher-level regulation.

M142

**Negative Surface Charge Defines the State of Cell Cortex and Regulates Excitable Dynamics in Amoeboid Migration and Macropinocytosis**

**T. Banerjee**[1], D. Biswas[2], D. S. Pal[1], Y. Miao[1], P. A. Iglesias[2], P. N. Devreotes[1]; [1]Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD, [2]Department of Electrical and Computer Engineering, Johns Hopkins University, Baltimore, MD.

The spatially distributed components of signal transduction excitable network (STEN) and cytoskeletal excitable network (CEN) in Dictyostelium and leucocyte cells generate autonomous waves in the substrate attached basal surface. One key event in this characteristic cortical wave formation is the symmetry breaking of membrane phospholipids and self-organization of STEN and CEN proteins to dynamically generate distinct regions in the cell cortex which corresponds to the typical front (or "activated") and back (or "inactivated") regions of a randomly migrating cell. An analogous pattern of organization is observed in chemotactic migration, micropinocytosis, phagocytosis, and cytokinesis of different types of eukaryotes cells. We have identified two novel unexpected features of these self-organizing patterns. First, we have found that three lipidated membrane proteins in Dictyostelium, PKBR1, Gßγ, and RasG, which are widely thought to be uniformly distributed over the membrane, show preferential localization in the back/inactivated regions of cell cortex. Our data indicates that besides conventional recruitment based mechanism used by many other components, a dynamic partitioning process may contribute to the self-organization pattern of components that remain associated with the membrane. Second, we have assessed the localization of the generic surface charge sensor that was previously shown to be tightly associated with the negatively charged inner surface. In Dictyostelium and RAW 264.7 cells, we found that the sensor partitioned to the back/inactivated region as did the

lipidated proteins. Our data suggested that high negative surface charge defines the back or inactivated region of the cell cortex and the cell migration and macropinocytosis processes are essentially guided by the alteration of surface potential on the inner leaflet to generate dynamic polarity.

M143

**Clics Are Ancient Conserved Regulators of $G_{\alpha}12/13$ and Rac Signaling in Angiogenesis and Tubulogenesis.**

A. Arena, **D. Shaye**, D. Mao, J. Kitajewski; Dept. of Physiology and Biophysics. University of Illinois at Chicago, Chicago, IL.

The *C. elegans* excretory canal (*ExCa*), a single-cell tube, is a tractable model to study tubulogenesis. Several conserved proteins required for *ExCa* tubulogenesis also play a role in angiogenesis. One example is EXC-4, a *C. elegans* ortholog of the Chloride Intracellular Channel (CLIC) family of proteins. After the initial discovery that *exc-4* regulates *ExCa* tubulogenesis we, and others, showed that human CLIC1 and CLIC4 function in endothelial cells to promote migration, growth, and tube formation. EXC-4 is localized to the plasma membrane in the *ExCa*, and this localization is critical for its function. In mammalian cells CLIC1 and CLIC4 are cytoplasmic, however, they are recruited to the plasma membrane upon activation of the S1P family of receptors (S1PRs), which are potent regulators of angiogenesis that act through $G_{\alpha}i$, $G_{\alpha}12/13$, RhoA and Rac1. The connection between regulation of CLIC localization and S1PR activity led us to hypothesize that CLICs have a conserved function in $G_{\alpha}$-Rho/Rac signaling in *C. elegans* and in endothelial cells. We isolated and characterized a new loss-of-function *exc-4* allele, which has allowed us to define genetic interactions between *exc-4/CLIC* and other genes in *ExCa* tubulogenesis. Using this new allele we found that that *exc-4* genetically interacts with *gpa-12/*$G_{\alpha 12/13}$ and two worm Rac orthologs, *ced-10* and *mig-2*, implicating $G_{\alpha}$-Rac signaling in *ExCa* tubulogenesis and indicating that EXC-4 interfaces with this pathway in *C. elegans*. Similarly, our work in endothelial cells indicates that CLIC1 and CLIC4 are required for S1P-induced Rac1 activation and Rac1-mediated cellular behaviors. These results define a new and conserved function for EXC-4/CLIC proteins in $G_{\alpha}$-Rac signaling.

M144

**A Template for Actin Organization At the Leading Edge**

**A. Pipathsouk**, R. Brunetti, J. Town, O. Weiner; University of California, San Francisco, San Francisco, CA.

How local interactions of actin regulators yield large-scale organization of cell shape is not well understood. For example, why does the WAVE complex build lamellipodia, broad sheet-like protrusions that power cell migration, whereas the highly related actin regulator N-WASP forms finger-like actin networks? From in vitro reconstitutions, sheet-like actin structures require spatially organized actin nucleation along a linear structural template. What is the endogenous basis of a template for lamellipodia in living cells? To investigate whether WAVE complex self-organizes into a template that sculpts lamellipodia, we studied its nanoscale organization at the plasma membrane. Our previous work showed that WAVE complex self-associates into small foci following actin depolymerization. At conventional resolution, these appeared to be amorphous blobs, similar to droplet-like phase transitions exhibited by N-WASP. Using super-resolution microscopy, we found the WAVE complex “foci” are actually highly ordered rings, 280nm wide, which can elongate into micron-length spirals. These linear structures indicate that WAVE complex self-assembles into a linear structure that could represent the

physical template for lamellipodial formation; actin assembly perpendicular to these linear arrays of WAVE complex could generate the actin sheets that are the basic unit of lamellipodia organization. Analysis of the membrane organization around these rings provided further insight into the role of geometry in cell morphogenesis. These rings localize to necks of membrane invaginations, which are saddle point geometries (positive curvature in one axis and negative curvature in the other axis). This suggests a specific geometric input into WAVE complex's localization. This saddle preference for the WAVE complex could explain emergent cell behaviors such as the expanding self-straightening behavior of lamellipodia as well as the ability of endothelial cells to seal transcellular holes. To investigate the molecular mechanism of saddle curvature recognition, we show that the WAVE complex and IRSp53, an inverse BAR domain protein, collaborate together to recognize saddle geometry that neither can recognize on its own. Our work highlights how partnering protein interactions enable complex shape sensing and how feedback between cell shape and actin regulators yields self-organized cell morphogenesis.

M145

**Patterning of Membrane-associated Proteins through Membrane Flows**

**V. Gerganova**[1], I. Lamas[1], D. Rutkowski[2], A. Vjestica[1], D. Vavylonis[2], S. G. Martin[1]; [1]University of Lausanne, Lausanne, SWITZERLAND, [2]Lehigh University, Bethlehem, PA.

How cells pattern and polarize in response to internal and external cues is a fundamental biological problem. In the rod-shaped fission yeast cell, the polar and lateral plasma membranes are distinct in lipid and protein composition. Polar zones are defined by local Cdc42 GTPase activity, which directs polarized secretion. By contrast, how the lateral membrane is patterned and how membrane-associated proteins, such as the Cdc42 GAP Rga4, accumulate laterally is less well understood. We have recently developed a light-inducible tool based on the CRY2PHR-CIBN system to acutely recruit proteins to the plasma membrane upon blue light photo-stimulation. Blue light activation triggers the formation of large CRY2PHR-CIBN complexes at the cell cortex, which are initially homogeneously distributed. Remarkably, after photo-activation, CRY2PHR-CIBN complexes are rapidly cleared from the growing pole(s). We hypothesize that exocytosis at the center of a broader zone of endocytosis at sites of growth acts as a driver of membrane flows causing this cell patterning. Mathematical simulations indeed show that the density of plasma membrane-associated proteins at the pole decreases according to membrane affinity (Koff). Experiments confirm a concentrated zone of exocytosis within a broader zone of endocytosis at zones of future CRY2PHR-CIBN clearance and disruption of the phenomenon by blocking polarized secretion. Empirical modulation of membrane binding affinity using both synthetic probes and the endogenous Rga4 further demonstrate that this parameter defines their cortical distribution. These results suggest that membrane flows created by the specific location of polarized secretion and endocytosis pattern the lateral distribution of membrane-associated proteins.

M146

**An Asymmetric Mechanoresponse At Cadherin Junctions Ensures Epithelial Integrity During Mitotic Rounding**

**M. Gloerich**[1], J. Monster[1], L. Donker[1], M. Vliem[1], Z. Win[2], J. De Rooij[1], B. Baum[2]; [1]UMC Utrecht, Center for Molecular Medicine, Utrecht, NETHERLANDS, [2]UCL, London, UNITED KINGDOM.

Epithelia are continuously self-renewed, but how epithelial integrity is maintained while cells undergo dramatic shape changes during mitosis is not fully understood. Here, we show that mitotic entry coincides with an increase in tensile forces on adhesions between mitotic cells and their neighbours. By physically confining epithelia in height, we demonstrate that the increase in junctional tension is a direct consequence of mitotic cell rounding. To withstand intercellular forces, cadherin-based cell-cell adhesions can trigger an adaptive response to reinforce junctions through recruitment of the actin binding protein Vinculin. Surprisingly, we find that Vinculin is not recruited to cadherin in the mitotic cells but only in their neighbours, resulting in asymmetry in the composition of cadherin junctions. When junctional Vinculin recruitment is inhibited in mitotic neighbours, this results in junctional breakage and a weakened epithelial barrier upon mitotic entry. Conversely, preventing junctional release of Vinculin in mitotic cells attenuate the ability of cells to round up in mitosis. Our data thus identify an asymmetric mechanoresponse at cadherin adhesions during mitosis, which is essential to maintain epithelial integrity while at the same time enable the shape changes of mitotic cells.

M147

**CD2AP Links Actin to PI3 Kinase Activity to Extend Epithelial Cell Height and Constrain Cell Area**

**Y. Wang**, W. M. Brieher; Department of Cell and Developmental Biology, University of Illinois, Urbana-Champaign, IL.

Epithelial cells generate three different membrane domains (apical, basal, and lateral) that perform distinct functions. The ratios of apical and basal membrane to lateral membrane varies between cell types depending on physiological function, but little is known as to what controls the height of the lateral membrane relative to the area of the apical and basal domains. Here, we show that the actin-binding scaffold protein, CD2AP, is a critical determinant of epithelial proportions. Depletion of CD2AP or PI3-kinase inhibition results in loss of F-actin and expansion of apical-basal domains which comes at the expense of lateral membrane height in MDCK cells. Immunofluorescence staining showed that CD2AP co-localized with PI3K p85α. CD2AP is required for PI3K p85α and p110γ recruitment and PtdIns(3,4,5)$P_3$ synthesis along lateral membranes. Thermophoresis results showed that SH3 domains (1-329 a.a.) of CD2AP binds to the N-terminus of PI3K p85α (1-315 a.a.) which contains two proline-rich motifs. Targeting SH3 domains (1-329 a.a.) of CD2AP to lateral membrane was sufficient to restore p85α and p110γ membrane levels. CD2AP 1-329 a.a. or PI3K p110 γ membrane tethering both recovered F-actin accumulation and apical expansion in CD2AP-knockdown MDCK cells. SH3 domains of CD2AP contributes to both PH-GFP fluorescence signals generation along cell borders and constraining cell spreading area. Thus, CD2AP recruits PI3K to regulate cell height. We also showed that CD2AP is not actin monomer sequester, but directly associates with F-actin and regulates actin dynamics *in vitro*. Single filament elongation experiments showed that both NT- and CT- of CD2AP decreased actin polymerization rates. *In vivo*, Latruculin B treatment attenuated both CD2AP and PI3K levels, i.e. CD2AP and PI3K are both upstream and downstream of actin polymerization. Thus we hypothesized that CD2AP bridges actin assembly to PI3K activation to form a positive feedback loop to support lateral membrane

extension. Our results may provide insights into squamous to cuboidal to columnar epithelial transitions seen in complex epithelial tissues *in vivo*.

M148

**MAPK Feedback Phosphorylation of RGS Controls Its Spatiotemporal Localization and Alters Endocytosis through the Kelch Repeat Protein Kel1 During the Yeast Pheromone Response**

W. C. Simke, **J. B. Kelley**, A. J. Hart; University of Maine, Orono, ME.

G-protein coupled receptor (GPCR) signaling pathways regulate myriad cellular processes that require detection of an extracellular signal and conversion of that signal into a cytosolic response. The budding yeast, *Saccharomyces cerevisiae*, grows towards potential mating partners using a GPCR pathway to track the external gradient by initiating Rho-mediated cell polarization. This pathway makes use of two branches, a Gβγ branch that leads to Rho polarization and a MAPK cascade, and a less well understood Gα branch that anchors MAPK to the membrane to regulate formin activity. The mating pathway is desensitized by the regulator of G-protein signaling (RGS), Sst2. It has long been known that there is a MAPK feedback phosphorylation to the RGS, but this modification was found to have no effect on signaling from the Gβγ branch of the pathway. We hypothesized that the phosphorylation event serves to regulate the spatial and temporal dynamics of the RGS during the pheromone response to control where MAPK is active. Using a combination of live cell imaging in microfluidic devices and computational image analysis, we show that phosphorylation of the RGS results in loss of its localization to the site of polarization, and alters the pattern of active Gα-MAPK complex at the leading edge of the cell. In addition to the changes in localization of the RGS and its target Gα, we also came across two surprising results: 1) the ability to phosphorylate the RGS is required for the cell to successfully complete cytokinesis prior to beginning the mating response and 2) phosphorylated RGS leads to a decrease in endocytosis. These results lead us to consider the role of the formin regulator and member of the mitotic exit network, Kel1. Kel1 is a kelch repeat containing protein that has previously been found to interact with the RGS. Here we show that Kel1 is required for proper RGS localization, and provide evidence that RGS regulates Kel1 in a phosphorylation dependent manner. We propose that this newly discovered role for an RGS protein in the regulation of vesicle trafficking may function to fine tune the balance of endocytosis and exocytosis to promote the cell growth required for pheromone induced morphogenesis.

M149

**RhoA mediates epithelial cell shape changes via mechanosensitive endocytosis**

**K. Cavanaugh**[1,2], M. Staddon[3], E. Munro[1,4], S. Banerjee[3], M. Gardel[4,5]; [1]Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, [2]Committee on Development, Regeneration and Stem Cell Biology, University of Chicago, Chicago, IL, [3]Department of Physics and Astronomy, and Institute for the Physics of Living Systems, University College London, London, UNITED KINGDOM, [4]Institute for Biophysical Dynamics, University of Chicago, Chicago, IL, [5]James Franck Institute, and Department of Physics, University of Chicago, Chicago, IL.

RhoA mediates epithelial cell shape changes via mechanosensitive endocytosisKate E. Cavanaugh[1,2], Michael F. Staddon[3],Ed Munro[1,4], Shiladitya Banerjee[3], Margaret L. Gardel[4, 51 Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637, USA, [2]Committee on Development, Regeneration and Stem Cell Biology, University of Chicago, Chicago, IL 60637, USA, [3]Department of

Physics and Astronomy, and Institute for the Physics of Living Systems, University College London, London WC1E 6BT, United Kingdom, [4]Institute for Biophysical Dynamics, University of Chicago, Chicago 60637 IL, USA, [5]James Franck Institute, and Department of Physics, University of Chicago, Chicago 60637 IL, USA.Abstract: Epithelial remodeling involves ratcheting behavior whereby periodic contractility produces transient changes in cell-cell contact lengths, which stabilize to produce lasting morphogenetic changes. Pulsatile RhoA activity is thought to underlie many morphogenetic ratchets, but how RhoA governs transient changes in junction length, and how these changes are rectified to produce irreversible deformation, remains poorly understood. Here, we use optogenetics to characterize responses to pulsatile RhoA in model epithelium. Short RhoA pulses drive reversible junction contractions, while longer pulses produce irreversible junction length changes that saturate with prolonged pulse durations. Using an enhanced vertex model, we show that this is explained by two effects: thresholded tension remodeling and continuous strain relaxation. Our model predicts that structuring RhoA into multiple pulses overcomes the saturation of contractility and we confirm this experimentally. Junction remodeling also requires formin-mediated E-cadherin clustering and dynamin-dependent endocytosis. Thus, irreversible junction deformations are regulated by RhoA-mediated contractility, membrane trafficking, and adhesion receptor remodeling.

## Minisymposium 15: Cytoskeleton In Vitro

M150

**A Structural and Mechanistic Model for the Regulation of LRRK2'S Interaction with Microtubules**

**J. Salogiannis**; University of California San Diego, San Diego, CA.

Parkinson's Disease (PD) is the second most prevalent neurodegenerative disease, affecting ~10 million people worldwide. One of the most commonly mutated genes in PD is **L**eucine **R**ich **R**epeat **K**inase **2** (*LRRK2*). Autosomal dominant mutations in *LRRK2* cause familial PD, while *LRRK2* mutations are also associated with sporadic PD. LRRK2 is a large protein with multiple domains, including a kinase and GTPase domain. A unifying theme for *LRRK2* and many other PD genes is that they function in intracellular trafficking. For example, LRRK2 co-localizes with microtubules, an association that is enhanced by the majority of familial PD mutations and has been shown to disrupt trafficking in model systems. We set out to determine the structure of LRRK2, how LRRK2 interacts with microtubules, and why interactions with microtubules could be detrimental.Using pure components in an in vitro reconstituted system we show that LRRK2 directly binds microtubules. Furthermore, low nanomolar concentrations of LRRK2 inhibit the motility of the microtubule-based motor kinesin-1. Using cryo-electron microscopy, we solved a 3.5Å structure of the catalytic half of LRRK2, which contains its kinase and GTPase domains. Using cryo-electron tomography we solved an 18Å structure of the microtubule-associated filaments formed by LRRK2 in cells. Based on these structures, we built an atomic model of microtubule-associated LRRK2. This model led us to hypothesize that the conformational state of LRRK2's kinase domain is critical for filament formation on microtubules. In support of this model, we find that kinase inhibitors favoring a closed-kinase conformation negatively impact kinesin motility, while kinase inhibitors favoring an open-kinase confirmation relieve the inhibition of kinesin motility caused by LRRK2. In cells, we observe similar trends; with kinase inhibitors that favor an open conformation reducing LRRK2 microtubule-associated filament formation and those favoring a closed conformation enhancing LRRK2 filaments formation. We also find that LRRK2's kinase activity towards the GTPase Rab10 is stimulated by microtubules in vitro. Together, our work leads us to propose that

PD-linked LRRK2 uses microtubules as an intracellular scaffold to act as a roadblock for molecular motors and phosphorylate Rab proteins.

M151

**Mitochondria-adaptor TRAK Enables Kinesin-1 Driven Transport in Crowded Environments**

**V. Henrichs**[1,2], Z. Nahácka[1], J. Rohlena[1], C. Bařinka[1], J. Neužil[1], S. Diez[3,4], M. Braun[1], Z. Lánský[1]; [1]Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, Vestec u Prahy, CZECH REPUBLIC, [2]Faculty of Science, Charles University in Prague, Prague, CZECH REPUBLIC, [3]B CUBE - Center of Molecular Bioengineering, Technische Universität Dresden, Dresden, GERMANY, [4]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, GERMANY.

Intracellular trafficking of organelles driven by kinesin-1 stepping along microtubules underpins essential processes, such as neuronal activity. Numerous experiments in the absence of other proteins show that during a single microtubule attachment kinesin-1 performs hundreds of steps resulting in micron-long runs along the microtubule. The presence of other microtubule-associated proteins, however, decreases drastically the lengths of kinesin-1 runs. How can kinesin-1 act as an efficient transporter in the crowded cellular environment thus remains largely unclear. Here, we *in vitro* show that the interaction of TRAK (Milton), an adaptor protein essential for mitochondrial trafficking, activates kinesin-1 and enables kinesin-1-based transport in crowded environments. The binding of TRAK to kinesin-1 i) increased the probability of kinesin-1 passing through cohesive envelopes of tau molecules formed on the microtubule surface, ii) facilitated kinesin-1 navigation around obstacles on microtubules and iii) increased the run length of kinesin-1 in cell lysate. Moreover, TRAK enabled kinesin-1-based long-distance transport of isolated mitochondria along microtubules in vitro. We explain this enhanced transport activity of kinesin-1 by an observed interaction of TRAK with microtubules, which provides an additional foothold for the stepping of the TRAK-kinesin-1 complex. We show that anchoring of kinesin-1 to the microtubule by an adaptor protein can turn kinesin-1 into an efficient long-range transporter in crowded environments.

M152

**Structure of the Dynein-2 Complex and Its Assembly with Intraflagellar Transport Trains**

K. Toropova[1], **A. J. Roberts**[1], R. Zalyte[2], A. G. Mukhopadhyay[1], M. Mladenov[1], A. Carter[2]; [1]Birkbeck, University of London, London, UNITED KINGDOM, [2]Medical Research Council Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM.

Cilia are antenna-like organelles that project from the surface of virtually every cell type in the body. To assemble and execute their essential functions in sensing and motility, cilia use a conserved system to transport cargoes and signalling molecules along their length. This process of intraflagellar transport (IFT) involves multi-megadalton polymers, termed IFT “trains", which move under the power of oppositely-directed microtubule motors dynein-2 and kinesin-II. Here we recombinantly expressed the ~1.4 MDa human dynein-2 complex and solved its cryo-EM structure to near-atomic resolution. The two identical copies of the dynein-2 heavy chain are contorted into different conformations by a WDR60-WDR34 heterodimer and a block of two RB and six LC8 light chains. One heavy chain is steered into a zig-zag, which matches the periodicity of the anterograde IFT-B train. Our results converge on a model in which an unusual stoichiometry of non-motor subunits control dynein-2 assembly and asymmetry,

giving mechanistic insight into dynein-2's interaction with IFT trains and the origin of diverse functions in the dynein family.

M153

**Physical Integration Within *Xenopus* Egg Extract Microtubule Asters Suggests Aster Movement Is Driven by Dynein-dependent Surface Forces and Actomyosin Contraction**

**J. Pelletier**[1,2,3], C. Field[1,2], S. Fürthauer[4], N. Fakhri[3], T. Mitchison[1,2]; [1]Harvard Medical School, Boston, MA, [2]Marine Biological Laboratory, Woods Hole, MA, [3]Massachusetts Institute of Technology, Cambridge, MA, [4]Flatiron Institute, New York, NY.

The eukaryotic cytoplasm includes dynamic networks of cytoskeletal polymers and membranous organelles that mutually interpenetrate and mechanically interact via steric and molecular interactions. During telophase, forces on astral microtubules cause centrosomes to move away from the division plane to partition into daughter cells and position the cleavage furrow. In *Xenopus laevis* eggs, centrosomes travel hundreds of microns away from the midplane after anaphase of first mitosis. This occurs before astral microtubules reach the cortex, so forces must be generated within bulk cytoplasm. Two related questions are unanswered: How are asymmetric forces on asters generated, and do asters move relative to other cytoplasmic components, or collectively with them? Using *Xenopus* egg extracts, we co-imaged microtubules, F-actin, endoplasmic reticulum (ER), mitochondria, and a soluble probe in interphase asters as they grew, interacted, and moved. Aster movement was triggered in two ways: away from boundaries where asters interacted, and by dynein attached to the coverslip, which caused periodic motion under some conditions. Within moving asters, multiple cytoplasmic networks tended to move in the same direction at the same velocity, suggesting physical integration. Photorelease of soluble fluorescein showed that even small molecules are advected with the moving aster network. Dynein-mediated transport did cause inwards movement of ER and mitochondria. Inwards velocity was maximal at aster surfaces, suggesting force generation by organelle-bound dynein is mostly restricted to the aster surface. Centrosomes moved predictably away from aster boundaries, leading to regular spacing of the centrosomes. We expected this movement to be dynein-dependent, but inhibition experiments revealed a large role for actomyosin contraction away from regions of low actin density at aster boundaries. Our results inform a multicomponent active gel model of aster movement, in which dynamic, interpenetrating cytoplasmic networks mechanically interact to separate centrosomes and position the egg cleavage plane.

M154

**How to Make Microtubules and Build the Mitotic Spindle**

**S. Petry**; Princeton University, Princeton, NJ.

The mission of my lab is to understand how cells obtain their shape, position organelles, move materials, and segregate chromosomes during cell division. Each of these functions relies on a specific architecture of the microtubule (MT) cytoskeleton. Despite its central biological role, it is not well understood how such a particular MT architecture is established in a cell. Moreover, we still lack a basic understanding of how a cell makes microtubules in the first place, although their building block tubulin was discovered in the 1960s. In this talk, I will discuss how we recently solved a long-standing mystery of how microtubules are nucleated in cells by identifying a novel MT nucleation factor which is part of the universal module that makes MTs. Furthermore, we uncovered how the MT nucleation module can be

recruited to the right location and turned on at the right time to give rise to a cellular MT structure. Last, we use microtubule substructures as pieces of the puzzle to assemble cellular MT networks, such as the mitotic spindle, and thereby further our understanding of how the MT nucleation machinery builds cellular structures in health and disease.

M155

**The Mitotic Spindle Protein Ckap2 Is a Potent Microtubule Assembly Factor**

T. McAlear, **S. Bechstedt**; McGill University, Montreal, QC, CANADA.

Microtubule dynamics are modified throughout the cell cycle, including an increase in both microtubule nucleation and growth rates when cells begin to enter mitosis. To date, only one microtubule associated protein family, XMAP215/chTOG, has been shown to significantly speed up microtubule growth. We have identified a second microtubule growth factor, the mitotic spindle protein Cytoskeleton Associated Protein 2 (CKAP2). To determine how CKAP2 affects microtubule dynamics, we use in vitro biophysical assays to reconstitute microtubule dynamics with purified CKAP2 and tubulin. In microtubule growth assays, we show CKAP2 increases the association rate constant of tubulin to the growing microtubule end by 50 fold. Additionally, CKAP2 nucleates microtubule growth at 100 fold lower tubulin concentrations compared to controls. In fact, CKAP2 seems to push microtubule growth to the physical limit, as defined by diffusion of the tubulin dimer to the growing microtubule end. Further, CKAP2 lowers microtubule catastrophe rates by 30 fold. Notably, CKAP2 is predicted to be highly disordered, and lacks characterized domains. These results suggest that CKAP2 acts as microtubule assembly factor, likely contributing to increased growth and nucleation of microtubules in the mitotic spindle. Our findings help explain CKAP2s role as a proliferation marker and potential oncogene

M156

**Multi-component in *Vitro* Reconstitution Induces Robust Microtubule Treadmilling**

**M. Zanic**, G. Arpag, E. J. Lawrence; Vanderbilt University, Nashville, TN.

Dynamic instability, in which a microtubule end switches between phases of growth and shrinkage, is the hallmark behavior of microtubules. Microtubule treadmilling, in which the plus end grows while the minus end shrinks, is also observed in cells when minus ends are detached from nucleating centers. While microtubule dynamic instability has been widely studied *in vitro*, the conditions that lead to microtubule treadmilling are not well understood. To elucidate the mechanisms underlying microtubule treadmilling, we first characterized microtubule dynamics *in vitro* with purified tubulin over a range of tubulin concentrations, and analytically calculated a net assembly/disassembly rate at both polymer ends. Our population-level results predicted that microtubules assembled with tubulin alone can treadmill, albeit with minus ends leading, consistent with earlier reports. We then used an *in vitro* assay that allows observation of treadmilling on a single-microtubule level. In conditions predicted for treadmilling, we observed a significant fraction of microtubules that could be classified as treadmilling, with the majority exhibiting minus-end directionality. However, not only was the treadmilling direction different from that observed in cells, the overall flux rates of these microtubules were an order of magnitude smaller than measured cellular rates. We hypothesized that this discrepancy is due to the regulatory effects of Microtubule Associated Proteins (MAPs) in cellular environments. To test this hypothesis, we explored the combined effects of MAPs on microtubule assembly flux rates using computer simulations, constrained by published experimental observations. Our *in silico* experiments

predicted that a combination of four MAPs (EB1, XMAP215, CLASP2 and MCAK) could promote plus-end-leading treadmilling with high flux rates. Finally, we tested the predictions of our computational model using the multi-MAP *in vitro* microtubule assembly assay, and found that with this minimal *in vitro* system, we could indeed reconstitute robust and fast plus-end-leading treadmilling, consistent with observations in cells.

M157

**Reconstitution of Dynamic Actin Cables From Purified Components**

**L. W. Pollard**, M. V. Garabedian, S. L. Alioto, B. L. Goode; Brandeis University, Waltham, MA.

A central unanswered question in biology is how cells assemble large protein structures that have precise sizes and shapes tailored to their functions and simultaneously undergo dynamic turnover. *Saccharomyces cerevisiae* actin cables provide an ideal system for addressing this question, because their essential function in cargo transport demands that they simultaneously undergo rapid assembly and turnover while maintaining well-defined lengths. Genetic studies have identified a molecular parts list required for proper cable morphology and function *in vivo*, but a minimal set of components sufficient to assemble a dynamic cable has not yet been established, and it has remained unclear how each component contributes to cable formation. Here, using only eight purified *S. cerevisiae* proteins (actin, profilin, formin, capping protein, tropomyosin, cofilin, coronin, and AIP1), we were able to assemble linear actin structures that undergo steady-state polarized turnover while maintaining lengths similar to cables *in vivo*. Cables were polymerized at one end by formins attached to beads, in a profilin-dependent manner, and disassembled at the other end by cofilin, coronin, and AIP1. Cable assembly also required tropomyosin, which antagonized disassembly-promoting factors. Capping protein restricted actin polymerization to the formin-coated beads and limited cable network density. Remarkably, removal of individual proteins from the *in vitro* system led to cable defects similar to the phenotypes observed *in vivo* for disrupting the corresponding genes. Thus, our reconstitution system closely mirrors cellular requirements for cable formation and demonstrates that a minimal set of nanoscale components can self-organize into a dynamic, microns-long cellular structure.

M158

**Cell Division Proteins Follow Treadmilling FtsZ Filaments by Diffusion-and-Capture**

**N. Baranova**[1], M. Loose[1], P. Radler[1], V. M. Hernández-Rocamora[2], W. Vollmer[2]; [1]Institute of Science and Technology Austria, Klosterneuburg, AUSTRIA, [2]Newcastle University, Newcastle, UNITED KINGDOM.

The tubulin homolog FtsZ plays a key role for bacterial cytokinesis, but the molecular mechanisms underlying its function are largely unknown. Treadmilling filaments of FtsZ are thought to actively move proteins within the division plane to distribute cell wall synthesis for the generation of the two new cell poles. To study how FtsZ filament dynamics are coupled to cell wall synthesis, we reconstituted part of the bacterial cell division machinery using its purified components FtsZ, FtsA and truncated transmembrane proteins essential for cell division. We found that the membrane-bound cytosolic peptides of FtsN and FtsQ co-migrated with treadmilling FtsZ-FtsA filaments. Remarkably, despite their directed behavior on the ensemble level, individual peptides showed random motion and transient confinement. Our work suggests that divisome proteins dynamically follow treadmilling FtsZ filaments at the cell division site by a diffusion-and-capture mechanism and highlights the importance of transient interactions for the self-organization of complex biological structures.

M159

**Phosphoinositides Regulate Force-independent Interactions between Talin, Vinculin, and Actin in Vitro**

**C. Kelley**, T. Litschel, D. Dedden, S. Schumacher, P. Schwille, N. Mizuno; Max Planck Institute of Biochemistry, Martinsried, GERMANY.

The focal adhesion (FA) proteins talin and vinculin connect integrin receptors in the membrane to contractile actomyosin networks, acting as the mechanosensitive core of FA complexes. Both proteins have been shown to directly bind F-actin, the phosphoinositide PI(4,5)$P_2$, and each other. Though these interactions are critical to FA formation, stability, and dynamics, the underlying mechanisms of regulation for talin and vinculin within FA complexes remain unclear.Here, talin-vinculin-actin assemblies have been reconstituted in vitro using various synthetic membrane systems, including liposomes, supported lipid bilayers, and encapsulation within giant unilamellar vesicles. Using biochemistry, light microscopy, and cryo-EM, we show that interactions between full-length talin and full-length vinculin are mediated by membrane binding. Previously, it has been suggested that force application is required for release of talin autoinhibition, leading to vinculin binding. Here, we show that the addition of phosphoinositide-rich membranes alone is sufficient to trigger talin-vinculin interactions, even in the absence of force. Additionally, membrane-bound talin and vinculin coordinate the recruitment and reorganization of F-actin at membrane surfaces, suggesting that local regulation of membrane composition could directly control the recruitment, activation, and engagement of proteins, such as talin and vinculin, within FAs.

## Minisymposium 16: Dynamics of Morphogenesis in Cells, Tissues and Organisms

M160

**The Role of Cell and Tissue Morphology in Neuroepithelial Nuclear Positioning**

**C. Norden**[1,2]; [1]MPI-CBG, Dresden, GERMANY, [2]Instituto Gulbenkian de Ciência, Oeiras, PORTUGAL.

Correct nuclear positioning is important for cellular function and developmental programs in diverse contexts. A particular nuclear positioning process occurs in diverse neuroepithelia, in which nuclei must travel to apical positions before mitosis a step essential for proper tissue maturation. While the cell biological mechanisms of these movements have been addressed in diverse neuroepithelia, how nuclear positioning is linked to tissue morphology is less clear. Using zebrafish as a model system, and performing quantitative single cell and tissue-wide imaging, we find that kinetics and actin-dependent mechanisms of nuclear positioning vary in neuroepithelia of different shape. In straight neuroepithelia nuclear positioning is controlled by Rho-ROCK-dependent myosin contractility. In contrast, in basally constricted neuroepithelia a novel formin-dependent pushing mechanism is found. These mechanistic differences are conserved in other tissues, morphologically comparable to retina and hindbrain, and migration modes can change when cell and tissue shape changes are induced. Thus, for neuroepithelial cells, in which nuclear positioning is tightly linked to successful tissue development, this important end justifies the many means.

M161

**An Adhesion Code Enables Robust Pattern Formation in the Zebrafish Spinal Cord**

**T. Tsai**[1], M. Sikora[2], C. Heisenberg[2], S. Megason[1]; [1]Harvard Medical School, Boston, MA, [2]Institute of Science and Technology, Klosterneuberg, AUSTRIA.

An outstanding question in embryonic development is how different cell types reach their final positions correctly, despite large scale cellular re-arrangement during tissue morphogenesis. To achieve this, cell fate specification needs to be coordinated with regulated cell migration and adhesion, yet the mechanisms are not well-understood. In the zebrafish spinal cord, thirteen neural progenitor domains, each consisting of distinct cell type, are specified along the ventral-to-dorsal axis by opposing gradients of Shh and BMP/Wnt. The Shh signal is noisy, resulting in cells specified in a mixed pattern, requiring that individual cells be sorted to resolve this mixed pattern into well-separated domains.Here we set out to test if differential adhesion plays a role in assisting pattern formation during zebrafish spinal cord development. We developed two cell-based mechanical assays based on micropipette aspiration to measure adhesion forces and adhesion preferences among three types of neural progenitors (p3, pMN, and p0 cells). Interestingly, each cell type exhibited preference to selectively stabilize homotypic contact and adhered more strongly to cells of the same type. A subsequent transcriptomic and genetic analysis revealed three adhesion molecules (N-cadherin, Cadherin 11, and Protocadherin 19) that are differentially expressed among the three cell types, forming a three-molecule adhesion code. When the expression levels of these adhesion molecules are perturbed, the adhesion preference to cells of the same type is lost, and the neural progenitor pattern in the spinal cord is disrupted *in vivo*. These findings allow us to propose an “adhesion code” mechanism that promotes cell sorting during tissue scale patterning. While the conventional view of spinal cord patterning is heavily focusing on interpretation of biochemical signals and transcriptional regulation, our findings suggest cell adhesion is a critical contributor to enable precise patterning in a tissue that is undergoing dramatic morphogenetic movement.

M162

**Differentiation of Structurally- and Optically- Distinct Types of Iridophores Is Required for Stripe Formation in Zebrafish**

**D. Gur**[1,2], E. Bain[3], D. Parichy[3], J. Lippincott-Schwartz[2]; [1]NIH/NICHD, Bethesda, MD, [2]Janelia Research Campus, Ashburn, VA, [3]University of Virginia, Charlottesville, VA.

Color patterns are prominent features of many animals, they evolve rapidly and have important functions in protection against UV irradiation, camouflage, kin recognition, shoaling and mate choice. The alternating blue stripe and golden interstripe of adult zebrafish (Danio rerio), a leading model of vertebrate pattern formation, are produced by a mosaic of black melanophores, yellow xanthophores and light reflecting iridophores that distribute in the dermis during juvenile development. It has been proposed that the zebrafish stripe formation depends on dispersal and patterned aggregation of a single differentiated iridophore cell type. Using a combination of X-ray diffraction, Raman spectroscopy, genetic manipulations, and cryogenic scanning electron microscopy, we found that there are several distinct types of iridophores in the stripe versus interstripe zones, contrary to the existing model. The different iridophore cell types had distinct crystal morphologies, ultrastructural organizations and optical properties associated with them. Single cell transcriptomic analysis confirmed the multiplicity of distinct iridophore cell types in stripe and interstripe zones. Based on these data, we propose that the

zebrafish skin's striped pattern results from differentiation of optically-distinct types of iridophores: these arise from precursor cells that migrate to their appropriate stripe zone and then differentiate into the specific iridophore type.

M163

**FGF Signaling Controls Epithelial-mesenchymal Transition During Gastrulation through the Regulation of Cell Adhesion and Division**

**J. Sun**, V. A. Stepanik, A. Stathopoulos; Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA.

Gastrulation, the formation of the three germ layers, is said to be the most important event in life. This morphogenetic process involves extensive cell movements and dynamic cell interactions and is regulated by highly conserved signaling pathways. In *Drosophila*, gastrulation starts as the presumptive mesoderm cells invaginate to form a tube-like structure. Subsequently, mesoderm cells undergo an epithelial-to-mesenchymal transition (EMT) and migrate dorsally to form a monolayer on top of the ectoderm, where they receive a combination of Wingless, BMP and FGF signals to induce differentiation of distinct cell lineages including cardiac, visceral, and somatic muscles. Two FGF ligands, Pyramus (Pyr) and Thisbe (Ths), which both signal through FGF receptor Heartless (Htl), play partially redundant roles during the mesoderm EMT and migration processes. However, the individual function of each ligand is still unclear. We have previously shown that EMT is a prolonged process in which FGF signaling pathway functions to support collective cell movement by regulating progressive decrease in cell-cell attachments as well as by promoting synchronized cell division. Meanwhile, we found that blocking cell division in addition to FGF signaling results in an arrest of EMT: adherens junctions (AJs) fail to be dissembled from the apical center of the invaginated tube upon tube collapse. Here, we demonstrate that Pyr plays a major role in supporting EMT and in activating the downstream Ras-MAPK intracellular signaling cascade. In contrast to secreted Ths, Pyr functions locally, stimulates a stronger receptor response, and its localization and temporal expression are tightly regulated through a novel post-translational mechanism that will be described.

M164

**Acute Rho1 Activation Reveals that Ventral Epithelial Cells of the *Drosophila* Embryo Are Specifically Predisposed for Coordinated Anisotropic Constriction During Gastrulation**

**A. Rich**, R. Fehon, M. Glotzer; University of Chicago, Chicago, IL.

Many morphogenetic events, including convergent extension and tube formation, require modulation of the actomyosin cytoskeleton. Ventral furrow formation in Drosophila embryos is one such morphogenetic event; it results when extracellular signals activate two transcription factors, Snail and Twist, in a subset of ventral epithelial cells. These regulators then drive the expression of multiple proteins which ultimately induce Rho1 activation, apical constriction, and invagination of cells into the embryo. These apical constrictions are anisotropic and appear coordinated, but the basis for this anisotropy and coordination is unknown.To address this and related questions, we utilized optogenetics to control Rho1 activity in the embryo. Acute Rho1 activation at the onset of gastrulation induces ectopic invaginations in both the dorsal and ventral embryonic epithelium. Rho1 activation induces apical constriction in both dorsal and ventral cells, but ventral cell constriction is stronger and more anisotropic. Strikingly, ectopic Rho1 activation induces non-cell autonomous deformations outside the

activation zone only in the ventral epithelium. Thus, we demonstrate that ventral cells are specifically predisposed to respond to Rho1 activation with anisotropic and coordinated deformations.To identify the factors required for ventral cell specific behavior, we analyzed acute Rho1 activation in embryos deficient in factors required for ventral furrowing. Ventral cells depleted of RhoGEF2 exhibit anisotropic apical constriction, suggesting a molecular specialization of ventral cells beyond their ability to activate high levels of Rho1. Ventral cells lacking Twist exhibit weaker and less anisotropic apical constrictions, indicating a previously unknown role of Twist. Unlike wildtype or twist embryos, Rho1-induced deformations persist in RhoGEF2-depleted embryos.Collectively, our results demonstrate that while Rho1 is sufficient to initiate invagination throughout the embryonic epidermis, the individual cell shape changes accompanying these invaginations differ between dorsal and ventral cells. Additionally, ventral cells specifically can propagate the response to Rho1 activation outside of the zone of optogenetic activation. Experiments are in progress to identify the factors required for these ventral specific behaviors.

M165

**Distinct Prepatterns of RhoA Activity and F-actin Levels Promote Tissue Folding**

**M. Denk-Lobnig**, N. C. Heer, A. C. Martin; MIT, Cambridge, MA.

The emergence of organism and organ shape during development requires coordinated forces that change tissue shape. One way that planar epithelia are sculpted is through bending/folding of the sheet to generate curvature, forming structures like invaginations or tubes. How epithelial sheets regulate curvature is unknown. We used the invagination of the *Drosophila* presumptive mesoderm to determine that distinct multicellular patterns of RhoA activation and F-actin levels generate proper tissue curvature. First, we showed that mechanical connections between cells are not required for graded myosin activation, suggesting that a prepattern of transcriptional activation establishes the myosin activation gradient. Second, we showed a gradient in RhoA activity that is established by proper balance between a GTPase activating protein, C-GAP and a Guanine Nucleotide Exchange factor, RhoGEF2, which establishes proper tissue curvature. Finally, we show that cells that stretch in the marginal mesoderm exhibit depleted F-actin levels compared to ventral midline cells and the ectoderm, suggesting that a zone of F-actin depletion allows cell stretching at the tissue margin to promote apical constriction and folding at the ventral midline. Thus, transcriptional patterning of contractility and F-actin levels across a tissue determines tissue curvature, which could explain the diverse shapes in different folding processes throughout development.

M166

**A Single-cell Reconstruction of Planarian Regeneration Identifies Wound-induced Transcriptional States Required for Tissue Repair**

**B. Benham-Pyle**[1], C. Brewster[1], A. Kent[1], S. Chen[1], F. Mann[1,2], A. Scott[1], A. Box[1], A. Sánchez Alvarado[1,2]; [1]Stowers Institute for Biomedical Research, Kansas City, MO, [2]Howard Hughes Medical Institute, Chevy Chase, MD.

Wound-healing and tissue regeneration require the coordination of diverse cells and their functions across multiple tissue lineages. In particular, differentiated somatic cells signal to proliferative cells to initiate the growth and patterning of new tissue while maintaining and remodeling existing tissue. During planarian whole-body regeneration, pluripotent adult stem cells - cNeoblasts - drive new tissue

growth and several molecules required for tissue patterning, stem cell proliferation, and lineage specification have been characterized. However, the minimum number and types of stem and somatic cells required to coordinate regeneration has not been defined. By characterizing the regenerative competency of biopsies 0.50mm - 1.50mm in diameter, we determined that planarian regeneration requires a healthy tissue fragment 1.00mm in diameter (~10,000 cells). We then used single-cell RNA sequencing to characterize the dynamics of cellular composition in minimal tissue biopsies taken from un-irradiated, sub-lethally irradiated, and lethally irradiated animals, thereby modulating the number and diversity of stem cells and progenitors present at the time of injury. We profiled ~300,000 single-cell transcriptomes over two weeks of regeneration and identified 90 transcriptional cell states from eight previously described planarian tissue classes, some of which contained additional sub-cluster diversity. Significantly, several somatic cell states occurred only transiently during regeneration or displayed irradiation-dependent dynamics, thereby confirming that complex changes in cellular composition are associated with adult tissue remodeling upstream and downstream of stem cell proliferation. To test the functional role of wound-induced cell states, enriched genes were selected from each cluster of interest, visualized *in vivo* by *in situ* hybridization, and depleted by RNAi-mediated knockdown. Depletion of wound-induced genes produced a variety of phenotypes, including loss of tissue regeneration and reduced stem cell maintenance. Altogether, these results characterize whole-animal tissue composition changes after injury with unprecedented cellular detail. Moreover, we identified novel cell type-specific gene expression signatures generated by somatic tissues and required for stem cell maintenance and whole-body regeneration.

M167

**Simple Geometric Rules Define the Shape and Stability of Epithelial Lumens**

**C. G. Vasquez**, V. T. Vachharajani, A. R. Dunn; Stanford University, Stanford, CA.

A continuous sheet of epithelial cells surrounding a hollow opening, or lumen, defines the basic topology of numerous organs. While prior work has described the hydrostatic pressure-driven expansion of lumens when they are large, the physical mechanisms that promote the formation and maintenance of lumens while still small are less explored. In particular, models that rely solely on pressure-driven expansion face a potential challenge in that the Laplace pressure, which resists lumen expansion, is predicted to scale inversely with lumen radius. We therefore sought to understand how cells might avoid this 'Laplace paradox' in the context of *de novo* lumen formation. We investigated the physical forces responsible for stabilizing the initial stages of lumen growth using a 3D culture system in which Madin Darby Canine Kidney (MDCK) epithelial cells spontaneously form hollow lumens. Our experiments revealed that neither the actomyosin nor microtubule cytoskeletons were required to stabilize lumen geometry, and that a positive intraluminal pressure was not necessary for lumen stability. Instead, our observations are in agreement with a quantitative model in which cells can maintain lumen shape due to topological and geometrical factors tied to the establishment of apico-basolateral polarity. In addition, the shape of small lumens, as parameterized by the ratio of surface area to volume, could be described almost entirely by a geometric rule based on the positioning of cell nuclei within the spheroid. Our findings highlight a pressure-independent method for lumen growth that, to our knowledge, has been largely overlooked. We suggest that this model may provide a general physical mechanism for the formation and growth of luminal openings in a variety of physiological contexts.The advantages of pressure independent lumen growth remain to be firmly established in an *in vivo* model. However, we note that this mechanism is predicted to be physically robust and energy efficient relative to pressure-

driven growth, which exposes tissues to pressure-driven rupture. Importantly, when lumens achieve a sufficient diameter (~20 µm), a pressure-driven transition to circular cross-sections will dominate, providing a simple way to generate regular, space-filling shapes like spheres and tubes. The two mechanisms for dictating lumen shape thus coexist and fulfill complementary functions in driving embryonic growth and tissue morphogenesis.

M168

**Cells in Intermediate States During EMT Are Characterized by Specific Geometrical and Mechanical Intra-cellular Architectures.**

Y. Margaron[1], L. Guyon[2], L. Kurzawa[3], A. Morel[4], A. Pinhiero[5], L. Blanchoin[3], F. Reyal[5], A. Puisieux[4], **M. Thery**[6]; [1]CytoMorpho Lab, IRIG, Paris, FRANCE, [2]Interdisciplinary Research Institute of Grenoble (IRIG), Grenoble, FRANCE, [3]CytoMorpho Lab, IRIG, Grenoble, FRANCE, [4]Cancer Research Center of Lyon, Lyon, FRANCE, [5]RT2Lab, PSL Research University, Paris, FRANCE, [6]CytoMorpho Lab, Hopital Saint Louis, Paris, FRANCE.

The epithelial to mesenchymal transition (EMT) allows mammary breast cancer cells to dissociate from the primary tumour and form metastases. Recent works have revealed that the *dangerous* cells that have acquired augmented capacity to migrate and transdifferentiate into several cell types are in an intermediate stage between the epithelial and the mesenchymal state. It is now required to unravel the molecular and cellular mechanisms responsible for the acquisition of these properties and to identify early and specific structural markers characterizing these stages. Here we induced the expression of ZEB1, a transcription factor responsible for EMT initiation, to generate intermediate stages of EMT in human mamary epithelial cells and stimulation with TGFβ to push further the transition to the mesenchymal state. We measured and compared the architecture, internal organisation and mechanical properties of each state. We found that the lack of inter-cellular cohesiveness in intermediate and later stages of EMT can be detected early by microtubule destabilisation and the repositioning of the centrosome from the cell junction to the cell center. Consistent with their high migration velocities, we found that cells in intermediate state of EMT were in a low tensional state compared to epithelial and mesenchymal cells. The high contractility of mesenchymal cells powered a retrograde flow pushing the nucleus away from cell adhesion to the extra-cellular matrix. These measures revealed how defined structural and mechanical rearrangement in intermediate stages of EMT confered them specific dissociation and migration properties that distinguish them from epithelial and mesenchymal states.

M169

**Membrane Tension Regulates Fgf Driven Fate Choice in Embryonic Stem Cells**

**H. De Belly**[1], P. H. Jones[2], K. J. Chalut[3], E. K. Paluch[4,1]; [1]MRC Laboratory for Molecular Cell Biology, University College London, Gower Street, London, WC1E 6BT, UK., London, UNITED KINGDOM, [2]Department of Physics & Astronomy, University College London, Gower Street, London WC1E 6BT, London, UNITED KINGDOM, [3]Wellcome Trust/Medical Research Council Cambridge Stem Cell Research Institute, Tennis Court Road, University of Cambridge, Cambridge, CB2 1QR, UK., Cambridge, UNITED KINGDOM, [4]Department of Physiology, Development and Neuroscience, Downing Street, University of Cambridge, Cambridge, CB2 3DY, UK., Cambridge, UNITED KINGDOM.

Changes in cell shape and mechanics frequently accompany cell fate transitions. Yet how mechanics affects the regulatory pathways controlling cell fate is poorly understood. To probe the interplay

between shape, mechanics and fate, we used embryonic stem (ES) cells, which spread as they undergo early differentiation. We found that this spreading is regulated by a decrease in plasma membrane tension, and preventing this decrease obstructs early differentiation of ES cells. Moreover, we found that if cell membrane tension is not decreased, endocytosis of FGF signaling components, which direct the exit from the ES cell state, is significantly inhibited. Strikingly, the early differentiation defects we observed can be rescued by increasing Rab5a-facilitated endocytosis. Thus, we show that a mechanically-triggered increase in endocytosis regulates fate transitions. Our findings are of fundamental importance for understanding how cell mechanics regulates biochemical signaling, and therefore cell fate.

## Minisymposium 17: Nucleus Structure and Dynamics

M170

**Cytoplasmic Volume and Limiting Nucleoplasmin Scale Nuclear Size During *Xenopus Laevis* Development by Altering Chromatin Organization**

**P. Chen**, M. Tomschik, K. Nelson, J. Oakey, J. C. Gatlin IV, D. L. Levy; University of Wyoming, Laramie, WY.

A basic question in cell biology is how the size of the nucleus is regulated with respect to cell size. During early *Xenopus laevis* embryogenesis, there are dramatic reductions in both cell and nuclear sizes. This reproducible scaling of nuclear size provides a robust system with which to characterize and identify mechanisms of nuclear size regulation. To test if the volume of embryonic cytoplasm is limiting for nuclear growth, we used microfluidic technology to encapsulate gastrula stage *X. laevis* extract and nuclei in droplets of defined volume and shape. We found that cytoplasmic volume and composition, but not cytoplasmic shape, contribute to nuclear size scaling. To identify cytoplasmic components limiting for nuclear growth, we biochemically fractionated *X. laevis* egg extract and identified the histone chaperone nucleoplasmin (Npm2) as a putative nuclear size-scaling factor. Nuclear size increased in extract droplets supplemented with Npm2 protein, and embryos microinjected with Npm2 exhibited larger nuclei at gastrulation. Consistent with Npm2 being a nuclear-size scaling factor, per cell amounts of nuclear Npm2 and histone H2B decrease over development. Chromatin staining and micrococcal nuclease digestion assays showed that Npm2 increases chromatin compaction and nucleosome packing while decreasing the amount of euchromatin. We propose that Npm2 drives nuclear growth by altering chromatin organization. To further investigate how chromatin organization might influence nuclear size, we studied nuclei assembled de novo in *X. laevis* egg extract. Nuclei treated with DNase or MNase were still import-competent but failed to expand and properly localize lamins to the inner nuclear membrane, suggesting that nuclear import is not sufficient for nuclear growth. We are currently testing the model that chromatin stiffness produces intranuclear forces that contribute to nuclear growth. In summary, we propose that reductions in cell volume with concomitant decreases in the amounts of Npm2 and nuclear histones contribute to developmental nuclear size-scaling by affecting chromatin organization.

M171

**Prostaglandins Restrict Nuclear Actin to Control the Nucleolus**

D. Wineland[1], **T. Tootle, 52242**[1], G. Kimble[1], D. Kelpsch[2]; [1]University of Iowa-Carver Coll Med, Iowa City, IA, [2]Carnegie Institution for Science, Baltimore, MD.

Prostaglandins (PGs), lipid signals produced downstream of cyclooxygenase enzymes, have numerous physiological and pathological functions, including pain and inflammation, reproduction, heart health and disease, and cancer development and progression. One means by which PGs act is through regulating the actin cytoskeleton. Actin is not solely cytoplasmic, but localizes and functions within the nucleus. Using the robust genetic system of Drosophila and the well-characterized developmental process of oogenesis, we discovered multiple pools of nuclear actin that exhibit distinct developmental and subnuclear localizations. Specifically, fluorescently conjugated DNase I labels monomeric actin in the nucleolus of every cell. Anti-actin C4 labels a monomeric, nucleolar actin in a subset of cells during early oogenesis and recognizes polymeric nuclear actin in both the oocyte and undifferentiated germline cells. Anti-actin AC15 labels polymeric nuclear actin that localizes to the chromatin starting at mid-oogenesis in both germline and somatic cells. Additionally, ectopic expression of GFP-Actin results in nuclear actin rod formation during early oogenesis. We find that PGs are required to limit the levels of the different pools of nuclear actin. Loss of the Drosophila cyclooxygenase enzyme, Pxt, results in increased GFP-Actin rod formation, an increased frequency of cells exhibiting C4 nuclear actin, and premature and increased levels of AC15 nuclear actin. Thus, PG signaling is a critical negative regulator of nuclear actin. Our findings that both DNase I and C4 label actin within the nucleolus, PGs regulate C4 nuclear actin, and PGs regulate the structure of the nucleolus, led us to hypothesize that PGs tightly control nuclear actin to regulate the nucleolus. Supporting this loss of Exportin 6, which mediates the nuclear export of actin, results in defects in nucleolar structure that are similar to those observed for loss of Pxt. These data suggest that nuclear actin plays a critical role in regulating nucleolar structure. Together our findings lead to the model that PGs tightly control nuclear actin to regulate nucleolar structure and likely function. As both the nucleolar localization and functions of actin and the role of PGs in regulating the nucleolus are conserved across organisms, our findings reveal a novel pathway that may play critical roles in both normal and pathological instances. Indeed, high levels of PGs, increased nuclear actin, and nucleolar changes are each independently associated with cancer development and progression.

M172

**Examining the Modular Architecture of the Nuclear Pore Complex through Targeted Degradation**

**S. Regmi**, R. Kaufhold, V. Aksenova, S. Chen, H. Lee, E. Turcotte, A. Arnaoutov, M. Dasso; National Institutes of Health, Bethesda, MD.

The nuclear pore complex (NPC) is a channel through the nuclear envelope that allows macromolecular transport between the cytoplasm and nucleus. Disrupted NPCs and nucleocytoplasmic transport are hallmarks of many neurodegenerative diseases, including Huntington's and amyotrophic lateral sclerosis. NPCs contain roughly 30 proteins called Nups; Nup mutations are found in many tumors, further underscoring the need to better understand both individual Nups and NPC function overall. 32 copies of the vertebrate nonameric Y-complex assemble into rings that gird the inner and outer faces of the NPC. These rings are assumed to act as scaffolds of NPC architecture. To understand the roles of individual Y-complex subunits, we used CRISPR/Cas9 to add Auxin-Induced Degron (AID) tags at the

genomic loci encoding Nup160, Nup133, Nup107, Nup96 and Nup85 in human DLD-1 cells. The tagged Nups assemble into functional NPCs and degrade rapidly (<4 hours) after auxin addition. In the resultant cell lines, we assessed how each Nup contributes to NPC structure and function. After Nup160, Nup133 or Nup85 depletion, NPCs retained non-tagged Y-complex members and stayed surprisingly intact and functional for transport. At the same time, post-mitotic NPC assembly was disrupted in their absence, suggesting that they are required for NPC formation but can be removed from intact NPCs without catastrophic consequences. By contrast, Nup96 or Nup107 depletion caused rapid dispersion of non-tagged Y-complex subunits and other Nups, particularly those from the cytoplasmic face of the NPC. Loss of Nup96 or Nup107 was also associated with substantially slower nuclear import. There results indicate that Nup96 and Nup107 are essential for maintenance of Y-complex ring structures and functional NPCs. Interestingly, mass spectrometry of nuclear envelope fractions from cells depleted Nup96 or Nup107 showed that not all Nups are dispersed upon their loss, and that members of the inner ring complex (IRC) were largely retained. To examine the relationship between the Y-complex and these retained Nups, we tagged Nup188, a member of the IRC. Depletion of Nup188 resulted in a pattern that inverse to the patterns generated after Nup96 or Nup107 loss, showing retention of the Y-complex but loss of most IRC components. These findings lead us to propose a model in which the NPC has a modular architecture with interwoven pieces that can nevertheless stand in relative isolation from each other.

M173

**The Mechanosensitivity of Nucleocytoplasmic Transport Is Governed by Increased Active Transport Both Into and Out of the Nucleus**

**I. Andreu**[1], I. Granero-Moya[1], M. Molina[1], V. González-Tarragó[1], J. Kechagia[1], P. Roca-Cusachs[1,2]; [1]IBEC, Barcelona, SPAIN, [2]UB and Ciber, Barcelona, SPAIN.

Mechanical force drives fundamental processes in health and disease, and regulate transcription. Increasing evidence shows that the cell nucleus responds to force, for instance by increasing nuclear import of the transcriptional regulator YAP [1]. However, whether nucleocytoplasmic transport is a mechanosensitive process, the mechanism involved, its applicability to different cargoes, and its molecular specificity remain unknown. Here we show that nuclear force promotes active versus passive transport in both directions, making nucleocytoplasmic transport mechanosensitive. We carried out static and dynamic measurements on proteins of different molecular weights (thereby regulating diffusivity through nuclear pores) containing nuclear import signals (NLS) or nuclear export signals (NES) of different strengths. We observe that both active import and export increase with nuclear force, either applied directly (via AFM) or indirectly (by plating cells on substrates of different rigidities). Further, we show that the force-dependent steady state localization of the protein depends on a balance between active transport and passive diffusion. Finally, we show the applicability of this mechanism to different transcriptional regulators that enter or exit the nucleus with force. Our study shows a general mechanism by which cargoes specifically respond to nuclear force by entering or exiting the nucleus, which the cell uses as a control of mechanosensitive transcription.[1] Elosegui-Artola et al., Force Triggers YAP Nuclear Entry by Regulating Transport across Nuclear Pores, Cell (2017), https://doi.org/10.1016/j.cell.2017.10.008

M174

**The Giant KASH Protein ANC-1 Functions with and Without LINC Complexes to Position Nuclei and Other Organelles**

**H. Hao**, S. Kalra, L. Jameson, L. Herrera, N. Cain, D. Starr; University California-Davis, Davis, CA.

Nesprin-1 and 2 are outer nuclear membrane KASH proteins that interact with SUN proteins to form Linker of nucleoskeleton and cytoskeleton (LINC) complexes to position nuclei. LINC is important for many cellular and developmental processes; defects in LINC lead to disease. We use the *C. elegans* hypodermis as a model because it contains 139 syncytial nuclei that are normally evenly spaced apart. In *C. elegans*, ANC-1 is an ortholog of Nesprin 1 and 2 that is over 8500 residues with a conserved N-terminal calponin homology (CH) domain that binds actin and a C-terminal KASH domain that interacts with SUN protein UNC-84. Mutations in *anc-1* or *unc-84* disrupt nuclear anchorage. In our working model, ANC-1 tethers nuclei to the actin cytoskeleton. However, our new genetic data do not fit with the model. First, *anc-1(null)* mutants have much more severe nuclear anchorage defects than *unc-84(null)* or *anc-1(Δkash)* mutants, suggesting an unknown, LINC-independent role of ANC-1. Second, CRISPR deletion of the N-terminal CH domains had no nuclear anchorage defects, indicating that a shorter isoform, *anc-1b*, without the CH domain is sufficient to position nuclei in the hyp7. In support of this, early stop codons and RNAi targeted against the first 1500 residues of *anc-1* do not cause nuclear anchorage defects and we isolated a cDNA representing *anc-1b*. Interestingly, CRISPR-mediated deletion of the large repeats in the middle of ANC-1 or the N-terminal residues of ANC-1b caused severe nuclear anchorage defects, implicating an essential role for the cytoplasmic domains of ANC-1 in nuclear positioning. Using CRISPR editing, we show that GFP::ANC-1b localizes to the nuclear envelope and what appears to be the ER. In addition, mitochondria and lipid droplets are also mislocalized in *anc-1(null)* mutants. We hypothesize that the cytoplasmic domains of ANC-1 regulate the global cytoskeleton and *anc-1* mutants disrupt the positioning of nuclei, mitochondria, lipid droplets, and other organelles. Finally, deletion of both the transmembrane domain and the KASH domain caused significantly more severe nuclear anchorage defects compared with *anc-1(Δkash)*, indicating that targeting ANC-1 to membranes is important for its LINC-independent function. In summary, we propose a new role for the giant KASH protein ANC-1 in organizing the cytoplasm to position multiple organelles.

M175

**Nuclear Membrane Stability in Micronuclei Determined by Chromatin Content**

H. Z. Huang, **E. M. Hatch**, E. M. Choo; Fred Hutchinson Cancer Research Center, Seattle, WA.

Micronucleation of missegregated chromosomes has been shown to cause aneuploidy, DNA damage, chromosome rearrangement, and activation of innate immune signaling pathways. These consequences have been linked to persistent loss of micronucleus compartmentalization. Our previous work demonstrated that the micronuclear membrane has a high probability of rupture during interphase and rarely repairs, which results in extended mislocalization of the missegregated chromatin to the cytosol. This rupture occurs in part due to defects in nuclear lamina assembly, which result in gaps in the lamina network where the membrane can rupture under stress. However, it is unclear why nuclear lamina assembly is so often disrupted in micronuclei. To determine whether intrinsic features of the chromatin affect nuclear envelope assembly and stability, we assessed rupture frequency of eight chromosomes after micronucleation. Chromosome missegregation was induced in a euploid human cell line then fixed cells containing single chromosome micronuclei were assessed for missegregated chromosome identity

and membrane rupture by IF-FISH. We found that rupture frequency varied widely between individual chromosomes at early timepoints after mitosis, but that the proportion of intact micronuclei decreased for all chromosomes as interphase progressed. This suggests that chromatin properties mainly affect initial nuclear envelope assembly, and not the maintenance of membrane integrity over time. We next assessed the correlation of micronucleus rupture frequency with several chromosome characteristics, length, gene density, rDNA repeats, and centromere length, and identified a positive correlation between membrane stability and both chromosome length and gene density. Chromosome length was directly correlated with micronucleus size and, consistent with larger micronuclei having more stable membranes, entrapment of multiple chromosomes into a single micronucleus significantly decreased rupture frequency. Our analysis identified gene density as the strongest predictor of initial micronucleus stability, with low gene density chromosomes rupturing the most frequently. Our current model is that chromatin content determines nuclear lamina assembly in micronuclei, which in turn affects initial membrane stability, but is insufficient to support maintenance of proper nuclear lamina organization over many hours. We are currently working to test these hypotheses to better understand what regulates nuclear membrane stability and the relationship between chromatin characteristics and nuclear lamina organization.

M176

**Structure-function Analysis of Heh1(LEM2) and Chm7 Suggests Role for Direct-PA-binding in Nuclear Envelope Surveillance**

**D. J. Thaller**[1], C. J. Marklew[2], B. Ciani[2], C. P. Lusk[1]; [1]Yale School of Medicine, New Haven, CT, [2]University of Sheffield, Sheffield, UNITED KINGDOM.

The endosomal sorting complexes required for transport (ESCRT) protein Chm7 has been implicated in a nuclear envelope surveillance pathway that directly monitors the cytosolic-exposure of the integral inner nuclear membrane protein Heh1 (LEM2) in budding yeast[1-3]. To establish additional genetic tools to functionally interrogate this pathway, we have performed a systematic deletion and point-mutation analysis of both Heh1 and Chm7. We have explored how these alleles impact Chm7 nuclear envelope targeting, “activation” and functional complementation in genetic backgrounds where *CHM7* and *HEH1* are essential. In so doing, we uncovered a previously unidentified role for a short hydrophobic stretch in Chm7 that confers specific binding to phosphatidic acid (PA). An analogous sequence in human CHMP7 binds non-specifically to lipids[4]. Consistent with the idea that PA-binding might be important for Chm7 function, the use of a gain-of-function chm7 protein results in the formation of a PA-rich domain at the inner nuclear membrane. Together, our data indicate that Chm7 binds to both Heh1 and PA-rich membranes and suggests a model where nuclear envelope surveillance might require changes to local membrane composition.References:1. Webster, B. M., Colombi, P., Jäger, J. & Lusk, C. P. Surveillance of nuclear pore complex assembly by ESCRT-III/Vps4. *Cell* **159**, 388-401 (2014).2. Webster, B. M. *et al.* Chm7 and Heh1 collaborate to link nuclear pore complex quality control with nuclear envelope sealing. *EMBO J.* **35**, 2447-67 (2016).3. Thaller, D. J. *et al.* An ESCRT-LEM protein surveillance system is poised to directly monitor the nuclear envelope and nuclear transport system. *Elife* **8**, (2019).4. Olmos, Y., Perdrix-Rosell, A. & Carlton, J. G. Membrane Binding by CHMP7 Coordinates ESCRT-III-Dependent Nuclear Envelope Reformation. *Curr. Biol.* **26**, 2635-2641 (2016).

M177

**Maternal and Paternal Genome Mixing in the *C. Elegans* Zygote Involves Stepwise Pronuclear Fusion and Fenestration of Pronuclear Membranes**

**M. M. Rahman**[1], A. Harned[2], I. Chang[2], R. Maheshwari[1], K. Narayan[2], O. Cohen-Fix[1]; [1]National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, [2]Frederick National Laboratory for Cancer Research, NCI, NIH, Frederick, MD.

In a fertilized embryo, maternal and paternal genome that are initially present in two separate pronuclei must mix in a process that requires nuclear envelope breakdown (NEBD). NEBD in the zygote is poorly understood, especially in mammals where visualizing the process is challenging. It is assumed that once the parental pronuclei are in close apposition NEBD is initiated. In *C. elegans,* once the maternal and paternal pronuclei meet, a gap is formed in the juxtaposed membranes, close to the metaphase-aligned chromosomes. This membrane gap is critical for parental genome mixing. Recently, we reported that in *C. elegans,* partial depletion of the conserved Polo-like kinase, PLK-1, leads to the formation of paired-nuclei with separated parental genomes due to a failure in gap formation in the NEs between the two pronuclei. The structure of NE membrane gap and the signals that promote its formation are unknown. To understand this process we used two techniques, live-cell fluorescence microscopy and electron microscopy, specifically Focused Ion Beam - Scanning Electron Microscopy (FIB-SEM).Live-cell fluorescence images revealed that a stable interface is formed between two juxtaposed pronuclear membranes, and that at metaphase the interface retains both inner and outer nuclear membrane proteins. Zygotes were collected at different stages of mitosis and subjected to high-pressure freezing prior to FIB-SEM. Interface membranes reconstructed from SEM images taken at 9 nm resolution revealed that the interface is composed of only two membranes that are flanked by a novel type of membrane junction, a 3-way sheet junction, present between the opposing pronuclei at metaphase. Three-way sheet junctions reduced the number of pronuclear membranes in the interface from four parental membranes to two. Analysis of multiple prometaphase- and metaphase-stage embryos by FIB-SEM uncovered a second type of membrane junction between the outer nuclear membranes of the two opposing pronuclei. In contrast, *plk-1* mutant zygotes were devoid of junctions, indicating that junction formation is a prerequisite for mixing of parental genomes. We hypothesized that proteins involved in endoplasmic reticulum (ER) remodeling may be involved in junction formation. Our preliminary data suggest that Atlastin, a protein known for its role in ER-ER homotypic fusion, is required for the process.

M178

**Mutant Lamins Cause Nuclear Envelope Rupture and DNA Damage in Skeletal Muscle Cells**

**T. Kirby**[1], A. Earle[1], G. Fedorchak[1], P. Isermann[1], J. Patel[1], S. Iruvanti[1], S. Moore[2], G. Bonne[3], L. Wallrath[2], J. Lammerding[1]; [1]Cornell University, Ithaca, NY, [2]University of Iowa, Iowa City, IA, [3]Sorbonne Université, Center of Research in Myology, Association Institute of Myology, Paris, FRANCE.

Mutations in the human *LMNA* gene, which encodes the nuclear envelope proteins lamins A and C, cause autosomal dominant Emery-Dreifuss muscular dystrophy, congenital muscular dystrophy, limb-girdle muscular dystrophy, and other diseases collectively known as laminopathies. The molecular mechanisms responsible for these diseases remain incompletely understood, but the muscle-specific defects suggest that mutations may render nuclei more susceptible to mechanical stress. Using three mouse models of muscle laminopathies, we found that *Lmna* mutations caused extensive nuclear envelope damage, consisting of chromatin protrusions and transient rupture of the nuclear envelope, in

skeletal muscle cells *in vitro* and *in vivo*. The nuclear envelope damage was associated with progressive DNA damage, activation of DNA damage response pathways, and reduced viability. Intriguingly, nuclear envelope damage resulted from nuclear movement in maturing skeletal muscle cells, rather than actomyosin contractility, and the nuclear damage was reversed by either depletion of kinesin-1 or disruption of the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex. Depletion of kinesin-1 or LINC complex disruption reduced DNA damage, indicating that DNA damage is the result of nuclear envelope damage. Furthermore, LINC complex disruption rescued myofiber function and viability, indicating that the myofiber dysfunction is the result of mechanically induced nuclear envelope damage. The extent of nuclear envelope damage and DNA damage in the different *Lmna* mouse models strongly correlated with the disease onset and severity *in vivo*, and inducing DNA damage in wild-type muscle cells was sufficient to phenocopy the reduced cell viability of lamin A/C-deficient muscle cells, suggesting a causative role of DNA damage in disease pathogenesis. Corroborating the mouse model data, muscle biopsies from patients with *LMNA* associated muscular dystrophy similarly revealed significant DNA damage compared to age-matched controls, particularly in severe cases of the disease. Taken together, these findings point to a new and important role of nuclear envelope rupture and DNA damage as pathogenic contributors for these skeletal muscle diseases.

M179

**Phase-separated Heterochromatin Domains Impart Mechanical Stiffness to the Nucleus**

**M. C. King**[1], J. F. Williams[1], I. V. Surovtsev[1], A. Nguyen[2], S. G. J. Mochrie[3]; [1]Yale School of Medicine, New Haven, CT, [2]Pomona College, Claremont, CA, [3]Yale University, New Haven, CT.

The association of heterochromatin with the nuclear periphery has long been suggested to reflect its transcriptional repression. However, our prior work revealed that chromatin, particularly regions of the genome associated with the inner nuclear membrane, also play an integral role in supporting the mechanical robustness of the nucleus through mechanisms that remain poorly understood. In light of new insights into the potential for heterochromatin to form a distinct liquid-like phase, we sought to define the underlying basis for the biophysical contribution of heterochromatin to nuclear mechanics. Our approach employs an image reconstruction software we developed to quantitatively measure dynamic nuclear envelope fluctuations, a novel force spectroscopy assay that employs optical tweezers to directly measure the viscoelastic properties of isolated nuclei, and microscopy approaches to monitor the dynamics of heterochromatin foci in living cells. We find that loss of the histone H3K9me2/3 binding protein Swi6, an HP1 orthologue found to undergo liquid-liquid phase separation *in vitro*, results in increased nuclear deformability *in vivo* and a 50 percent drop in the nuclear spring constant *in vitro*. Accordingly, deletion of the histone H3K9 demethylase Epe1, which drives heterochromatin spreading, results in stiffer nuclei. As cytoplasmic microtubules are coupled to the centromeres in fission yeast, we characterized the deformation of the centromeric heterochromatin in response to force, which reinforces the liquid-like behavior of heterochromatic foci. This behavior is altered in cells harboring an allele of Swi6 with a mutation in the critical “hinge” domain; this mutated protein associates normally with H3K9me2/3-rich chromatin domains but fails to induce silencing, suggesting that this allele may uncouple the phase separation behavior of Swi6 from its other biochemical activities. Accordingly, both this allele and cells lacking Swi6 demonstrate defects in the coalescence of heterochromatic foci. Taken together, these results suggest that liquid-liquid phase separation can contribute to cellular mechanics, in this case by contributing to nuclear stiffness.

## Minisymposium 18: Cell Biological Aspects of Immunity

M180

**Structural Basis for Influenza Virus Ns1 Protein Block of Mrna Nuclear Export**

**K. Zhang**[1], Y. Xie[2], R. Muñoz-Moreno[3], J. Wang[1], L. Zhang[4], M. Esparza[1], B. M. A. Fontoura[1], Y. Ren[2]; [1]University of Texas Southwestern Medical Center, Dallas, TX, [2]Vanderbilt University School of Medicine, Nashville, TN, [3]Icahn School of Medicine at Mount Sinai, New York, NY, [4]School of Life Sciences, Xiamen University, Xiamen, CHINA.

Influenza viruses antagonize key immune defence mechanisms via the virulence factor non-structural protein 1 (NS1). A key mechanism of virulence by NS1 is blocking nuclear export of host messenger RNAs, including those encoding immune factors; however, the direct cellular target of NS1 and the mechanism of host mRNA export inhibition are not known. Here, we identify the target of NS1 as the mRNA export receptor complex, nuclear RNA export factor 1-nuclear transport factor 2-related export protein 1 (NXF1-NXT1), which is the principal receptor mediating docking and translocation of mRNAs through the nuclear pore complex via interactions with nucleoporins. We determined the crystal structure of NS1 in complex with NXF1-NXT1 at 3.8 Å resolution. The structure reveals that NS1 prevents binding of NXF1-NXT1 to nucleoporins, thereby inhibiting mRNA export through the nuclear pore complex into the cytoplasm for translation. We demonstrate that a mutant influenza virus deficient in binding NXF1-NXT1 does not block host mRNA export and is attenuated. This attenuation is marked by the release of mRNAs encoding immune factors from the nucleus. In sum, our study uncovers the molecular basis of a major nuclear function of influenza NS1 protein that causes potent blockage of host gene expression and contributes to inhibition of host immunity.

M181

**Moves and Countermoves: Viral-driven Evolution of Necroptotic Cell Death**

**S. N. Palmer**, D. C. Hancks; University of Texas Southwestern Medical Center, Dallas, TX.

Viral systems have been instrumental in elucidating principles and regulatory mechanisms including species-specific evolutionary innovations of cell death. Recently a novel cell death pathway referred to as programmed necrosis - hereafter necroptosis - mediated by the RIPK3/MLKL axis has been implicated as a host countermeasure to viral-mediated inhibition of apoptosis. While some viral-encoded inhibitors have been identified, how pathogen-mediated antagonism has shaped cellular effectors of necroptosis is unknown. We report elevated rates of amino acid substitutions - a hallmark of positive selection - across multiple primate lineages for RIPK3 and MLKL but not upstream activators such as RIPK1 or TRIF. RIPK3 and MLKL rapid evolution is recurrent and signatures are distributed throughout both proteins, suggesting the existence of multiple inhibitors. While the viral factors driving this evolution is unclear, we speculate RIPK3 and MLKL pseudosubstrates may in part play a role based on clustering of rapidly evolving sites in the RIPK3 active site. Indeed, we find viral orthologs - virologs - of MLKL encoded by two-thirds of known poxviruses (20 spp. total) including species like myxoma that lack the poxvirus master regulator E3L, a known inhibitor of necroptosis. Consistent with action as mimics and decoys, these v(iral)MLKLs lack an N-terminus but maintain the C-terminal portion of the protein corresponding to the RIPK3 substrate. Evolutionary analysis indicates repeated gains and losses of vMLKLs across poxvirus genomes including three vMLKLs in eptesipox. These observations might signal genomic accordion dynamics for vMLKL similar to what has been reported for vaccinia in cell culture. We are

currently testing these mimics for functional inhibition of necroptosis and exploring host range in cell-based assays. Notably, vMLKLs are conserved in several poxviruses thought to exclusively infect birds where the upstream kinase RIPK3 is absent from all sequenced avian genomes, foreshadowing the existence of an undefined kinase which phosphorylates MLKL. This observation suggests these mimics may reveal ongoing adaptations in necroptosis as well as novel battlefronts during infection. Our finding has significant implications as influenza triggers necroptosis and transmits between aves and mammals. These data illustrate how viruses have shaped the evolution of this key host defense and how a viro-perspective of cell biology can be exploited to inform fundamental responses to pathogens.

M182

**Stem Cell Immunoengineering for Universal Cardiac Therapy Via CRISPR-Cas9**

**L. Randolph**, X. Lian; Pennsylvania State University, University Park, PA.

Heart transplant is currently the only clinical option for heart failure, a common form of cardiovascular disease, which is a leading cause of death globally. Heart transplant may not be an ideal solution due to required lifetime immunosuppressive therapies. Additionally, donor organ availability is dwarfed by demand and further limited by HLA matching between the donor and patient. Induced pluripotent stem cell (iPSC) technology and directed differentiation strategies provide the potential to de novo generate theoretically unlimited quantities of cardiomyocytes from a patient's own cells. However, research has shown iPSC-derived cardiomyocytes will elicit an immune response even when transplanted autologously, indicating unique immunogenic properties of in vitro differentiated cardiomyocytes. To develop improved therapeutic options, we generated universal donor stem cells (UDSCs) to evade innate and adaptive immune detection by engineering human leukocyte antigen (HLA) expression.Class I HLA molecules display intracellular proteins to cytotoxic T cells, which if unrecognized will trigger cell lysis. Using CRISPR-Cas9 technology, we knocked out beta 2 microglobulin (B2M), a required protein for cell surface expression of class I HLA molecules, in 2 human pluripotent stem cell (hPSC) lines to prevent T-cell detection. However, this strategy leaves our engineered cells vulnerable to lysis by natural killer (NK) cells, which will attack cells lacking all HLA molecule expression. To overcome this, we designed two fusion proteins made up of HLA-E or HLA-G, both NK cell inhibitors, fused to B2M and integrated this vector into the genome to allow functional cell surface expression of only HLA-E or HLA-G. Our UDSCs showed normal hPSC morphology and pluripotent marker expression and differentiated to all 3 germ layers.To test the immunogenicity of these cells we differentiated them into cardiomyocytes and performed lysis and degranulation assays with NK cells. We found that while the wild type cells did not elicit a lytic response from NK cells, the B2M KO cells triggered the expected missing-self reaction and the expression of HLA-E or HLA-G restored protection from NK cell attack. We expect lysis assays with cytotoxic T-cells to show a strong lytic response to wild type cells and no response to our B2M KO or UDSCs. Our UDSCs have great potential for transplantation medicine for cardiovascular disease and have broad applications to other cell types affected by degenerative or autoimmune diseases.

M183

**Antibody-dependent Macrophage Phagocytosis Is Inhibited by Tall Bystander Proteins**

**A. Joffe**, M. Bakalar, J. Hasnain, P. Geissler, D. Fletcher; UC Berkeley, Berkeley, CA.

Macrophages are able to detect and destroy cells opsonized in antibodies through the process of antibody-dependent phagocytosis (ADP). Complementing antibody-dependent cellular cytotoxicity

(ADCC) carried out by T-cells and NK-cells, macrophage ADP has been shown to be a contributor to efficient clearance of cancer cells during treatment with cancer immunotherapies. In our previous work, we measured phagocytosis of reconstituted target particles with defined surface proteins and used the system to investigate the effect of antigen height on ADP. We concluded that ADP signaling is most efficient when antibodies are targeted to short antigens due to size-based segregation of CD45 phosphatase from the phagocytic interface, which is needed for Fc receptor activation. In our current work, we have increased the complexity of our experimental system by adding non-antigen bystander proteins of varying heights and densities. We find that these bystander proteins inhibit phagocytosis of reconstituted target particles with increasing height and density of the bystander proteins. Using a combination of experimental and computational methods, we determined that this effect is due to bystander proteins sterically blocking antibody access and, therefore, blocking binding of the macrophage to its target. These findings suggest that it is useful to frame ADP as a multistep process where (i) macrophages overcome the steric barrier presented by bystander proteins to bind to antibody on a target and (ii) macrophages undergo phagocytic signaling upon receptor activation at the interface. In the first step, target binding is favored for tall antigens that are more accessible, especially those that display antibodies above the surrounding bystander proteins. The second step, however, is favored for short antigens (< 10nm), which lead to size-based segregation of inhibitory phosphatases and subsequent activation of Fc receptors. This picture of ADP may inform future design of cancer immunotherapies by choosing antigens that are neither too short to inhibit target binding nor too tall to inhibit phagocytic signaling.

M184

**Hepatokine Induction by Colchicine Prevents Systemic Inflammation Via Activating PTPN6 Inhibitory Signaling**

**J. WENG**[1], P. Koch[2], K. Shimada[1], T. J. Mitchison[1]; [1]Harvard Medical School, Boston, MA, [2]Massachusetts General Hospital, Boston, MA.

Inflammation is a protective response to challenges including infection, injury, and tissue stresses. However, failure to resolve inflammation could lead to various diseases. The liver controls tissue metabolism and energy homeostasis by releasing secretory proteins called hepatokines. It is not clear whether liver-hepatokine-leukocyte communication contributes to inflammation regulation. Colchicine, an anti-microtubule and anti-inflammatory medication, is frequently prescribed for gout, Familial Mediterranean Fever, pericarditis, and is being clinically tested for a wide spectrum of diseases. Yet, its usage is limited by its unclear systemic action.By measuring biomarkers of microtubule perturbation and using hepatocyte-specific siRNAs, we uncovered hepatocytes are preferentially targeted by colchicine. Colchicine depolymerizes microtubules of hepatocytes and activates the transcription factor Nrf2, which regulates expression of protective genes, through promoting P62-dependent Keap1 degradation. Targeted hepatocytes subsequently secrete a novel hepatokine, GDF15, into circulation. As a divergent member of the TGF-b family, GDF15 activates inhibitory protein tyrosine phosphatase PTPN6 and inhibits inflammation via blocking leukocyte adhesion, extravasation, and IL-1b production. Our work indicates the liver mediates the anti-inflammatory activity of colchicine and opens the possibility of using anti-inflammatory hepatokines for prevention or treatment of inflammatory diseases, starting with GDF15.

M185

**Molecular Mechanisms of STING Pathway Activation and Inhibition**

**S. L. Ergun**, D. Fernandez, L. Li; BIOCHEMISTRY DEPT, STANFORD UNIVERSITY, Stanford, CA.

The STING (STimulator of INterferon Genes) pathway is an innate immune signaling cascade which promotes essential anti-cancer, anti-viral, and anti-bacterial responses. Conversely, STING overactivation is linked to several autoimmune and inflammatory diseases such as Lupus, Multiple Sclerosis, heart attack, and Parkinson's disease. Despite its significance in the disease context, the precise molecular mechanism of STING activation and attenuation remains unclear. Using structural biology and biochemistry, we report that the metazoan second messenger 2'3'-cGAMP induces closing of the human STING homodimer and release of the STING C-terminal tail, which exposes a polymerization interface on the STING dimer and leads to the formation of disulfide-linked polymers via cysteine residue 148. Disease-causing hyperactive STING mutations either flank C148 and depend on disulfide formation or reside in the C-terminal tail binding site and cause constitutive C-terminal tail release and polymerization. Finally, bacterial cyclic-di-GMP induces an alternative active STING conformation, activates STING in a cooperative manner, and acts as a partial antagonist of 2'3'-cGAMP signaling. Our insights explain the tight control of STING signaling given varying background activation signals and provide a novel therapeutic hypothesis for autoimmune syndrome treatment.

M186

**INPP5E Is Required for Recruitment of the TCR/CD3 Complex to the Immune Synapse Via the Ciliary Machinery**

**T. Chiu**, F. Yang, J. Liao; Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei, TAIWAN.

The primary cilia function as a sensory organelle in many eukaryotic cells. Recently several proteins have been seen to co-exist in primary cilia and in immune synapses of T cells, although how a rod-shaped cilium is related to a plate-shaped immune synapse remains elusive. Here we show that T-lymphocytes, which lack primary cilia, express inositol polyphosphate-5-phosphatase E (INPP5E), a ciliary-specific protein responsible for regulating phosphoinositide localization. We find that INPP5E colocalizes with the microtubule organizing center (MTOC) and the distal appendages in Jurkat T-cells. In addition, INPP5E accumulates at the immune synapse during antigen-specific conjugation. Knockdown of INPP5E results in abolished T-cell receptor (TCR) /CD3 recruitment at the immune synapse. Moreover, proximal TCR signaling, including CD3ζ and ZAP70 phosphorylation, is disrupted in INPP5E-deficient cells. The defects in INPP5E-deficient cells are contributed by phospholipid distributions at the supramolecular activation cluster (SMAC), which is modulated by the catalytic domain of INPP5E. Together, these results suggest a repurposed ciliary machinery at the immune synapse for the recruitment of the TCR/CD3 complex and regulated by controlling phospholipid distributions through INPP5E.

M187

**MISTR1 Is a Master Regulator of OXPHOS and Cell Death**

**M. Sorouri**[1,2], P. Jesudhasan[1], D. C. Hancks[1]; [1]UT Southwestern Medical Center, Dallas, TX, [2]Baylor University, Waco, TX.

As pathogens manipulate host-encoded master regulators, we hypothesized that molecular scars of host-pathogen conflicts - such as signatures of rapid evolution and viral mimics - could lead to the

discovery of novel cellular functions. Indeed, our evolution-guided screens have identified MItochondrial STress Response (MISTR), a cellular circuit conserved in vertebrates. MISTR1 is a constitutively expressed protein that functions as the central axis of MISTR, which associates with Complex IV (CIV) of the electron transport chain (ETC). In agreement with a role in oxidative phosphorylation (OXPHOS), *MISTR1* KO cells display reduced proliferation and cellular ATP levels compared to WT cells when switched from glucose- to galactose-containing media; yet, this defect is grossly attenuated when the cells are grown in galactose-containing media for several days prior to assay, indicating MISTR1 may act as a (metabolic) stress sensor. Consistently, we identify several functional microRNA response elements (MRE) encoded by the *MISTR1* 3'-UTR targeted by ultraconserved miRNAs induced by distinct stress signals. Unexpectedly, genetic analysis indicates that these miRNAs link MISTR1 to its paralogs MISTR_AV and MISTR_H - factors induced by infection and hypoxia, respectively. Induction of MISTR_AV by immune signals leads to upregulation of a miRNA, for which all 22 nucleotides are conserved in teleosts, embedded in the 3'-UTR of *MISTR_AV* (*miR-147B*) that targets an MRE in the 3'-UTR of *MISTR1*. Endogenous overexpression of *miR-147B* produced by a serendipitous CRISPR/CAS mutation triggers robust caspase-3 activation and PARP cleavage in response to apoptosis-inducing drugs presumably through *miR-147B*-mediated downregulation of MISTR1. Relatedly, *MISTR1* KO cells display increased sensitivity to STS-induced cell death. A recently published structure of CIV illustrates that MISTR1 lies at the dimeric interface of CIV homodimers which would preclude CIV dimerization. We posit that stress-induced, miRNA-mediated downregulation of MISTR1 allows the formation of higher-order ETC structures including CIV dimers and supercomplexes to augment OXPHOS. Notably, MISTR homologs are present in yeast, plasmodium, plants as well as diverse viruses. These data suggest MISTR1 is a master regulator of OXPHOS and cell death in a stress response circuit regulated by related ultraconserved miRNAs.

M188

**Ratio of Activating to Inhibitory Signaling Dominates Phagocytic Decision-Making**

**E. C. Suter**, E. M. Schmid, A. M. Joffe, D. A. Fletcher; UC Berkeley, Berkeley, CA.

Cancer immunotherapies involving macrophages broadly aim to modulate their effector function in one of two ways, either by increasing phagocytic activating signals or decreasing inhibitory signals. To increase activating signals, therapeutic antibodies targeting cancer antigens are introduced to drive antibody-dependent phagocytosis through activating Fc receptors (FcRs) on the macrophage. To decrease inhibitory signals, blocking antibodies targeting macrophage inhibitory receptor SIRPα (or its ligand CD47) are introduced to enable the macrophage to bypass this inhibitory checkpoint, thereby removing the brakes on macrophage effector function. In each strategy, the goal is to tip the balance of activating vs. inhibitory receptors present at the interface between the macrophage and target cell, either by promoting activating FcR engagement or by preventing inhibitory SIRPα engagement. However, little has been shown quantitatively about how macrophages integrate these competing receptor signals to ultimately determine phagocytic behavior. Here we demonstrate that macrophage phagocytic decisions are finely tuned to the ratio of activating ligand (antibody) to inhibitory ligand (CD47) present on the target cell. Using reconstituted cell-like target particles with defined surface proteins, we found that the same activation-inhibition ratio drives similar levels of phagocytosis across a broad range of absolute molecule densities. To test the dependence of this ratiometric decision-making on specific receptors, we examined a panel of synthetic activating and inhibitory receptors and found similar results, indicating this ratiometric paradigm applies broadly to both endogenous and artificial

(e.g. CAR) immune receptors. Our results demonstrate that ratio—not an absolute threshold—dominates macrophage phagocytosis decisions. This understanding of how macrophages integrate contradictory signals may be useful when designing combination immunotherapies. Additionally, this study suggests that characterization of tumor surface composition could be critical for determining patient-specific immunotherapy regiments.

M189

**Organelle Membrane Contact Sites At the Origin of Nlrp3 Inflammasome Activation**

**R. Ricci**, Z. Zhang, Z. Liu; IGBMC, Illkirch, FRANCE.

Inflammasomes are multimeric protein complexes that assemble in the cytosol upon exposure to PAMPs or DAMPs. They all serve as a scaffold to recruit the inactive zymogen pro-caspase-1, leading to auto-activation of caspase-1. Active caspase-1 cleaves the precursor cytokines pro-IL-1β and pro-IL-18 into mature IL-1β and IL-18, respectively. Inflammasome activation occurs when the sensor molecule senses or binds its activating factors. How this occurs is starting to be clarified for certain inflammasome-related sensor molecules. However, mechanisms leading to the assembly and activation of the NLRP3 inflammasome remain elusive. Recruitment of NLRP3 to mitochondria-associated endoplasmic reticulum membranes (MAMs) and its activation by MAM-derived effector molecules have been recently demonstrated. In our previous work, we have shown that the Golgi apparatus is critical for NLRP3 inflammasome activation. We have shown that clustering of organelles (mitochondria, ER membranes and Golgi) occurs upon NLRP3 inflammasome activation. Furthermore, we demonstrated that activation of protein kinase D (PKD) at the Golgi is required for NLRP3 inflammasome assembly. Using live cell imaging, we have recently generated new data strongly supporting a model in which NLRP3 activation requires membrane contact sites (MCS) between ER and the Trans-Golgi Network (TGN). Together with our previous work, we link MCS between TGN and ER to mechanisms of innate immunity and potentially inflammatory diseases.

## Minisymposium 19: Cell Migration Mechanisms in Development and Disease

M190

**E-cadherin Is An Invasion Suppressor, Survival Factor, and Metastasis Promoter in Multiple Models of Breast Cancer**

**V. Padmanaban**[1], I. Krol[2], Y. Suhail[1], B. Szczerba[1], N. Aceto[2], J. Bader[1], A. Ewald[1]; [1]Johns Hopkins University School of Medicine, Baltimore, MD, [2]University of Basel, Basel, SWITZERLAND.

The loss of E-cadherin (E-cad) has been linked to increased invasion of cancer cell lines and has been inferred to promote metastasis, leading to its classification as a tumor, invasion, and metastasis suppressor. However, about 90% of breast cancers retain E-cad expression in both the primary tumor and distant metastases. In this study, we test the requirement of E-cad for metastasis using mouse models of luminal and basal breast cancers that allow for an inducible deletion of E-cad. Loss of E-cad increases tumor invasion and dissemination in both 3D organotypic *ex vivo* assays and *in vivo* assays. In contrast, E-cad loss strongly inhibits metastasis. Careful analysis of intermediate stages of metastasis revealed that despite an increase in local invasion, E-cad loss is associated with a decrease in migratory persistence, decrease in circulating tumor cell counts, decrease in tumor seeding potential, and decrease in metastatic outgrowth, collectively causing a strong decrease in metastasis. Mechanistically,

we found a TGFβ-dependent enrichment of reactive oxygen species specifically within the disseminated E-cad- cancer cells, frequently leading to their apoptosis. Significantly, colony formation of E-cad-negative cells was rescued by treatment with a TGFβ-receptor inhibitor, an anti-oxidant, or an apoptosis inhibitor. These results suggest that E-cad acts as a survival factor during the detachment, systemic dissemination, and seeding phases of metastasis in these models of breast cancer.

M191

**A*Drosophila*Model Visualizing Dissemination of Transformed Epithelial Cells Into the Circulation**

**Y. Kwon**, J. Lee, A. Cabrera; University of Washington, Seattle, WA.

Cancer cells initiate metastasis by disseminating into the circulation. Despite its importance, how transformed cells move out from an intact tissue and enter the circulation is poorly understood, largely due to the lack of a proper *in vivo* system that allows molecular dissection of the process. Here, we use a fully developed tissue, *Drosophila* midgut, and describe the morphologically distinct steps and the molecular events occurring over the course of $Ras^{V12}$-transformed cell dissemination. $Ras^{V12}$-transformed cells breached extracellular matrix and visceral muscle layers by forming invadopodia exclusively at the basal side. Then, these cells transmigrated through the ruptures using bleb-driven amoeboid movement. Our observations indicate that dissemination of $Ras^{V12}$-transformed cells is an opportunistic event, requiring the mechanosensitive channel Piezo to exploit the chance created by the novel action of invadopodia. Collectively, our study uncovers an *in vivo* mode of cell dissemination and provides unique insights into the role of Piezo in adopting an appropriate migratory strategy in response to the microenvironmental rearrangements.

M192

**Tuning Cell Contractility and Vinculin Localization At Cell Junctions Is Required for Basal Epithelial Cell Extrusion**

c. Villeneuve, E. Lagoutte, S. Mathieu, L. De Plater, J. Maitre, J. Manneville, P. Chavrier, **C. Rosse**; Institut Curie, Paris, FRANCE.

Metastasis is the main cause of cancer-related deaths. How these cancer cell alterations evolve within tightly regulated tissue remains elusive. We show that an oncogenic mammary epithelial cell surrounded by normal cells could *in vivo* basally extrude and disseminate at very early stage before the formation of a primary tumor. The key polarity protein atypical PKC iota (aPKCi) overexpression is crucial for oncogenic basal cell extrusion *in vivo*, a new mechanism for early breast tumor cell dissemination. Moreover, we highlight the importance of the different mechanical properties between the oncogenic aPKCi overexpressing cells and the surrounded normal cells associated with the decrease of vinculin at the cell junction which triggers cell segregation, first step promoting and controling the direction of cell extrusion. In fact, by combining biophysical approaches, we show an increase in cell tension at the interface between aPKCi+ and WT cells dependent on myosin II activity, associated with a relocation of vinculin from cell-cell junctions to focal adhesions in aPKCi+ cells, leading to the acquisition of pro-migratory features in these oncogenic cells. We identify aPKCi and vinculin as new regulators of cell segregation and propose that a balance between cell contractility and cell-cell adhesion at the interface between normal and oncogenic aPKCi+ cells is crucial for promoting basal cell extrusion. We anticipate that this mechanism may be conserved in other carcinomas, promoting early cancer cell dissemination.

M193

**Epigenetic Heterogeneity Within the Collective Invasion Pack Promotes Myo10-dependent Fibronectin Micropatterning by Leader Cells**

**E. R. Summerbell**, J. K. Mouw, J. S. K. Bell, J. L. Arnst, T. O. Khatib, J. Konen, B. Dwivedi, S. Seby, J. Kowalski, P. M. Vertino, A. I. Marcus; Emory University, Atlanta, GA.

Tumor heterogeneity drives disease progression, treatment resistance, and patient relapse, yet remains largely under-explored in metastatic evolution. Polyclonal metastatic lesions can originate from heterogeneous clusters of collectively invading cells. These invasive cell packs can contain phenotypically-distinct invasive “leader” and noninvasive “follower” cell populations. Despite a growing understanding of the underlying genotypic and transcriptomic differences distinguishing intratumor heterogeneity, little is known about epigenetic factors that govern heterogeneous phenotypes within collectively invading tumors. As such, we investigated epigenetic heterogeneity within collective invasion packs by integrating genome-wide DNA methylation and gene expression data using purified lung cancer leader and follower cell populations.We show the first evidence of DNA methylation heterogeneity between leader and follower cells that influences their unique gene expression. Integration of DNA methylation array data and RNAseq data identified the filopodia motor protein myosin-X (MYO10) at the intersection of epigenetic regulation and collective cancer cell invasion. MYO10 is unmethylated at its promoter and overexpressed in leader cells, and MYO10 is necessary for collective invasion of multiple lung cancer cell lines. Furthermore, we show the first evidence that Jag1 transcriptionally regulates MYO10, expanding the role of the Notch pathway in collective invasion.Experimental deconstruction of MYO10 function in leader cells shows that MYO10-driven filopodia micropattern extracellular fibronectin (FN) into linear tracks at the leading edge of collective invasion packs, where fibronectin is essential for 3D invasion. Live cell imaging of spheroid collective invasion showed that MYO10-driven filopodia grow longer in length and time than MYO10-knockdown filopodia. Nascent rhodamine-FN puncta form in line with filopodia shafts behind the MYO10+ filopodia tip and then elongate proximally towards the cell body through fibrillogenesis. MYO10-knockdown cells lose the ability to remodel endogenous FN. Thus, we show that MYO10-driven filopodia are necessary for FN micropatterning during collective cancer cell invasion.In total, our data demonstrate that epigenetic heterogeneity and Jag1/Notch signaling jointly drive transcriptional activation of MYO10 in leader cells, resulting in linearized fibronectin micropatterning by filopodia within the collective invasion pack.

M194

**A Motile Sheath of Migratory Cells Supports Collective Migration of Epithelial Cells in Confinement During Migration of the Zebrafish Posterior Lateral Line Primordium**

**D. Dalle Nogare**, A. Chitnis; NICHD, NIH, Bethesda, MD.

During embryonic development, cells must navigate through a complex three-dimensional environment robustly and reproducibly. The zebrafish posterior lateral line primordium (PLLp), a group of approximately 120 cells which migrates from the otic vesicle to the tip of the tail, spearheading the development of the lateral line sensory system, is an excellent model to study such collective migration in an in vivo context. This system migrates in a channel formed by the underlying horizontal myoseptum and somites, and the overlying skin. While cells in the leading part of the PLLp are flat and have a more mesenchymal morphology, cells in the trailing part progressively reorganize to form epithelial rosettes,

called protoneuromasts. These epithelial cells extend basal cryptic lamellipodia in the direction of migration in response to both chemokine and FGF signals. In this study, we show that, in addition to these cryptic lamellipodia, the core epithelial cells are in fact surrounded by a population of motile cells which extend actin-rich migratory processes apposed to the overlying skin. These thin cells wrap around the protoneuromasts, forming a continuous sheath of cells around the apical and lateral surface of the PLLp. The processes extended by these cells are highly polarized in the direction of migration and this directionality, like that of the basal lamellipodia, is dependent on FGF signaling. Consistent with interactions of sheath cells with the overlying skin contributing to migration, removal of the skin stalls migration. However, this is accompanied by some surprising changes. There is a profound change in the morphology of the sheath cells, with directional superficial lamellipodia being replaced with the appearance of undirected blebs or ruffles. Furthermore, removal of the skin not only affects underlying lamellipodia, it simultaneously alters the morphology and behavior of the deeper basal cryptic lamellipodia, even though these cells do not directly contact the skin. Directional actin-rich protrusions on both the apical and basal surface and migration are completely and simultaneously restored upon regrowth of the skin over the PLLp. We suggest that this system utilizes a circumferential sheath of motile cells to allow the internal epithelial cells to migrate collectively in the confined space of the horizontal myopseptum and that elastic confinement provided by the overlying skin is essential for effective collective migratory behavior of primordium cells.

M195

**A Mechanistic Model of PLC/PKC Signaling Implicates Phosphatidic Acid as a Key Amplifier of Chemotactic Gradient Sensing**

**J. L. Nosbisch**[1], A. Rahman[1], K. Mohan[1], T. C. Elston[2], J. E. Bear[3,2], J. M. Haugh[1]; [1]North Carolina State University, Raleigh, NC, [2]University of North Carolina School of Medicine, Chapel Hill, NC, [3]UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC.

Chemotaxis of fibroblasts and other mesenchymal cells is critical for embryonic development and wound healing. Fibroblast chemotaxis directed by a gradient of platelet-derived growth factor (PDGF) requires signaling through the phospholipase C (PLC)/protein kinase C (PKC) pathway. Diacylglycerol (DAG), the lipid product of PLC that activates conventional PKCs, is focally enriched at the up-gradient leading edge of fibroblasts responding to a shallow gradient of PDGF (Asokan et al., PMID 25482883), suggesting that an internal amplification mechanism is at play.In previous work, a mechanistic, reaction-diffusion model of the PLC/PKC signaling pathway was developed to identify possible mechanisms responsible for signal amplification (Mohan et al., PMID 28700916). We found that phosphorylation of myrisotylated alanine-rich C kinase substrate (MARCKS) by membrane-localized PKC constitutes a positive feedback loop sufficient for local amplification of DAG and active PKC at the leading edge. However, by itself, the MARCKS feedback only weakly amplifies the signal in shallow PDGF gradients, and the system lacks robustness to modest changes in the midpoint concentration of PDGF.The new model includes phosphatidic acid (PA), a lipid intermediate in the metabolism of DAG, identifying roles in two other positive feedback loops. Model simulations show that the MARCKS feedback mechanism synergizes with these new feedback loops to polarize PKC signaling in response to shallow PDGF gradients and over an appreciable range of midpoint PDGF concentrations. DAG kinases and phospholipase D, the enzymes that produce PA, are implicated as key regulators, and testable predictions related to perturbation of DAG kinase activity are most promising.

M196

**Connectivity Analysis of GEF/GTPase Networks in Living Cells**

**D. J. Marston**[1], M. Vilela[2], J. Ren[1], G. Glekas[1], M. L. Azoitei[1], G. Danuser[2], J. Sondek[1], K. M. Hahn[1]; [1]University of North Carolina-Chapel Hill, Chapel Hill, NC, [2]University of Texas Southwestern Medical Center, Dallas, TX.

Fluorescent biosensors are powerful tools to map the activation of signaling molecules in space and time. However, it has not been possible to quantify how different activation events, visualized at specific times and subcellular positions, affect one another or contribute to cell behavior. Here we demonstrate the use of partial correlation analysis to parse out such relationships from multiplexed biosensor imaging data. We show that these approaches can quantify the fraction of a Rho GTPase signal that results from a particular RhoGEF, the relative contribution a RhoGEF makes to each of the downstream GTPases it interacts with, and importantly determine these relationships with respect to output, e.g. cell motility vs other downstream functions.First, we produced a new series of biosensors to visualize activation patterns of six diverse Dbl family RhoGEFs. We then focused on a pair of these, Vav2 and Asef, and correlated their activity with edge dynamics. To allow simultaneous imaging, we then red-shifted the emission wavelengths of GTPase biosensors and used these new sensors to image and correlate the activation of GEFs and GTPases concurrently in the same cell. Using partial correlation analysis, we identified when and where the GEF Asef regulates the GTPases Cdc42 and Rac1 to control cell edge dynamics. We found that Asef activity is strongly coupled to cell protrusion dynamics as compared to Vav2. Furthermore, Asef strongly activates Cdc42 signals related to cell edge movement. In contrast, the interaction between Asef and Rac1 is weaker and probably indirect, and not related to edge movement. This approach exemplifies a powerful means to elucidate the real-time connectivity of signal transduction networks.

M197

**The $LTB_4$-BLT1 Signaling Axis Coordinates Actomyosin Dynamics and Beta-2 Integrin Trafficking to Drive Intravascular Neutrophil Response to Infection**

**B. Subramanian**[1], N. Melis[1], D. Chen[1], W. Wang[1], D. Gallardo[1], R. Weigert[1], C. Parent[2]; [1]National Cancer Institute, NIH, Bethesda, MD, [2]University of Michigan, Ann Arbor, MI.

The eicosanoid Leukotriene $B_4$ ($LTB_4$), relays chemotactic signals to direct neutrophil interstitial migration in response to injury through its receptor, BLT1. However, whether the $LTB_4$-BLT1 axis relays signals during intravascular neutrophil response has not been directly addressed. To test this issue, we developed a sterile bacterial infection model in the mouse footpad and used intravital microscopy to directly visualize the impact of $LTB_4$-BLT1 axis on the intravascular neutrophil dynamics. Here, we report that $LTB_4$ produced by neutrophils acts as an autocrine/paracrine signal via BLT1 to drive their recruitment, arrest, and extravasation during infection in living mice. To understand the mechanism behind these findings, we utilized Intravital Subcellular Microscopy (ISMic) and discovered that $LTB_4$ elicits cell adhesion and polarization during neutrophil arrest *in vivo*. Specifically, $LTB_4$ signaling coordinates the dynamic redistribution of - 1) non-muscle Myosin IIA (NMIIA) to the back of cells, and 2) $ß_2$-integrin (Itgb2) to the neutrophil-endothelial interface, whose retention on the cell surface promotes neutrophil arrest and subsequent extravasation. Additionally, using an *in vitro* adhesion assay, we found that blocking $LTB_4$-BLT1 signaling or NMIIA activation results in a reduction of Itgb2 recycling to the plasma membrane in primary human neutrophils. Notably, we also discovered that neutrophils shed

extracellular vesicles (EVs) *in vivo* and that blocking their biogenesis/release prevents the autocrine/paracrine actions of $LTB_4$ signaling therefore inhibiting neutrophil arrest and extravasation response. Overall, our study unravels a crucial function for $LTB_4$ in promoting neutrophil communication in the vasculature during early response to infection.

M198

**Osmolarity-independent Cues Guide Rapid Cell Migration to Injury in Zebrafish Epidermis**

**A. S. Kennard**[1,2], J. A. Theriot[2,3]; [1]Stanford University, Stanford, CA, [2]University of Washington, Seattle, WA, [3]Howard Hughes Medical Institute, Seattle, WA.

During wound healing cells must respond rapidly to changes in their environment in order to mitigate tissue damage, on timescales too short to be controlled by changes in gene expression. To disentangle the effects of different environmental cues on wound-induced cell migration, we have studied wound healing in larval zebrafish: when the tailfin is lacerated with a needle, basal epidermal stem cells up to 300µm away from the wound polarize their cytoskeleton within tens of seconds and migrate toward the wound over the span of about 15 minutes. One cue that has been suggested to induce cell migration after injury is a sudden shift in osmolarity due to mixing of interstitial fluid and the external medium at the wound (Gault 2014). By changing the composition and concentration of ions in the external medium, we discovered that, although a change in osmolarity did promote cell migration, it was not necessary for actin polarization: cell migration was partially inhibited in all isosmotic media, but the actin cytoskeleton still polarized toward the wound in all media except isosmotic sodium chloride. By measuring cell volume after wound healing we confirmed that changes in cell volume were not sufficient to explain this differential cytoskeletal response in isosmotic media. This suggests that cells can sense tissue damage via non-osmotic, ion-specific mechanisms. Electric fields, arising from short-circuiting ion transport across the epidermis at the wound site, are a possible alternative signal of tissue damage, but it is unknown whether electric fields are able to induce cell polarization or migration towards a wound *in vivo* at short (<30 min) timescales. Consistent with this possibility, we found that applying an electric field *in vivo* is sufficient to stimulate actin polarization and rapid cell migration in the absence of a wound. Work is ongoing to directly measure the transepithelial potential across the epidermis and determine if this could be the source of an electric field that guides cell migration during wound healing. These results suggest that the overall physiology of the epidermis, including the transport of specific ionic species, has an important and unappreciated role in rapidly guiding cells towards damage.Reference: Gault WJ *et al.* (2014). Osmotic surveillance mediates rapid wound closure through nucleotide release. *J Cell Biol. 207*(6): 767-782.

M199

**Migrating Neutrophils Execute Front Protrusion and Rear Retraction Programs with Certainty Until Doubt or Completion**

**A. Hadjitheodorou**[1], G. R. R. Bell[2], F. Ellett[3], D. Irimia[3], R. Tibshirani[4], S. R. Collins[2], J. A. Theriot[5]; [1]Department of Bioengineering, Stanford University, Stanford, CA, [2]Department of Microbiology and Molecular Genetics, University of California Davis, Davis, CA, [3]Department of Surgery, BioMEMS Resource Center Massachusetts General Hospital, Harvard Medical School, Boston, MA, [4]Department of Statistics, Stanford University, Stanford, CA, [5]Department of Biology, University of Washington, Seattle, WA.

Chemotaxing neutrophils continuously engage in directional decisions as they navigate complex environments. During interactions with obstacles, neutrophils often develop multiple competing fronts, raising the question of how the cell is able to select which front to maintain and which front(s) to abandon.To tackle this question, we challenged HL60 neutrophil-like cells with an oval-shaped obstacle positioned symmetrically in 6 x 3 um microfluidic channels. Cells respond to the mechanical challenge by creating two equivalent competing fronts that encounter identical chemotactic gradients. The symmetry breaks when one front starts retracting allowing the dominant front to drive the cell around the obstacle.From time-lapse microscopy data, we extracted hundreds of image features that report on cell shape and distributions of cytoskeletal components. Through a supervised statistical learning approach, we identified a small subset of features that carry predictive power; using these we asked how far back in time could we accurately forecast the direction outcome. Among the examined features, cell shape and actin features appear to have the largest predictive power. Surprisingly, we could predict the cell's turning direction with accuracy greater than 70% only during the last third of the decision-making process (about 18 sec before the initiation of retraction). In this context, cell decision-making does not apparently require amplification of pre-existing asymmetries.Previous work (Yang*, Collins* & Meyer, 2016, *Nat. Cell Biol.* **18**: 121) showed that Cdc42, a Rho-family GTPase, is predictive of cell turning in chemotaxing neutrophil-like cells. To determine if Cdc42 activity gives improved prediction of cell decision-making, we combined our microfluidic assay with cells expressing an optogenetic G-protein coupled receptor and a spectrally compatible FRET biosensor for Cdc42 activity. This system enables light-stimulated neutrophil "chemotaxis" while measuring downstream GTPase activity in the same cells. Repetitive stimulation to one of the competing fronts is sufficient to bias the cell's direction with a 90% success rate. Interestingly, administering stimulation only during the early phase of competition did not bias the cell's stochastic choice. This suggests that inducing a transient asymmetry at an early stage is not sufficient to drive the system out of its steady state. We hypothesize that the two fronts are independently executing their protrusion programs and only late in the competition they start "raising doubt" and are amenable to re-programming. In addition, we found that once a cell has made a decision, the losing front enters a refractory period that requires complete retraction to the cell body before stimulation can encourage a new protrusion.

## Minisymposium 20: Principles of Organelle Spatial Organization and Interactions

M200

**Single Molecule Characterization of Protein Dynamics At Endoplasmic Reticulum-Organelle Contact Sites**

**J. Nixon-Abell**[1], C. J. Obara[1], F. Riccio[2], J. Lippincott-Schwartz[1], C. Blackstone[3]; [1]Janelia Research Campus, Ashburn, VA, [2]Kings College London, London, UNITED KINGDOM, [3]National Institutes of Health (NINDS), Bethesda, MD.

The endoplasmic reticulum (ER), present in all eukaryotic cells, consists of a complex membranous network stretching from the nuclear envelope to the plasma membrane. This remarkably versatile organelle plays crucial roles in numerous cellular functions, from calcium signaling to protein and lipid synthesis. Recent work has also highlighted an emerging role for the ER as a key regulator of the morphology and function of other subcellular organelles, with ER-organelle contact sites mediating the exchange of crucial signaling components and biomolecules. Several candidate proteins have been proposed to act as molecular tethers to facilitate specific ER-organelle interactions. However, the

dynamics and spatial regulation of these tethering proteins within the ER membrane remains unclear.Here, we introduce a high-speed, correlative single molecule imaging approach to study molecular behavior at ER-organelle contact sites. With this approach, we track the motion of several ER-organelle tethering proteins in live cells, including VAP-A, VAP-B, Mfn1, Mfn2, and PDZD8. We demonstrate that each tether exhibits a unique dynamic signature and spatial confinement pattern, with tethering complexes assembling and disassembling on subsecond timescales. Based on these measurements, we conclude that distinct subtypes of contact site exist, and can be grouped according to the dynamic properties of specific tethers. We observe heterogeneity in tethering protein dynamics across different cell types and at spatially discrete ER-organelle contacts within the same cell. Furthermore, several tethering proteins exhibit specific alterations to their dynamic behavior in response to nutrient deprivation or ER calcium depletion, suggesting that the physiological state of cells impacts ER-organelle contacts by changing tethering complex dynamics. Fascinatingly, we find that molecular tethers harboring certain disease-associated mutations demonstrate altered dynamics at the single molecule level, with this impaired behavior correlating with downstream cellular pathogenesis.Taken together, this work provides initial insights into the single-molecule dynamics of tethering proteins at ER-organelle contact sites. Alterations in these dynamics appear to be a key component in dictating the functional heterogeneity of contact sites, and potentially underlie certain ER-associated disease states.

M201

**Reticulon 3 Directly Regulates Late Endosome Trafficking on Microtubules**

**H. Wu**, G. Voeltz; University of Colorado Boulder, Boulder, CO.

Four paralogous reticulon proteins (Rtn1-4) in animal cells share a highly conserved C-terminal transmembrane Reticulon Homology Domain (RHD), but their cytoplasmic domains are highly variable. The shared RHD localizes Rtn proteins to regions of high membrane curvature including ER tubules and the edges of ER sheets. However, the diversity in the cytoplasmic domains of different Rtn proteins could contribute to distinct functions. Rtn4 generates the shape of ER tubules, but we find that Rtn3 does not share this function. Rtn3 deletion does not alter ER shape or dynamics. Rtn3 also has a unique localization. While Rtn4 distributes evenly along ER tubules, Rtn3 concentrates at a striking location where ER tubules and microtubules (MTs) intersect. When Rtn3 puncta are trafficking along MTs, they localize to tripartite junctions between an ER tubule, a MT, and a membrane contact site (MCS) with a late endosome (LE). When Rtn3 is deleted, LEs accumulate in the cell periphery and fail to properly sort cargo. This phenotype can be rescued by reintroduction of exogenous Rtn3. To test if Rtn3 can directly tether LEs to the ER, we expressed the cytoplasmic domain of Rtn3 that contains a mitochondrial targeting signal (mito-Rtn3). We find that mito-Rtn3 is sufficient to tether LEs to mitochondria. Together our data support a role for Rtn3 in tethering an ER MCS that loads LEs onto MTs for proper endosomal trafficking.

M202

**Rna Granules Hitchhike on Lysosomes for Long-distance Transport, Using Annexin A11 as a Molecular Tether**

**Y. Liao**[1], M. Fernandopulle[2], G. Wang[3], H. Choi[1], L. Hao[2], C. Drerup[4], R. Patel[2], S. Qamar[3], J. Nixon-Abell[3], Y. Shen[5], W. Meadows[3], M. Vendruscolo[5], T. Knowles[5,6], M. Nelson[2], M. Czekalska[3], G. Musteikyte[3], M. A. Gachechiladze[2], C. Stephens[2], H. Pasolli[1], L. Forrest[2], P. S. George-Hyslop[3,7], J. Lippincott-Schwartz[1], M. E.

Ward[2]; [1]HHMI Janelia Research Campus, Ashburn, VA, [2]NINDS, NIH, Bethesda, MD, [3]Cambridge Institute for Medical Research, Department of Clinical Neurosciences, University of Cambridge, Cambridge, UNITED KINGDOM, [4]NICHD, NIH, Bethesda, MD, [5]Department of Chemistry, University of Cambridge, Cambridge, UNITED KINGDOM, [6]Cavendish Laboratory, Department of Physics, University of Cambridge, Cambridge, UNITED KINGDOM, [7]Department of Medicine (Division of Neurology), University of Toronto and University Health Network, Toronto, ON, CANADA.

Long-distance RNA transport plays a critical role in cells by enabling local protein translation at metabolically-active sites distant from the nucleus. This ensures an appropriate spatial organization of proteins, vital to polarized cells such as neurons. Here, we present a mechanism for RNA transport in non-polarized cells and neurons, in which RNA granules indirectly "hitchhike" on moving lysosomes. *In vitro* biophysical modeling, live-cell microscopy, and unbiased proximity labeling proteomics reveal that annexin A11 (ANXA11), an RNA granule-associated phosphoinositide-binding protein, acts as an adaptor between RNA granules and lysosomes. ANXA11 possesses an N-terminal low complexity domain, facilitating its phase separation into membraneless RNA granules, and a C-terminal membrane binding domain, enabling interactions with lysosomes. Mutations in ANXA11, which are associated with familial amyotrophic lateral sclerosis (ALS), decrease long-range transport of RNA granules in neurons by disrupting their docking onto lysosomes. Thus, ANXA11 enables neuronal RNA transport via lysosomal hitchhiking of RNA granules, performing a critical cellular function that is disrupted in ALS.

M203

**The Significance of Sequestering H2A, H2Av and H2B on Lipid Droplets**

**R. A. Stephenson**[1], L. Chen[1], J. M. Thomalla[1], M. Beller[2], M. A. Welte[1]; [1]University of Rochester, Rochester, NY, [2]Heinrich Heine University Düsseldorf, Düsseldorf, GERMANY.

Although histones are essential for life, too little and too much histones results in cellular defects. Thus, new histone synthesis is usually tightly coupled to DNA replication. Yet, many animal embryos face a unique dilemma: they start with a single diploid nucleus but need large, maternal histone stores for rapid early development, creating an imbalance between histones and DNA. *Drosophila* embryos solve this problem by sequestering excess histones H2B, H2A, and H2Av on lipid droplets (LDs), fat storage organelles in the cytoplasm. However, it remains unclear why histones are stored on LDs rather than on other organelles or in the cytoplasm. Histones are bound to LDs via the Jabba protein. Newly laid *Jabba*$^{-/-}$ embryos lack maternal H2A, H2Av, and H2B protein. To understand their absence, we first examined how the maternal histone supply normally arises. LDs are generated in nurse cells (NCs); later, NC cytoplasm, including LDs, is transferred to the oocyte. Using flies expressing endogenously regulated H2Av-RFP or H2B-mEOS, we quantified histone levels in the ooplasm. In the wild type (WT), H2Av and H2B levels increased steadily, with a dramatic rise after transfer from NCs was completed, arguing for substantial histone synthesis in the oocyte. In *Jabba*$^{-/-}$, histone levels in the ooplasm decrease as oocytes mature, suggesting that histones are unstable. Western analysis confirmed that stage 14 *Jabba*$^{-/-}$ oocytes have less H2Av than WT. This divergent behavior is not due to differences in transcription or message stability, as histone mRNA levels were similar in mutant and WT. Intriguingly, Jabba expression was increased as WT oocytes mature. We propose that Jabba levels determine how much H2Av can accumulate. Consistent with this notion, H2Av accumulation is intermediate in oocytes expressing one copy of Jabba and increased beyond WT levels in oocytes expressing four copies of Jabba. Previously, we identified motifs in Jabba important for LD targeting and histone binding. To determine how Jabba

influences the H2Av pool, we assessed the fate of maternal histones when Jabba-histone association is abolished. In *Jabba* mutants that lack histone binding ability, H2Av levels are indistinguishable to *Jabba*$^{-/-}$. This data suggests that Jabba confers physical protection to support the H2Av pool. We then examined if LD binding is necessary to maintain histones in the ooplasm. Jabba[193-320], which binds histones but not LDs, was enriched in NC nuclei and rare to undetectable in oocytes. Ooplasmic H2Av levels at stage 14 were comparable to *Jabba*$^{-/-}$. We propose that Jabba binding to LDs retains both Jabba and histones in the cytoplasm and promotes their transport from NCs to the oocyte, where Jabba prevents histone degradation.

M204

**Structure, Biogenesis, and Engineering of the Eukaryotic $CO_2$-concentrating Organelle, the Pyrenoid**

M. Meyer[1], **M. Jonikas**[1], S. He[1], E. Freeman Rosenzweig[2], A. Itakura[2], N. Atkinson[3], H. Chou[4], T. Mettler-Altmann[5], W. Patena[1], T. Wunder[6], J. Lau[7], D. Matthies[4], G. Yates[7], V. Chen[2], T. Olusola[1], U. Goodenough[8], M. Stitt[9], B. Engel[10], O. Mueller-Cajar[6], Z. Yu[4], A. Smith[11], H. Griffiths[12], A. McCormick[3], L. Mackinder[7]; [1]Princeton University, Princeton, NJ, [2]Stanford University, Stanford, CA, [3]University of Edinburgh, Edinburgh, UNITED KINGDOM, [4]Howard Hughes Medical Institute, Ashburn, VA, [5]University of Dusseldorf, Dusseldorf, GERMANY, [6]Nanyang Technological University, Singapore, SINGAPORE, [7]University of York, York, UNITED KINGDOM, [8]Washington University in St. Louis, St. Louis, MO, [9]Max Planck Institute of Molecular Plant Physiology, Golm, GERMANY, [10]Max Planck Institute of Biochemistry, engelben@biochem.mpg.de, GERMANY, [11]John Innes Centre, Norwich, UNITED KINGDOM, [12]Cambridge University, Cambridge, UNITED KINGDOM.

Approximately one-third of global carbon-fixation occurs in an overlooked algal organelle called the pyrenoid. The pyrenoid contains the $CO_2$-fixing enzyme Rubisco, and enhances carbon-fixation by supplying Rubisco with a high concentration of $CO_2$. The molecular structure and biogenesis of this ecologically fundamental organelle have remained enigmatic. By using high-throughput localization of proteins and identification of protein-protein interactions in the model alga *Chlamydomonas reinhardtii*, we increased the number of known pyrenoid components from 6 to over 80, and discovered the existence of three new protein layers in the pyrenoid: a plate-like layer, a mesh layer, and a punctate layer. We discovered that an abundant pyrenoid protein, Essential Pyrenoid Component 1 (EPYC1), works as a molecular glue that binds Rubisco holoenzymes together to form the matrix at the core of the pyrenoid. We then found that a simple mechanism involving a Rubisco-binding motif explains both targeting of proteins to the pyrenoid and the overall architecture of the three pyrenoid sub-compartments. Finally, contrary to longstanding belief that the pyrenoid matrix is a solid structure, we discovered that the matrix behaves as a liquid droplet, which mixes internally, divides by fission, and dissolves and condenses during the cell cycle. Our data provide insights into pyrenoid protein composition, structural organization and biogenesis. Working with our collaborators in the Combining Algal and Plant Photosynthesis project, we aim to transfer algal pyrenoid components into higher plants to enhance carbon fixation and yields in crops.

M205

**Miga2 Links Mitochondria, the Er and Lipid Droplets and Promotes *De Novo* Lipogenesis in Adipocytes**

C. A. Freyre[1], **R. W. Klemm**[1], P. C. Rauher[1], C. S. Ejsing[2,3]; [1]University of Zurich, Zurich, SWITZERLAND, [2]University of Southern Denmark, Odense, DENMARK, [3]Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg, GERMANY.

Physical contact between organelles is vital to the function of eukaryotic cells. Lipid droplets (LDs) are dynamic organelles specialized in lipid storage, and interact physically with mitochondria in several cell types. The mechanisms coupling these organelles are, however, poorly understood, and the cell-biological function of their interaction remains largely unknown. Here, we discover in adipocytes that the outer mitochondrial membrane protein MIGA2 links mitochondria to LDs. We identify an amphipathic LD-targeting motif, and reveal that MIGA2 binds to the membrane-proteins VAP-A/B in the endoplasmic reticulum (ER). We find that in adipocytes MIGA2 is involved in promoting triglyceride (TAG) synthesis from non-lipid precursors. Our data indicate that MIGA2 links reactions of *de novo* lipogenesis in mitochondria to TAG production in the ER thereby facilitating efficient lipid storage in LDs. Based on its presence in many tissues MIGA2 is likely critical for lipid and energy homeostasis in a wide spectrum of cell-types.

M206

**Drosophila Adipocytes Maintain Spatially Distinct Lipid Droplet Sub-populations**

**M. Henne**, R. Ugrankar, J. Bowerman; UT Southwestern Medical Center, Dallas, TX.

Adipocytes store nutrients as lipid droplets (LDs), but how they organize their LD stores to balance lipid uptake, storage, and mobilization remains poorly understood. Here, using *Drosophila* fat body (FB) adipocytes we characterize spatially distinct LD populations that are maintained by different lipid pools. We identify peripheral LDs (pLDs) that make close contact with the plasma membrane (PM) and are maintained by lipophorin-dependent lipid trafficking. pLDs are distinct from larger cytoplasmic medial LDs (mLDs), which are maintained by FASN1-dependent *de novo* lipogenesis. We find that sorting nexin CG1514/Snazarus (Snz) associates with pLDs and regulates LD homeostasis at ER-PM contact sites. Loss of *SNZ* perturbs pLD organization whereas Snz over-expression drives LD expansion, triacylglyceride production, starvation resistance, and lifespan extension through a DESAT1-dependent pathway. We propose that *Drosophila* adipocytes maintain spatially distinct LD populations, and identify Snz as a regulator of LD organization and inter-organelle crosstalk.

M207

**Two Forms of Opa1 Coordinate to Induce Mitochondrial Inner Membrane Fusion**

**Y. Ge**[1], S. Boopathy[1], X. Shi[2], A. Smith[3], L. H. Chao[1]; [1]The Massachusetts General Hospital, Boston, MA, [2]Case Western Reserve University, Cleveland, OH, [3]University of Akron, Akron, OH.

Mitochondrial membrane dynamics is a cellular rheostat that relates organelle morphology and metabolic function. Using an *in vitro* reconstitution system, we describe a mechanism for how mitochondrial inner-membrane fusion is regulated by the ratio of two forms of Opa1. We found that the long-form of Opa1 (l-Opa1) is sufficient for membrane docking, hemifusion and basal levels of content release. However, stoichiometric levels of the processed, short form of Opa1 (s-Opa1) work together with l-Opa1 to mediate efficient and fast membrane pore opening. Additionally, we found that excess levels of s-Opa1 inhibit fusion activity, as seen in conditions of abnormal cellular proteostasis. These observations describe a tunable mechanism for gating membrane fusion.

M208

**A Mitochondrial Translocon Subunit Promotes Assembly of the Cristae Organizing MICOS Complex in Proximity to ER Contact Sites**

P. Tirrell, **J. Friedman**, R. Lohray, K. Nguyen; UT Southwestern, Dallas, TX.

Mitochondrial cristae organization is regulated by the highly conserved MICOS complex. MICOS, an approximately megadalton complex comprised of six conserved subunits, localizes to cristae junctions, sites of membrane invagination on the mitochondrial inner membrane. The principal MICOS component, Mic60, assembles into structures that persist in the absence of other complex members and in mitochondria devoid of cristae, suggesting it marks sites of MICOS assembly and cristae biogenesis. However, there are no known upstream determinants of Mic60 assembly and positioning. Using an unbiased visual screen, we find that a subunit of the translocase of the outer mitochondrial membrane (TOM) complex regulates Mic60 assembly into structures that predominantly localize in proximity to endoplasmic reticulum (ER) membrane contact sites. Mic60 assemblies are more generally linked to inter-organelle contact sites as they reposition in proximity to mitochondrial-vacuole contacts in a yeast strain where the ER-mitochondrial tether is deleted and bypassed. These data reveal that positioning of the MICOS complex is contingent on extra-mitochondrial spatial cues and are consistent with a model that a phospholipid micro-environment on the inner mitochondrial membrane generated by inter-organelle contact sites regulates MICOS assembly and dictates cristae biogenesis sites.

M209

**Quality Control of Protein Complex Assembly by a Transmembrane Recognition Factor**

**P. Carvalho**, N. Natarajan, O. Foresti; Sir William Dunn School of Pathology, University of Oxford, oxford, UNITED KINGDOM.

The inner nuclear membrane (INM) is continuous with the endoplasmic reticulum (ER) but harbors a distinctive proteome essential for nuclear functions. In yeast, the Asi1/Asi2/Asi3 ubiquitin ligase complex safeguards the INM proteome through clearance of mislocalized ER membrane proteins. How the Asi complex selectively targets mislocalized proteins and its activity is coordinated with other ER functions, such as protein biogenesis, are unclear. Here, we uncover a link between INM proteome identity and membrane protein complex assembly in the remaining ER. We show that lone proteins and complex subunits failing to assemble in the ER, access the INM for Asi-mediated degradation. Substrates are recognized by direct binding of Asi2 to their transmembrane domains for subsequent ubiquitination by Asi1/Asi3 and membrane extraction. Our data suggests a model in which spatial segregation of membrane protein complex assembly and quality control facilitates assembly efficiency and reduces levels of orphan subunits.

## Minisymposium 21: Regulation of Cell Division

M210

**Waves of Erk Activity Orchestrate Osteoblast Tissue Growth in Zebrafish Bone Regeneration**

**A. De Simone**, M. Evanitsky, L. Hayden, B. Cox, J. Wang, A. Chao, K. Poss, S. Di Talia, 27705; Duke University, Durham, NC.

Regeneration is the spectacular process in which a lost body part regrows to its original form and function. How are cellular behaviors such as proliferation, hypertrophy and migration orchestrated across the regenerating tissue to form a body part of the correct size and shape? Gradients of diffusible morphogens regulate cell behavior in many multicellular systems, but it is unclear how such gradients could coordinate tissue growth across the large spatial scales - millimeters or centimeters - of regenerating adult tissues. Self-organizing chemical signals can provide an effective mechanism of communication across large distance, but their role in regulating regeneration remains largely unexplored, owing to difficulties in imaging, analyzing and conceptualizing these complex systems. To overcome those barriers, we developed the regenerating scale of adult zebrafish as a quantitative platform to study tissue growth and morphogenesis. Scales are external millimeter-sized bony disks coated with a mono-layer of bone-depositing osteoblasts. Lost scales regenerate in just a few weeks. Regeneration starts with the formation of new osteoblast tissue by dermal trans-differentiation, followed by osteoblast proliferation and then hypertrophy. Scales are an ideal system to study tissue regeneration quantitatively owing to their simple organization and accessibility to live imaging. We discovered that traveling waves of Fgf-dependent Erk activity instruct hypertrophy of the regenerating osteoblast tissue. We find that these rings of Erk activity propagate across the millimeter-sized tissue as concentric trigger waves and induce patterned tissue growth, thus orchestrating scale morphogenesis. We find similar, yet slower, traveling waves in ontogenic scales, suggesting the reactivation of an ontogenic program in scale regeneration. Using a combination of theoretical and experimental analyses, we estimate that simple diffusion would take weeks to propagate Fgf signals across the entire tissue. Instead, concentric trigger waves deliver signals across the entire scale in 1-2 days. Induction of uniform oscillations of Erk activity slows down tissue growth and perturbs the geometry of the tissue, indicating a crucial role for signaling wave dynamics in ensuring that scales regain their shape during regeneration. We propose that signaling waves represent a general strategy to deliver growth factor signals and coordinate tissue geometry in large regenerating systems.

M211

**Mitogen-independent Cell Cycle Progression in B Lymphocytes.**

**A. Singh**[1], M. Spitzer[2], J. P. Joy[1], G. P. Nolan[2], R. Sen[1]; [1]Gene Regulation Section, Laboratory of Molecular Biology and Immunology, National Institute on Aging, Baltimore, MD, [2]Microbiology and Immunology - Baxter Labs, Center for Clinical Sciences Research, Stanford, CA.

Clonal expansion determines effectiveness of adaptive immune responses where activation of lymphocytes leads to selection of a tiny proportion of lymphocytes to proliferate robustly and execute the immune program. Proliferation studies in lymphocytes are carried out with persistent mitogenic stimulation, presumably reflecting our bias that mitogenic re-stimulation is necessary to get robust clonal expansion. The classical view, obtained largely from population analyses of fibroblasts, posits that a mitogenic signal is required at the end of each mitosis for cells to progress through the next G1 phase.

However, recent analyses of tumor cell lines have shown that a subset of cells within a population retains the ability to undergo G1 progression without additional signaling. For B lymphocytes the germinal centers (GC) proliferation shows good evidence of mitogen-independent proliferative phase in dark zone (DZ). Even, CD8+ T cells have been shown to have similar properties, suggesting that this may be a common feature of cell cycle regulation in lymphocytes. Despite its uniqueness and likely physiological relevance, this form of proliferation has received scant attention. Our study characterizes mitogen-independent proliferation in primary murine B lymphocytes. We demonstrate that, regardless of the initiating stimulus, commitment to DNA replication (S phase) programs B cells to undergo several rounds of cell division in the absence of overt mitogenic signaling and the extent of division is limited by cell death rather than by return to quiescence. High dimensional cytometry identifies mitogen-independent cell cycle progression is driven by unique characteristics of the G1 phase of cells that have divided once, large cell size, low levels of p27 and phosphorylated-Rb. In contrast to studies in cell lines, B cell division under these conditions requires CDK4/6 activity to traverse the second G1 phase. Transcriptional and protein analyses revealed up-regulation of survivin (Birc5) in the G1 phase of B cells past first mitosis and pharmacological inhibition of survivin function blocked G1 progression of cells undergoing mitogen-independent proliferation, but not of naïve B cells stimulated with mitogens. These observations indicate that, in contrast to textbook models of the cell cycle, B cells inherit a partially active G1 phase after cell division that permits them to move quickly to the next S phase in the absence of exogenous G1 progression signals. We propose that these mechanisms may assist in rapid cell division without differentiation that is required for clonal expansion in response to antigen and in B cell proliferation in the GC-DZ.

M212

**Temporal Control of Cell Division: Switches, Feedback Control and Refractory Periods**

A. Araujo[1], **S. Santos**[1], L. Gelens[2], J. Ferrell[3]; [1]The Francis Crick Institute, London, UNITED KINGDOM, [2]University of Leuven, Leuven, BELGIUM, [3]Stanford University, Palo Alto, CA.

Mitosis is one of the most dramatic cellular events and the transition from interphase to mitosis is temporally tightly regulated to ensure that two copies of the newly replicated DNA are evenly segregated to two daughter cells. Mitosis is triggered by the activation of Cdk1-cyclin B1 and its translocation from the cytoplasm to the nucleus. Positive feedback loops regulate the activation of Cdk1-cyclin B1 and help make the onset of mitosis irreversible and all-or-none in character. In addition, we recently showed that an analogous process, spatial positive feedback, regulates Cdk1-cyclin B1 redistribution at the onset of mitosis (*Santos, SDM et al Cell 2012*). Triggering spatial positive feedback by chemical biology approaches, promoted both the rapid translocation of Cdk1-cyclin B1 to the nucleus and induced premature mitosis in the majority of free cycling cells. Surprisingly, we observed that even G1-cells underwent mitosis. In other words, even cells in which DNA was not fully replicated were able to undergo cell division. These observations raised the question *on when do cells become competent to undergo a new division cycle and how is this mechanism controlled*. By combining live cell imaging of cell cycle biosensors with computational approaches in mammalian and hES cells we found that feedback regulation in the Cdk1-cyclin B1 core network imposes a refractory period in which cells are unable to respond to a mitotic trigger. If feedback is weakened, the refractory period is shortened and cells can re-enter a new division cycle soon after dividing. Our results suggest that feedback regulation may be the basis for a long and complete interphase and is essential for maintenance of chromosome number and

for reliable segregation of genetic information to daughter cells. Understanding system-level feedback regulation proved to be fundamental to understanding the basic features of transitioning from interphase to mitiosis in mammalian cells.

M213

**Transient Hysteresis in Cdk4/6 Activity Underlies Passage of the Restriction Point in G1**

**T. Meyer**, M. Chung, C. Liu; Stanford University School of Medicine, Stanford, CA.

Cells escape the need for mitogens at a restriction point several hours before entering S phase. The restriction point has been proposed to result from CDK4/6 initiating partial Rb phosphorylation to trigger a bistable switch whereby cyclin E/A-CDK and Rb mutually reinforce each other to induce Rb hyperphosphorylation. Here, using single live-cell analysis, we unexpectedly found that cyclin E/A-CDK activity can only maintain Rb hyperphosphorylation at high levels normally achieved at the onset of S phase and that CDK4/6 activity is required not only to induce but also to continuously maintain Rb hyperphosphorylation throughout G1 phase. Mitogen removal in G1 leads to a gradual loss of CDK4/6 activity, which can sustain Rb hyperphosphorylation in many cells until S phase, at which point cyclin E/A-CDK activity takes over. Thus, it is short-term memory, or transient hysteresis, in CDK4/6 activity following mitogen removal that sustains Rb hyperphosphorylation, demonstrating a probabilistic, rather than irreversible molecular mechanism underlying the restriction point. In addition to these new insights into the nature of the mammalian restriction point, our recent development of a reporter for CDK4/6 activity revealed a variable temporary G1 extension in cycling cells that is inserted after mitosis and is marked by low CDK4 and CDK2 activities. Furthermore, these live-cell studies provide evidence that human cells can enter the cell cycle in two ways, by either taking a fast path mediated by CDK4/6 activation or a much slower and less efficient path where CDK2 is activated without a need for CDK4/6 activity.

M214

**Unconventional Growth and Division Patterns in Marine-derived Black Yeasts**

L. M. Y. Mitchison-Field[1,2], J. M. Vargas-Muñiz[1], B. M. Stormo[1], E. J. D. Vogt[1], J. F. Pelletier[3,2], **C. M. Field**[3,2], A. S. Gladfelter[1,2]; [1]UNC Chapel Hill, Chapel Hill, NC, [2]Marine Biological Laboratory, Woods Hole, MA, [3]Harvard Med Sch, Boston, MA.

Fungi have relatively simple morphology and tractable genomes. Domesticated fungi, including budding and fission yeast and a few filamentous species, have long served as important model organisms for basic cell biology. In fungi, we can hope to understand how genomes encode growth and division patterns via cytoskeleton and secretion molecular systems. Studies in the laboratory yeasts *S. cerevisiae*and *S. pombe*defined “canonical rules” for budding and septation, but limited morphological descriptions of wild fungi suggest that these rules may not hold across all species. One source of novel fungi is the ocean, where the diversity and ecological roles of fungi are poorly understood. To explore marine fungal diversity, we cultured fungi from several marine environments in the vicinity of Woods Hole, MA and collected 36 unique culturable species across 20 genera.We then applied time-lapse differential interference contrast (DIC) microscopy to analyze growth and division morphology. A group of particular interest are the melanized fungi in the class Dothideomycetes, also known as black yeasts. Black yeast can withstand extreme environments such as deserts and hypersaline salterns. We observed diverse mixtures of budding and septation in black yeast species which challenge the rules established in

cerevisiae and pombe. We also found that individual black yeast species exhibited remarkable plasticity in cell size control, budding vs filamentous growth, and modes of cell division and polarization. Unexpected patterns included division through a combination of fission and budding, simultaneous production of multiple buds during vegetative growth, and cell division by sequential formation of orthogonal septa. To further explore these patterns, we are transforming black yeasts with fluorescent markers of chromatin, septa and microtubules. Black yeasts provide an exciting opportunity to explore how genomes encode morphological diversity, and the role of unusual growth and division patterns in adaptation to extreme environments.

M215

**Are Cancers Addicted to Aneuploidy?**

**J. Sheltzer**; Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Chromosome dosage imbalances are ubiquitous in cancer. Despite the prevalence of these alterations, we have little understanding of how they contribute to tumorigenesis. In particular, while multiple approaches have been developed to interrogate the function of single genes in cancer, our ability to manipulate the copy number of chromosomes and chromosome arms is extremely limited. Thus, our understanding of aneuploidy predominantly derives from correlative observations, and we lack a mechanistic understanding as to how large-scale copy number changes influences cancer progression.Cancers that arise from the same tissue tend to display recurrent copy number alterations. For instance, the amplification of chromosome 8 is frequently observed in sarcomas, while a majority of glioblastomas gain an extra copy of chromosome 7. We hypothesized that cancers may be “addicted” to these recurrent aneuploidies, in the same way that cancers can be addicted to BRAF or MYC expression. To test this, our lab has developed a novel, CRISPR-based, positive-negative selection strategy that allows us to precisely eliminate aneuploid chromosomes and chromosome arms from human cells. Using this approach, we have eliminated recurrent aneuploid chromosomes from multiple cancer cell lines. Removing aneuploid chromosomes decreases the expression of the genes found on those chromosomes and results in a profound set of transcriptional alterations *in trans*. “Aneuploidy-loss” cancers proliferate more slowly than their isogenic parental cells *in vitro* and display a pronounced cell cycle delay. Most strikingly, following aneuploidy elimination, the resultant cancer cells are almost entirely incapable of anchorage-independent growth or tumor formation. In total, these results indicate that chromosome dosage changes may represent a novel type of cancer “addiction” that can be targeted to incapacitate cancer cell growth. Moreover, our CRISPR-based methodology represents a robust approach to manipulate and study different gene dosage imbalances that are found in development and disease.

M216

**The Essential Plk1 Function in Centrosome Remodeling During Mitotic Entry Is Not PCM Expansion But γ-tubulin Complex Docking**

**M. Ota**, Z. Zhao, S. Wang, J. Harrison, D. Wu, A. Desai, K. Oegema; Ludwig Institute for Cancer Researc, University of California, San Diego, San Diego, CA.

Centrosomes are the major microtubule organizing centers of animal cells. In preparation for spindle assembly, centrosomes increase in size and nucleating capacity during mitotic entry in a process known as centrosome maturation. Polo like kinase 1 (Plk1) has a conserved role in centrosome maturation,

however its essential function in this process has been unclear. Here, we address this question using the *C. elegans* embryo where, in addition to PLK-1, centrosome maturation requires the kinase scaffold SPD-2 and the pericentriolar material (PCM) matrix component SPD-5. In prior work, we showed that phosphorylation by Plk1 of two sites (S653, S658) in SPD-5 are required for matrix expansion. To determine if the critical role of Plk1 in centrosome maturation is to drive matrix expansion, we compared the consequences of preventing Plk1 targeting to centrosomes (by mutating its docking site in SPD-2) to mutating the Plk1 target sites in SPD-5 required for matrix expansion. While both perturbations resulted in equally small mitotic centrosomes that formed small spindles, blocking Plk1 docking prevented chromosome segregation and was lethal, whereas blocking PCM matrix expansion did not prevent chromosome segregation or cause lethality. This result suggests that Plk1 has a critical function other than PCM matrix expansion. To identify this function, we performed scanning mutagenesis of predicted Plk1 phosphorylation sites in SPD-5. This effort identified two clusters in the SPD-5 N-terminus that, when mutated, led to penetrant embryonic lethality. Subsequent analysis revealed that these mutants have little effect on PCM expansion, but instead block recruitment of γ-tubulin-containing complexes to the expanding PCM matrix. The microtubule density of spindles formed in the γ-tubulin docking mutants appears similar to spindles in the PCM expansion-defective mutant, but they fail to segregate chromosomes. Reconstitution of phospho-dependent binding revealed that phosphorylation by Plk1 of sites in the SPD-5 N-terminus mediates direct binding of this region to the γ-tubulin complex. Based on these results, we conclude that the essential function of Plk1 in centrosome maturation is not PCM expansion but the generation of mitosis-specific docking sites for γ-tubulin-containing complexes that are necessary for spindle microtubule nucleation and chromosome segregation.

M217

**PCH-2/TRIP13 Regulates Spindle Checkpoint Strength**

L. Defachelles, A. Russo, C. Nelson, **N. Bhalla**; University of California, Santa Cruz, Santa Cruz, CA.

Spindle checkpoint strength is dictated by three criteria:the number of unattached kinetochores, cell volume and cell fate. We show that the conserved AAA-ATPase, PCH-2/TRIP13, which remodels the checkpoint effector Mad2 from an active conformation to an inactive one, controls checkpoint strength in*C. elegans*. When we genetically manipulate embryos to decrease cell volume, PCH-2 is no longer required for the spindle checkpoint or recruitment of Mad2 at unattached kinetochores in embryos with monopolar spindles. This role in checkpoint strength is not limited to large cells: the stronger checkpoint in germline precursor cells also depends on PCH-2. PCH-2 is enriched in germline precursor cells and this enrichment relies on a conserved factor that induces asymmetry in the early embryo. Finally, the stronger checkpoint in germline precursor cells is regulated by CMT-1, the *C. elegans*ortholog of $p31^{comet}$, which is required for both PCH-2's localization to unattached kinetochores and its enrichment in germline precursor cells. Thus, PCH-2, likely through its ability to regulate the availability of inactive Mad2 at and near unattached kinetochores, governs checkpoint strength.

M218

**ATP Availability Limits Mitotic Duration and Impacts Cell Fate Determination**

**C. Ferrás**[1], J. Oliveira[1], J. Monteiro[1], A. Santos[1], K. Bezstarosti[2], J. Demmers[2]; [1]IBMC/I3S, Porto, PORTUGAL, [2]Erasmus University Medical Center, Rotterdam, NETHERLANDS.

Little is known regarding the impact of energy metabolism on a prolonged mitosis and it remains unclear which processes are energy dependent under conditions that prevent SAC satisfaction (e.g. unattached kinetochores and/or in the presence of anti-mitotic chemotherapy drugs). Our data, from live cell imaging, shows that in different human cell lines mitotic energy content is a critical factor for the cell fate decision following a prolonged mitosis. We demonstrate that decreasing mitotic ATP levels, accompanied by increasing AMPK activation, leads to a switch in cell fate from mitotic cell death to slippage, thereby enhancing cell survival frequency. This increase in mitotic slippage results from a weakened SAC response, since cells treated with ATP synthesis inhibitors show a faster degradation of the mitotic substrate Cyclin B1. In agreement, inhibition of the anaphase-promoting complex/cyclosome (APC/C), the major regulator of Cyclin B1 levels during mitosis, rescues this phenotype, switching cell fate from mitotic slippage to death. We found that kinetochore localization of Mad1, CENP-E and Spindly was not significantly compromised, which suggests that the weakened SAC response was independent of kinetochore SAC signaling. Conversely, we found that *de novo* protein synthesis was decreased in energy deficient cells, and this was independent of AMPK signaling pathway. Importantly, CDC20 levels were significantly decreased which compromised mitotic checkpoint complex (MCC) production, thereby causing a premature activation of the APC/C and consequent mitotic slippage. In agreement with this, mitotic arrested cells treated with the global protein synthesis inhibitor cycloheximide phenocopy energy deficient conditions. Additionally, we found by mass spectrometry analysis and western blot analysis a global dephosphorylation of APC/C subunits 1 and 3 in energy deficient cells, suggesting a lower CDK1 activity. Critically, we demonstrated that APC3 requires continuous CDK1 dependent mitotic phosphorylation, which might contribute to strengthen SAC signaling. Overall, our data suggests that *de novo* ATP synthesis during a prolonged mitosis is required for continuous protein translation and CDK1 kinase activity. Our findings bring awareness to the limitations of using ATP deprivation as a therapeutic option in anti-tumoral strategies.

M219

**Loss of Epithelial Cell Size Regulation in Lung Cancer**

C. Sandlin, S. Gu, C. Deshpande, M. Feldman, **M. Good**; University of Pennsylvania, Philadelphia, PA.

How mammalian cells sense and regulate their sizes is poorly understood. It has been proposed that cells coordinate growth and cell division to achieve size homeostasis. However, it is debated which genes control cell size regulation and to whether alterations in cell size are sufficient to alter cell function and fate. A major challenge is that perturbations to cell size are poorly tolerated in cell culture. Therefore, few experimental systems exist that enable characterization of the consequences of cells growing inappropriately large or small. To identify factors that mediate cell size homeostasis and characterize the physiological impacts of cell size dysregulation, we screened through tumor tissues that exhibit enlarged cell sizes. We identified lung adenocarcinoma as a diseased cell state in which epithelial cells lose normal size regulation. Healthy AT2 cells in the lung exhibit a tight distribution of shapes and sizes. In contrast, using quantitative confocal imaging of whole-mount tumors we have found dramatic increases of the sizes of the nucleus and cell bodies for transformed AT2 cells. Importantly, these

enlarged cells appear to be proliferative and functional. They are mostly euploid and contain lamellar bodies and the cytosolic concentrations of markers appear similar to wildtype suggesting that they are not simply swollen with water. To determine whether their behavior is altered due to enlargement we have developed methods to isolate AT2 cells from single cell suspensions of lung tumors and separate them on the basis of cellular dimensions. We are currently performing gene profiling to identify gene regulatory programs or pathways that are altered in response to changes in cell size and that may contribute to the integrity of the tumor microenvironment and disease progression. By culturing these cells we propose that it is also feasible to identify genetic and epigenetic modifications required for epithelial cell size control.

## Minisymposium 22: Regulation of Cytoskeletal Dynamics and Transport

M220

**Reconstituting Cytoskeletal Assembly From Budding Yeast Extracts Reveals Basic Biophysical Properties of Septin Filament Polymerization**

**B. Woods**, K. Cannon, A. Gladfelter; University of North Carolina, Chapel Hill, NC.

Cells build large, micron-scaled structures from relatively small, nanometer-scaled proteins. The septin cytoskeleton is particularly adaptable in its ability to form micron-scale structures in a variety of shapes and sizes for various functions. Septins are GTPase proteins that self-assemble into oligomeric “rod-shaped” subunits 17-32 nm long, and are conserved from fungi to mammals. These oligomers can then polymerize into much larger filaments at the plasma membrane or in association with other cytoskeletal proteins. Filaments can be arranged into higher-order assemblies such as laterally associated bundles, crosslinked lattices, wrapped gauzes, and curved rings. Higher-order assemblies serve as platforms for protein localization including the polymerization of other cytoskeleton proteins, scaffolds for cytokinesis and cell signaling events during morphogenesis, and act as protein diffusion barriers. Septin assemblies go through substantial rearrangements throughout the cell cycle suggesting that septin filament polymerization is finely tuned and regulated.Much about what is known about actin and microtubule assembly came from seminal studies that uncovered biophysical properties underlying polymerization. A network of regulatory proteins modulate actin and microtubule biophysical properties to build and arrange higher-order assemblies. Very little is known about how cells control where and when septin filaments polymerize, and how filaments are arranged into higher-order assemblies. Genetic studies in yeast have identified a suite of potential septin regulators, including septin-associated kinases, cell polarity proteins, and cell cycle regulators. However, how these regulators mechanistically modulate septin filament polymerization or filament arrangements remains mysterious.To uncover the biophysical properties of septins and to investigate how regulators tune polymerization, we married a filament polymerization reconstitution system with cell extracts from budding yeast. Extracts are incubated on supported phospholipid bilayers and individual filaments are imaged by TIRF microscopy and SEM. We found that septin filament polymerization is most consistent with it being an isodesmic rather than cooperative process. We also found septins from extracts polymerize into distinct spatial patterns different from filaments polymerized from recombinant protein. Moreover, SEM has revealed a new, undescribed filament arrangement found from extracts. This combined extract-reconstitution approach enables us to dissect the underlying biophysical mechanisms of septin polymerization while simultaneously affording control of its regulators by utilizing the power of yeast genetics.

M221

**Plastin Promotes Fast Actin Filament Bundling Along with Formin-mediated Polymerization to Generate Rapid Filament Alignment During Contractile Ring Assembly**

**R. S. Kadzik**[1], Y. Li[2,3], D. Kovar[4,3], S. Wignall[1], E. Munro[3,2]; [1]Department of Molecular Biosciences, Northwestern University, Evanston, IL, [2]Committee on Development, Regeneration, and Stem Cell Biology, University of Chicago, Chicago, IL, [3]Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, [4]Department of Biochemistry and Molecular Biology, University of Chicago, Evanston, IL.

Cytokinesis is the process of physically dividing one cell into two, by means of a contractile ring made up of filamentous actin and non-muscle myosin. A key step in the assembly of the contractile ring is the rapid alignment of actin filaments into an organized array at the equator of the dividing cell. One proposed mechanism for local reorientation of actin filaments is compressive flow in the dividing cell, but whether this flow is sufficient to rapidly and robustly align filaments while the filaments are undergoing constant turnover is unclear. To evaluate what is required for timely assembly of the contractile ring, we used single molecule imaging and particle tracking analysis to measure local filament turnover and cortex contraction rates during the first cell division in the *C. Elegans* embryo. With these measurements and simple mathematical models, we found that compressive flow alone cannot account for the amount of filament alignment we observe during assembly of the contractile ring. Expanding on this model, we found that anisotropic assembly of actin filaments, along with slow realignment of filaments via myosin motor activity, could account for the rapid emergence and maintenance of a circumferentially aligned filament array. To evaluate if this mechanism of alignment operates in *C. elegans*, we imaged embryos expressing CYK-1/formin::GFP and LifeAct::mCherry and found that filament elongation is highly anisotropic at the equator of the cell, but not at the poles. Furthermore, a significant portion of CYK-1-polymerized filaments entering the equatorial region change direction to align with the arrayed filaments in the contractile ring. These results are consistent with templated assembly of actin filaments at the equator. We identified the *C. Elegans* homolog of the actin binding protein plastin (PLST-1) as a likely candidate to promote rapid bundling and alignment of fast-growing CYK-1 filaments in the embryo. Consistent with a role in assembly of the contractile ring, loss of PLST-1 results in slowed or abortive cytokinesis. Using multi-color TIRF microscopy to visualize actin polymerization by fluorescently labeled CYK-1 and crosslinking by fluorescently labeled PLST-1, we found that PLST-1 can rapidly bundle and cause realignment of CYK-1/formin polymerized actin filaments in-line with pre-existing actin filaments, consistent with our findings *in vivo*. Our results demonstrate a role for an actin bundling protein in promoting the templated elongation and alignment of actin filaments in the contractile ring and provide new insights into how the ring is rapidly and robustly assembled during cell division in animal cells.

M222

**Interplay Among Human EB1, APC, and Dia1 in Coordinating Microtubule and Actin Dynamics**

**M. A. Juanes**, R. Jaiswal, C. Fees, B. L. Goode; Brandeis University, Waltham, MA.

Human Adenomatous polyposis coli (APC) is a potent actin assembly-promoting factor, and uses its C-terminal 'Basic' domain (APC-C) to recruit actin monomers and nucleate actin assembly, alone or in collaboration with the formin Dia1. Recently, we generated a separation-of-function mutation in full-length APC (APC-m4) and showed that APC-mediated actin assembly is critical for directed cell migration

and focal adhesion turnover. Further, using polarization-resolved microscopy and FRAP analysis, we showed that APC maintains proper F-actin organization and dynamics at focal adhesions, which is essential for proper microtubule capture at focal adhesions and responsive induction of focal adhesion turnover. Here, we investigate how APC-mediated actin nucleation is influenced by interactions with the microtubule end-binding protein EB1. Interestingly, EB1 was first identified as a new in vivo binding partner of APC (Su et al., 1995), yet the functional significance of this interaction has remained unclear. Using in vitro TIRF microscopy and single molecule analysis, we show that EB1 directly inhibits APC-mediated actin nucleation, alone and together with Dia1, by blocking APC recruitment of actin monomers. Further, we identify several potential EB1-binding sites in the Basic domain of APC, which may underlie these regulatory effects. Consistent with these biochemical activities, knocking down EB1 in human U2OS cells leads to increased F-actin levels, which are dependent on endogenous APC. Conversely, overexpressing EB1 decreases F-actin levels, phenocopying the loss of either APC or Dia1. Overall, this work reveals a network of interactions and activities among EB1, APC, and Dia1 that coordinate microtubule plus-end dynamics with actin cytoskeleton organization and dynamics.

M223

**Clasp Mediates Microtubule Repair by Promoting Tubulin Incorporation Into Damaged Lattices**

**A. Aher**[1], D. Rai[1], L. Schaedel[2], J. Gaillard[2], K. John[2], L. Blanchoin[2,3], M. Thery[3,2], A. Akhmanova[1]; [1]Cell Biology, Department of Biology, Faculty of Science, Utrecht University, Utrecht, NETHERLANDS, [2]Univ. Grenoble-Alpes, CEA, CNRS, INRA, Biosciences & Biotechnology Institute of Grenoble, Laboratoire de Phyiologie Cellulaire & Végétale, CytoMorpho Lab, Grenoble, FRANCE, [3]Univ. Paris Diderot, INSERM, CEA, Hôpital Saint Louis, Institut Universitaire d'Hematologie, UMRS 1160, CytoMorpho Lab, Paris, France, Paris, FRANCE.

Microtubule network in cells plays a key role in processes like cell division, motility and intracellular trafficking. It is subjected to various mechanical stresses induced by acto-myosin contractility, motor based organelle transport and morphological changes during cell motility. Microtubule lattices have always been seen as relatively stable structures that mostly undergo turnover upon depolymerization from the plus ends due to dynamic instability. However recent evidence suggests that microtubules exchange tubulin dimers at the sites of lattice defects, which can either be induced by mechanical stress or occur spontaneously during polymerization. Tubulin incorporation restores microtubule integrity; moreover, "islands" of freshly incorporated, GTP-bound tubulin can slow down microtubule disassembly and promote rescues. Microtubule repair can occur in vitro in the presence of purified tubulin. However, in cells it is likely to be regulated by specific factors, the nature of which is currently unknown. CLASP is a known microtubule-stabilizing factor, which suppresses catastrophes and induces microtubule nucleation and rescue. Since CLASP promotes recovery of tapered growing plus ends with lagging protofilaments into complete ones, it is an interesting potential candidate to promote microtubule repair. Here, we used in vitro reconstitution assays combined with microfluidics and laser microsurgery to show that CLASP2α indeed stimulates microtubule lattice repair. CLASP2α promotes tubulin incorporation into damaged lattice sites thereby restoring the integrity of the tube. Furthermore, it induces the formation of complete tubes from partial protofilament assemblies and restores the stiffness of microtubules softened by hydrodynamic flow. A single CLASP2α domain, TOG2, which suppresses catastrophes when tethered to microtubules, was sufficient to stimulate microtubule repair, indicating that catastrophe suppression and lattice repair are mechanistically similar. Our results suggest

that the cellular machinery controlling microtubule nucleation and growth also protects microtubules against physiological insults.

M224

**A Combinatorial MAP Code Dictates Polarized Microtubule Transport**

B. Monroy[1], **K. M. Ori-McKenney**[1], T. Tan[1], J. Oclaman[1], D. W. Nowakowski[2], R. J. McKenney[1]; [1]University of California, Davis, Davis, CA, [2]N Molecular Systems Inc.,, Palo Alto, CA.

Many eukaryotic cells distribute their intracellular components through asymmetric, regulated active transport driven by molecular motors along microtubule tracks. A large variety of other proteins bind to microtubules, and as such, transport motors must encounter a number of non-enzymatic microtubule-associated proteins (MAPs) that decorate the microtubule cytoskeleton. How the binding activities of MAPs are coordinated and how this contributes to the balance and distribution of microtubule motor transport are open questions. Here, we utilize in vitro reconstitution of purified motor proteins and non-enzymatic MAPs to demonstrate that these MAPs have strongly differential effects on the motility of the three main classes of transport motors; kinesin-1, kinesin-3, and cytoplasmic dynein. Further, we dissect how combinations of MAPs with differential effects on motors give rise to novel behaviors, and reveal how transient interactions between MAPs and motors may give rise to these effects. From these data, we propose a "MAP code" that has the capacity to strongly bias directed movement along MTs and explain the intricate intracellular sorting observed in highly polarized cells such as neurons.

M225

**ARL8 Dependent Autoinhibition of SKIP Regulates Its Association with Kinesin-1**

**T. Keren-Kaplan**, J. S. Bonifacino; NIH/NICHD, Bethesda, MD.

Intracellular transport and positioning of organelles rely on coupling to motor proteins that move bidirectionally along microtubule tracks. Coupling of organelles to motors is mediated by specific adaptor proteins. The correct spatio-temporal functions of the motors are crucial to maintain cellular processes. Indeed, malfunctions in this machinery are the cause of numerous pathologies including amyotrophic-lateral-sclerosis and hereditary-spastic-paraplegia. Here, we focused on lysosomes, which are highly dynamic organelles. Recent evidence indicates that the precise positioning of lysosomes in the cell is important for autophagy, nutrient signaling and plasma membrane repair. The outward movement of lysosomes is regulated by a lysosome-associated complex named BORC that initiates a chain of interactions involving the small GTPase ARL8, the adaptor protein SKIP, and kinesin-1. We used bioinformatics, biochemical and cell biology tools to identify elements that regulate lysosome positioning, focusing on the adaptor protein SKIP. We found that dimerization of SKIP is required for its function. Most importantly, we discovered that SKIP is inhibited by an intra-molecular interaction between its N- and C-terminal parts. This inhibition is relieved by interaction with ARL8, indicating that ARL8 promotes conformational activation of SKIP for coupling of lysosomes to kinesin-1. More generally, these findings uncover a novel regulatory mechanism in which an organelle adaptor changes from an autoinhibited to an active conformation upon interaction with regulators on the organellar membrane.

M226

**Regulation of Mbp Local Translation Is Crucial for Adult Myelin Maintenance**

**M. Fu**, A. Valenzuela, B. Barres; Stanford University, Stanford, CA.

Efficient electrical signaling along axons depends on formation of compact myelin. This process of compaction requires myelin basic protein (MBP) to function as a molecular zipper to exclude cytoplasmic proteins and organelles from regions of compact myelin. *Mbp* is the most highly expressed mRNA in oligodendrocytes by 10-fold and it is transported along oligodendrocyte processes by kinesin and dynein before it is locally translated. Here, we further elucidate the mechanisms that regulate MBP local translation.First, ribosomal abundance inversely correlates with MBP protein levels. In primary oligodendrocytes, ribosomes accumulate slowly on the timescale of days prior to MBP protein translation. However, oligodendrocytes with high levels of MBP protein have few ribosomes, indicating that ribosomes may be degraded following MBP translation. In compartmentalized Boyden chambers, RNA isolated from oligodendrocyte processes contain high levels of ribosomal RNA, indicating that ribosomes may themselves be locally translated.Second, we ask whether transport of *Mbp* mRNA is required for MBP translation. In a proteomic screen using the RNA-binding reporter MS2, we found that *Mbp* mRNA associates with a myosin motor that is mutated in patients with distal myopathy and white matter lesions. We show that the myosin inhibitor blebbistatin causes *Mbp* mRNA to accumulate along microtubules, no longer distribute along actin, and also blocks MBP translation. We confirm this effect is specific to *Mbp* mRNA using a mouse model that replaces the *Mbp* 3'UTR with a stabilizing polyA sequence; these mice still express the *Mbp* CDS and thus are theoretically capable of translating MBP. Oligodendrocytes cultured from these mice strikingly lack *Mbp* mRNA granules outside the cell body, but have aberrant, large donut-shaped *Mbp* mRNAs outside the cell body that co-localize with late endosomes/lysosomes markers (Rab7, LAMP1). However, in both 2D and 3D microfiber cultures, these cells do translate MBP outside of the cell body. Thus, though 3'UTR-less *Mbp* mRNA can nonspecifically localize outside the cell body, they cannot be translated. This is consistent with the striking phenotypes of these adult mice - hypomyelination, tremors, and severe motor coordination defects.Third, we measure the dynamics of MBP translation. Using a photoactivatable reporter, we find that MBP translation does not occur gradually, but mostly in the fifth day of differentiation. Imaging with membrane-anchored GFP, we observe compaction events that occur rapidly, on the timescale of 3-5 minutes. Together, these experiments indicate that MBP translation is a highly regulated event that relies on the convergence of ribosome accumulation and regulation of *Mbp* mRNA transport and translation activation via the 3'UTR.

M227

**A Pair of E3 Ubiquitin Ligases Compete to Regulate Filopodial Dynamics and Axon Guidance**

N. P. Boyer, **S. Gupton**, L. McCormick, S. Menon, F. Urbina; University of North Carolina-Chapel Hill, Chapel Hill, NC.

Appropriate axon guidance is necessary to form accurate neuronal connections. The cytoskeletal rich growth cone at the tip of an extending axon senses and responds to extracellular guidance cues in order to navigate axon growth toward appropriate targets. Filopodia at the periphery of the growth cone have long been considered sensors for axon guidance cues, yet how they perceive and respond to extracellular cues remains ill-defined. Our work has found that in embryonic murine cortical neurons, the filopodial actin polymerase VASP is regulated via TRIM9-dependent non-degradative ubiquitination,

and that appropriate VASP ubiquitination and deubiquitination are required for axon turning in response to the guidance cue netrin-1. Here we show that the TRIM9-related E3 ubiquitin ligase TRIM67 antagonizes TRIM9-dependent VASP ubiquitination. Ubiquitination alters the dynamics of VASP at filopodia tips and the stability of growth cone filopodia. TRIM67 ligase activity is surprisingly required for antagonizing VASP ubiquitination, as well as its strong interaction with VASP, which outcomes TRIM9. By antagonizing VASP ubiquitination, TRIM67 increases filopodia stability and promotes responses to netrin. This antagonistic role in VASP ubiquitination is required for netrin-1 dependent filopodial and growth cone responses and netrin-dependent axon branching. Using a compartmentalized axon guidance microfluidic device, which exposes only axons to a stable gradient of netrin, we find that TRIM67 is required for netrin-dependent axon turning responses in vitro. In vivo, netrin is required for the formation of the corpus callosum, a major midline axon projection in the cortex. We find that deletion of *Trim67* delays the formation of this fiber tract, and the resultant commissure is thinner than in wildtype littermates. This phenotype is the opposite phenotype of mice in which *Trim9* is deleted. We suggest a novel yin yang model: coordinated regulation of non-degradative VASP ubiquitination by a pair of ligases is a critical element of netrin-dependent filopodial stability, growth cone response, and axon guidance.

M228

**Phosphorylation of the +TIP, TACC3, Modulates Its Interaction with Microtubules and Affects Axon Outgrowth and Guidance**

B. Erdogan[1], **L. Lowery**[1], G. Cammarata[1], R. St. Clair[2], B. Ballif[2]; [1]Boston College, Chestnut Hill, MA, [2]University of Vermont, Burlington, VT.

Decades of research have uncovered numerous key aspects of cytoskeletal regulation; however, we still lack a fundamental understanding of how upstream signaling pathways converge on cytoskeletal effectors to coordinate complex dynamics in multiple types of migrating cells. We examine this process in the embryonic neuronal growth cone, at the tip of the growing axon, in which both microtubules (MTs) and F-actin must be spatiotemporally coordinated in order to steer the growth cone in the right direction to form precise neuronal connections. Previously, we identified the protein TACC3 as a member of the +TIP family that regulates MT dynamics in *Xenopus laevis* and showed that TACC3 and its frequent binding partner, MT polymerase XMAP215, cooperate in the growth cone to promote axon outgrowth. We also found that both TACC3 and XMAP215 enable the growth cone to respond to a repellent axon guidance cue, Slit2. To determine how kinase signaling affects +TIP function, we have used mass spectrometry to identify several residues on TACC3 that are phosphorylated in response to the known axon guidance signaling kinase, Abelson tyrosine kinase. We have investigated the importance of these and other potential phosphorylation events by expressing either individual or combinatorial phosphomutants in growth cones and examined changes in MT regulation as well as growth cone behaviors. We find that phosphorylation of certain tyrosine residues (in the TACC domain) are indeed critical for TACC3 to bind to MTs and to promote TACC3's ability to drive persistent axon outgrowth. We also find that MT-F-actin interaction within the growth cone periphery is modulated by manipulation of the phosphorylation state of TACC3. Together, our data shed light upon how guidance cue signaling may spatiotemporally modulate TACC3 and XMAP215 dynamics to promote accurate growth cone steering during embryonic neural development.

M229

**Nestin Is a New Binding Partner of Dcx and Regulates Its Cdk5-mediated Phosphorylation and Dcx-dependent Growth Cone Morphology**

C. J. Bott[1], **B. Winckler**[1], J. M. Keil[2], L. McMahon[1], C. Yap[1], K. Y. Kwan[2]; [1]University of Virginia, University of Virginia, VA, [2]University of Michigan, University of Michigan, MI.

Nestin, an intermediate filament protein widely used as a marker of neural progenitors, was recently found to be expressed in developing neurons where it regulates growth cone morphology. Nestin, which is known to bind the active cdk5/p35 kinase, additionally affects responses to axon guidance cues upstream of cdk5. Changes in growth cone morphology require rearrangements of cytoskeletal elements, but the roles of intermediate filament proteins in this process are poorly understood. Here, we investigated the molecular mechanism by which nestin affects growth cone morphology. We found that nestin specifically interacts with the Lissencephaly-linked microtubule-binding protein doublecortin (DCX). Nestin facilitates phosphorylation of DCX by cdk5/p35 through a scaffolding mechanism, but the phosphorylation of other cdk5 substrates is not affected. Lastly, we show that nestin's effects on growth cone morphology are DCX-dependent, thus demonstrating a functional role for the DCX-nestin complex in neurons. We propose that nestin changes growth cone behavior by regulating the intracellular kinase signaling environment in developing neurons.

## Microsymposium 1: Autophagy, Protein Turnover & Quality Control

MS1

**The Role of Optineurin in Neuronal Mitophagy**

**C. S. Evans**, E. L. F. Holzbaur; University of Pennsylvania, Philadelphia, PA.

Mitophagy, the selective removal of damaged mitochondria, is thought to be critical to maintain neuronal homeostasis. Mutations in proteins implicated in mitophagy, including PINK1, Parkin, OPTN, and TBK1, cause Parkinson's disease or ALS, suggesting defective mitochondrial turnover contributes to neurodegeneration. To test this hypothesis, we used mild oxidative stress to induce low levels of mitochondrial damage in hippocampal neurons. We observed the sequential recruitment of Parkin, TBK1, and OPTN to depolarized mitochondria followed by their sequestration into autophagosomes, and determined this pathway was compartmentally restricted to the soma. Further, acidification of mitophagosomes was remarkably slow in neurons and overall was a rate-limiting step in the mitophagy pathway. Expression of an ALS-linked OPTN mutation disrupted the integrity of the mitochondrial network and this effect was exacerbated by oxidative stress. We propose that the slow kinetics of mitophagy enhance neuronal susceptibility to disease-associated mutations in the pathway, leading to neurodegeneration.

MS2

**Structural Mechanism of Folliculin-mediated Regulation of the Rag Gtpase Activation Cycle**

**R. Lawrence**, S. Fromm, Y. Fu, A. Yokom, D. Kim, A. Thelen, L. Young, J. Hurley, R. Zoncu; UC Berkeley, Berkeley, CA.

The decision to allocate resources toward cellular growth or quality control is critical for organismal survival. The mechanistic Target of Rapamycin Complex I (mTORC1) tunes the balance between cellular anabolism and catabolism, and is one of the most frequently mutated pathways in cancer. In response to nutrient stimulation, the Rag GTPases (Rags) recruit mTORC1 from the cytosol to the lysosomal surface, where its kinase function is activated. The Rags are obligate heterodimers (Rag A:RagC), whose nucleotide binding states respond to cellular nutrient status and determine their ability to bind to mTORC1. A key but poorly understood event in mTORC1 activation is conversion of RagC from the GTP- to the GDP-bound form. A complex of Folliculin (FLCN) and FLCN-interacting protein (FNIP) was proposed to promote mTORC1 activation by functioning as a RagC -specific GTPase Activating Protein (GAP), but how RagC is activated at the right time by FLCN:FNIP, and how it remains in check until needed, is one of the major unanswered questions in the mTORC1 regulation field. To determine the regulatory functions of FLCN:FNIP, we reconstituted a 'lysosomal FLCN complex' (LFC) containing FLCN:FNIP, the starved-state $RagA^{GDP}$:$RagC^{GTP}$ GTPases, and the lysosomal anchor Ragulator and determined its cryo-EM structure to 3.6Å. Surprisingly, this complex inhibits the RagC-GAP activity of FLCN by displacing the catalytic Arg finger of FLCN away from the RagC nucleotide pocket. Conversely, within the LFC, FLCN:FNIP protects starved-state $RagA^{GDP}$, providing a mechanism for stabilization of the inactive state Rag complex. Together, these data identify the LFC as a novel activation checkpoint for mTORC1, whereby FLCN:FNIP may help maintain the Rags in an inactive state under low nutrients, and yet is necessary for conversion to the active state when nutrient levels rise.

MS3

**Impaired Lysosome Transport to Distal Axons Contributes to Autophagic Stress in the Neurodegenerative Lysosomal Storage Disorder Niemann-Pick Type C**

**J. C. Roney**[1,2], T. Farfel-Becker[1], X. Cheng[1], F. M. Platt[2], Z. Sheng[1]; [1]Synaptic Function Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, [2]Department of Pharmacology, University of Oxford, Oxford, UNITED KINGDOM.

Niemann-Pick Type C (NPC) is a neurodegenerative lysosomal storage disorder characterized by accumulation of multiple lipids in late endosomes and lysosomes. An early pathologic feature of NPC is axonal dystrophy, which consists of bulbous swellings along axons that contain accumulated organelles associated with the autophagy-lysosomal pathway. Such changes occur before symptom-onset and neurodegeneration in NPC mice and suggest that defects in axonal organelle transport contribute to early NPC pathology. However, the mechanisms underlying these pathologic changes remain obscure. Our recent study characterized axon-targeted delivery of degradative lysosomes and demonstrated that the axon is an active compartment for local degradation (Farfel-Becker T et al., ***Cell Reports*** 2019). This work establishes a foundation for our current investigations into axonal lysosome trafficking and functionality in early NPC disease. Here we demonstrate that mature lysosome delivery to distal axons is significantly reduced in cortical neurons from Npc1 null mice, resulting in lower numbers of degradative lysosomes in NPC distal axons. Decreased axonal lysosome density leads to increased axonal autophagic stress that occurs without changes to autophagosome transport in NPC axons. The small GTPase Arl8b is a limiting factor in coupling lysosomes to the kinesin-1 motor for driving lysosomal transport, and we demonstrate that elevated Arl8b expression facilitates axonal delivery of mature active lysosomes in NPC. Rescuing axonal lysosome density by Arl8b expression reduces autophagic stress in axons from pre-symptomatic NPC mice. Collectively, these observations suggest a new pathological mechanism by which impaired lysosome transport disrupts maturation and progression of the autophagy-lysosomal pathway and contributes to altered axonal homeostasis in NPC. (Supported by the Intramural Research Program of NINDS, NIH)Farfel-Becker T, Roney J C, Cheng X-T, Li S, Cuddy S R, and Sheng Z-H. (2019). Neuronal soma-derived degradative lysosomes are continuously delivered to distal axons to maintain local degradation capacity. ***Cell Reports*** 28, 51-64.

MS4

**Mechanism of a Memory-enhancing Inhibitor of the Integrated Stress Response**

**A. A. Anand**[1], L. R. Kenner[2], J. C. Tsai[1], L. Miller-Vedam[2], H. C. Nguyen[2], P. Jaishankar[2], A. G. Myasnikov[2], C. J. Klose[1], L. A. McGeever[1], A. Frost[2], P. Walter[1]; [1]UCSF, Howard Hughes Medical Institute, San Francisco, CA, [2]UCSF, San Francisco, CA.

The integrated stress response (ISR) is an essential signaling network that enables cells to respond to intrinsic and extrinsic stresses by tuning mRNA translation. The ISR acts through the phosphorylation of the GTPase eIF2, a rate-limiting translation initiation factor. Phosphorylation converts eIF2 from a substrate into an inhibitor of its dedicated guanine nucleotide exchange factor, eIF2B, blocking translation. A drug-like eIF2B activator called ISRIB reverses the effects of eIF2 phosphorylation and restores translation during stress. Remarkably, in rodents, ISRIB enhances cognition and corrects cognitive deficits after brain injury without overt toxicity. To determine its mechanism of action, we solved by cryo-electron microscopy an atomic-resolution structure of ISRIB bound in a deep cleft within a decameric human eIF2B. Formation of fully active, decameric eIF2B holoenzyme depended on the

assembly of two identical tetrameric subcomplexes, and ISRIB promoted this step by cross-bridging a central symmetry interface. Structures of eIF2B bound to eIF2 in the phosphorylated and dephosphorylated state revealed eIF2B to be a static platform upon which substrate, inhibitor and drug bind to modulate nucleotide exchange. Based on these structures, an interrogation of the ISR in living cells provides a compelling explanation for both ISRIB's mechanism of activating eIF2B and its lack of toxicity. From this work, eIF2B assembly emerges as a paradigm for translational control and as a promising target for therapeutic intervention for a range of neurological diseases.

MS5

**A Close-up View of Mitophagy Using Mt-Keima and Fluorescence Lifetime Microscopy**

**D. Malide**[1], N. Sun[2,1], T. Finkel[3,1]; [1]National Institutes of Health, Bethesda, MD, [2]Ohio State University Wexner Medical Center, Columbus, OH, [3]Aging Institute, University of Pittsburgh Medical Center, Pittsburgh, PA.

Mitophagy is a cellular process that selectively removes damaged, old or dysfunctional mitochondria. Defective mitophagy is thought to contribute to normal aging and to various neurodegenerative and cardiovascular diseases. Previous methods used to detect mitophagy in vivo were cumbersome, insensitive and difficult to quantify. We created a transgenic mouse model that expresses the pH-dependent fluorescent protein mt-Keima in order to more readily assess mitophagy. Keima is a pH-sensitive, dual-excitation ratiometric fluorescent protein that also exhibits resistance to lysosomal proteases. At the physiological pH of the mitochondria (pH 8.0), the shorter-wavelength excitation predominates. Within the acidic lysosome (pH 4.5) after mitophagy, mt-Keima undergoes a gradual shift to longer-wavelength excitation. In addition to intensity imaging we describe here how to apply mt-Keima fluorescence lifetime microscopy (FLIM) to visualize mitophagy in live cells as well as various tissues including skeletal muscle, heart, liver, and kidney, obtained from mt-Keima transgenic mice. We observed that in control live cells mt-Keima fluorescence exhibits two components a short (0.4ns) lifetime corresponding to the mitophagic compartment and a longer (2.6ns) lifetime corresponding to normal mitochondria, in good correspondence to the intensity images. Interestingly, in the tissues the lifetime measurements reveal a heterogeneous mitophagic compartment containing in addition to the short (0.5ns) lifetime mt-Keima species an intermediary (1.2ns) longer lifetime component. Whether these 2 components correspond to different folding states, digestion products of the mt-Keima in the acidic environment remains to be elucidated. In conclusion FLIM provide a complementary approach to asses mitophagy in normal cells and tissues as well as in disease situations, or altered under environmental, genetic perturbations, or in aging.

MS6

**P27 Regulates the Autophagy-lysosomal Pathway Via the Control of Ragulator and MTOR Activity in Amino Acid Deprived Cells**

**A. Besson**, A. Nowosad; CNRS, Toulouse, FRANCE.

Autophagy is a catabolic process whereby cytoplasmic components are degraded within lysosomes, allowing cells to maintain energy homeostasis during nutrient depletion. Several studies have shown that the CDK inhibitor $p27^{Kip1}$ promotes starvation-induced autophagy. However, the underlying mechanism remains unknown. Here, we report that in amino acid deprived cells, p27 controls

autophagy via an mTORC1-dependent mechanism. During prolonged amino acid starvation, a fraction of p27 is recruited to lysosomes where it interacts with LAMTOR1, a component of the Ragulator complex required for mTORC1 lysosomal localization and activation. p27 binding to LAMTOR1 prevents Ragulator assembly and function and subsequent mTORC1 activation, thereby promoting autophagy. Conversely, upon amino acid withdrawal, $p27^{-/-}$ cells exhibit elevated mTORC1 signaling, impaired lysosomal activity and autophagy, and resistance to apoptosis. This is associated with sequestration of TFEB in the cytoplasm, preventing the induction of lysosomal genes required for lysosomal function. Silencing of LAMTOR1 or mTOR inhibition restores autophagy and induces apoptosis in $p27^{-/-}$ cells. Together, these results reveal a direct, coordinated regulation between the cell cycle and cell growth machineries.

## Microsymposium 2: Cell Polarity & Cilia Dynamics

MS7

**A Role for the Apical PAR Complex in Reorganizing Microtubules in Dividing Intestinal Cells**

**M. Sallee**, J. Feldman; Stanford University, Stanford, CA.

Dividing and differentiating cells require different arrangements of microtubules to function. Mitotic cells establish centrosomes as microtubule organizing centers (MTOCs), producing radial microtubule arrays that are critical for chromosome segregation. In contrast, polarized epithelial cells form parallel arrays of microtubules emanating from a non-centrosomal MTOC, the apical membrane, that promote cell polarity and intracellular transport. During development and tissue homeostasis, some polarized epithelial cells divide, presenting an important but poorly understood obstacle: microtubules must temporarily cycle between the apical surface and the centrosomes. The developing *C. elegans* intestine provides an excellent *in vivo* epithelial model to study how this microtubule reorganization is achieved. After the 16-cell embryonic intestine polarizes and establishes an apical MTOC, exactly four “E16*” cells divide again. The E16* divisions involve a rapid change in microtubule organization from apical to centrosomal as cells enter mitosis, and back to apical upon mitotic exit. Using fluorescent markers, genetic screens, and tissue-specific protein depletion with live imaging, we are testing the hypothesis that apical polarity proteins control microtubule reorganization during the E16* divisions. During mitosis, we observe that, like microtubules, MTOC-associated proteins also leave the apical membrane as the centrosome becomes the MTOC. However, the apical PAR polarity proteins remain at the apical membrane during the E16* divisions, suggesting that they may act as a memory mark and help direct the return of microtubules and MTOC proteins after division. Consistent with this model, we have found that intestine-specific depletion of the apical polarity proteins PAR-6 and PKC-3 disrupts apical MTOC reformation following the E16* division. A pilot forward genetic suppressor screen has isolated a suppressor of the MTOC defects caused by PAR-6 depletion. These experiments reveal a role for PAR proteins in returning MTOC function to the apical membrane following mitosis, a critical step in epithelial cell divisions across organisms.

MS8

**Integrating Neutrophil Fronts and Backs with the Mtorc2 Mechanotransduction Pathway**

**S. Saha**, O. D. Weiner; Cardiovascular Research Institute and Department of Biochemistry and Biophysics, UCSF, San Francisco, CA.

Neutrophils leverage feedback between mechanical forces and biochemical signaling to guide their polarity and motility during immune surveillance. For efficient movement, cells need to establish a single leading edge. We recently found that tension-based communication between actin protrusions (relayed by the PLD2/ mTORC2 pathway) are responsible for long-range competition between protrusions for proper control of polarity and movement. mTORC2 is an evolutionarily conserved regulator of cellular growth, proliferation and metabolism. Surprisingly, this signaling node has been co-opted for mechanochemical regulation of membrane homeostasis and cellular polarity or motility in systems ranging from yeast to Dictyostelium to neutrophils. Here we probe the molecular logic of how mTORC2 regulates the motility machinery.The kinase activity of mTORC2 is the most well-characterized route of regulating downstream effectors, but the complex is also thought to scaffold recruitment of some effectors in a kinase-independent fashion. Which of these activities links mTORC2 to the motility machinery is not well understood. Here we investigate this question using a combination of genetic and pharmacological approaches to selectively impair the kinase-dependent versus independent signaling roles of the complex. We find that the tension-based inhibition of Rac activation that enables competition between protrusions is gated by the kinase-independent role of the complex, whereas the mTORC2 kinase arm is essential for regulation of Myosin II activity at the trailing edge. With live cell imaging and Rac/Rho biosensors, we show the necessity of both of these branches of mTORC2 signaling for leading and trailing edge organization, cell polarity, movement, and guidance. Our results show how stretch activates the kinase dependent and independent arms of mTORC2 to integrate both the front (Rac) and back (Rho) polarity programs during neutrophil motility.

MS9

**A Novel Labeling Strategy Reveals that Neuronal Myosin V-labeled Vesicles Are Polarized to Dendrites**

**M. Frank**, M. Bentley; Department of Biological Sciences and The Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY.

The maintenance of the eukaryotic endomembrane system requires accurate trafficking of proteins between different cellular compartments by vesicle transport. This is particularly important for neurons, which have numerous subdomains that require specific complements of membrane proteins. Kinesins and dynein mediate long-range microtubule-based transport, while myosins mediate short-range actin-based transport. Myosin V is the primary processive motor that mediates transport towards the barbed end of actin, but little is known about its neuronal functions and its interactions with other motors. Identification of Myosin V cargoes is crucial for determining its neuronal function. One obvious approach to address this is to coexpress fluorescent Myosin V and candidate cargo proteins in the same cell. This is challenging because overexpression of full-length Myosin V results in a bright soluble pool that masks labeled vesicles. We recently developed a strategy to visualize vesicle-bound kinesins by expressing their vesicle-binding tail domains. Here we applied this approach to Myosin V in hippocampal neurons. Live-cell imaging found that Myosin V-labeled vesicles were polarized to the somatodendritic domain and many of these vesicles underwent long-range transport. To determine the identity of these vesicles, we coexpressed Myosin-V tail and important neuronal vesicle trafficking proteins. We found that Myosin V

co-transported with the dendritically polarized transferrin receptor, vesicles associated with Kinesin-3 family motors KIF13A and KIF13B. Myosin V also co-transported with a subset of vesicles carrying the dendritically polarized low-density lipoprotein receptor, vesicles associated with the Kinesin-3 motors KIF1A and KIF13B. Unpolarized dense-core granules labeled with brain-derived neurotrophic factor and axonally polarized vesicles labeled with neuron-glia cell adhesion molecule did not colocalize with Myosin V. We systematically measured overlap between Myosin V vesicles and important neuronal cargo proteins. Our results suggest that Myosin V binds to vesicles concurrently with several Kinesin-3 family members. This raises an important question: How are Myosin V and kinesin activity coordinated on the same vesicle? Our findings are also consistent with a previously proposed role for Myosin V in mediating retention of dendritically polarized vesicles in the proximal axon. The novel labeling strategy vastly enhances visualization of Myosin V on vesicles and should be broadly applicable for investigating Myosin V function in many cell types.

MS10

**Rab19 Mediates Formation of a Trafficking Super Complex that Regulates Primary Ciliogenesis**

**C. E. Jewett**, R. Prekeris; University of Colorado Anschutz Medical Campus, Aurora, CO.

The primary cilium is an antennae-like organelle protruding from the surface of most cell types and is required for signaling, cell cycle regulation, and sensing the extracellular environment. Defects in primary cilia formation result in a spectrum of diseases called ciliopathies. Over 50 years ago, Sorokin's seminal work identified two distinct pathways through which primary cilia may form depending on whether the centrosomes are located near the nucleus or cell surface. Despite considerable research characterizing the intracellular pathway of cilia formation used by cells with centrosomes close to the nucleus, the alternative pathway utilized by polarized epithelia with apically localized centrosomes is less understood. We have identified the small GTPase, Rab19, as a novel regulator of primary ciliogenesis in polarized cultures of renal epithelial cells. Rab19 loss of function cells show severe (95%) loss of primary cilia formation. Using super resolution microscopy, we found that Rab19 forms a punctate ring around the mother centriole in ciliated cells. To identify potential interacting partners of Rab19, we performed proteomics and found that Rab19 interacts with the Rab GTPase activating protein TBC1D4 and the HOPS late endosomal tethering complex. Knockout of TBC1D4 also eliminates primary cilia formation, suggesting that Rab19 and TBC1D4 function in the same pathway. Moreover, TBC1D4 localization appears to partially overlap with Rab19 and shows more dispersed localization at centriolar satellites. Rab19 and TBC1D4 localization is cell cycle-dependent such that centrosomal localization disappears during mitosis. In vitro biochemistry reveals that Rab19 mediates formation of a Rab19-TBC1D4-HOPS super complex, and we propose that this super complex is required for primary ciliogenesis in polarized epithelial cells.

MS11

**A Centriole-less Pericentriolar Material Serves as the Base of *C.elegans* Sensory Cilia**

**S. Eskinazi**, J. Magescas, J. L. Feldman; Stanford University, Stanford, CA.

Cilia are microtubule-based cellular appendages that assemble on basal bodies, which in some cell types are derived from centrioles. Perturbation of cilia related genes leads to a large scope of diseases, ciliopathies, such as retinal degeneration and polycystic kidney disease. While the assembly of cilia by basal bodies is shared among unicellular organisms and animal cells, in the nematode *Caenorhabditis*

*elegans* basal bodies are necessary for ciliogenesis, but are degraded upon cilia maturation. This raises the question of how ciliary microtubules are maintained in the absence of the basal bodies from which they are templated. We used CRISPR-generated endogenously tagged alleles to assess the localization of centrosomal proteins. Surprisingly, although the base of cilia lacks an association with centriole proteins or the PCM protein SPD-2/CEP192, SPD-5, ZYG-9/XMAP-215, TAC-1/TACC3 and the conserved microtubule nucleating γ-tubulin ring complex (γ-TuRC) localize to this region. Superresolution microscopy revealed distinct subdomains of these proteins, with a subset localizing more proximal to the ciliary axoneme and others localizing more distally to a region nucleating the assembly of dynamic microtubules into the cell body, confirming that the base of cilia is a microtubule organizing center (MTOC). Using tissue-specific degradation, we tested the role of γ-TuRC and SPD-5 at the base of cilia at different time points in development. Degradation of SPD-5 and the γ-TuRC component GIP-1/GCP3 after cilia maturation did not grossly impact cilia structure or function and γ-TuRC was not required for SPD-5 localization to the base of cilia. However, degradation of SPD-5 during ciliogenesis perturbed cilia structure, suggesting that SPD-5 is required to maintain the axoneme in the absence of a canonical basal body. Unlike at the centrosome, the localization and regulation of SPD-5 appears to be independent of mitotic kinases, as CDK-1/CDK1 and PLK-1/PLK1 do not localize to the base of cilia. Further degradation and imaging studies will reveal a more complete map of the base of cilia and explain the molecular control of both ciliogenesis and MTOC function. Additionally, we hypothesize that the base of cilia represents a pure MTOC structure stripped from the centrosome and so these studies are likely to reveal novel regulation of MTOC function at the centrosome.

MS12

**Cilia Development in Zebrafish Organ of Asymmetry**

**J. Manikas**, L. Rathbun, J. Freshour, H. Hehnly; Syracuse University, Syracuse, NY.

*In vitro* studies have identified two methods for ciliogenesis. The first, the centrosome/basal body constructs a cilium in the cell body where it then fuses with the plasma membrane. The second, the centrosome/basal body docks at the plasma membrane where it then assembles a cilium. The goal of our studies is to identify during *in vivo* organogenesis how cells construct a cilium. We have chosen to examine Kupffer's Vesicle (KV) development of *Danio rerio* (zebrafish) due to the transparency of the zebrafish embryo for live-cell 4-dimensional imaging. KV is an organ of asymmetry that is required to place visceral and abdominal organs with respect to the two main body axes. KV development requires cells to self-assemble into a rosette where cells then establish apicobasal polarity termed apical clustering. The rosette then transitions into a sphere with a fluid filled lumen. At this stage, cilia are projected into the lumen. Our studies have identified that early on in KV development, before a lumen has formed, cilia are being constructed in the cell volume (100%). In later stages of KV development (lumen expansion), we only see 1.3% of cilia still in the cell volume where the remaining population is projected into the lumen. These studies suggest that cilia first form in the cell body and then protrude into the extracellular space once a lumen is formed. To further identify the molecular mechanisms required to generate KV cilia we are analyzing the role of apically localized actin and the small GTPases Rab11 and Rab8. We propose a model that after the cilia is constructed and surrounded by a ciliary membrane cap, actin is organized along the apical membrane where it can generate tension to regulate when a cilium should protrude into the lumen. Based on the literature and our preliminary studies, Rab11-endosomes likely contribute to the formation of a ciliary cap and the recruitment of Rab8 that is required for cilia elongation. In efforts to further understand the role of Rab11 and Rab8 in ciliogenesis,

we are utilizing an optogenetic approach to disrupt either Rab11 or Rab8 and quantify its effect on cilia development and maturation in vivo.

## Microsymposium 3: Immune and Subcellular Response

MS13

**The *Chlamydia Trachomatis* Effector TepP Reprograms the Function of the F-actin Regulator Eps8 to Mediate the Transient Disassembly of Epithelial Cell-cell Junctions to Regulate Innate Immune Responses**

**L. Dolat**, R. Valdivia; Duke University Medical Center, Durham, NC.

*Chlamydia trachomatis* is the most prevalent sexually transmitted human bacterial pathogen. Chronic infections are associated with pelvic inflammatory disease and cancers of the cervix and uterus, but the molecular mechanism underlying these pathologies is poorly understood. *Chlamydia* employs a Type III secretion system to deliver a cohort of effector proteins into target epithelial cells to modify the cytoskeleton and stimulate invasion. We previously determined that the effector TepP (translocated early phosphoprotein) alters the tyrosine phosphorylation status of endocervical epithelial proteins during invasion to promote infection. Through a phosphoproteomic analysis of *Chlamydia*-infected epithelial cells, we determined that TepP promotes the Src-dependent tyrosine phosphorylation of EPS8, a filamentous actin-binding protein that localizes to epithelial tight junctions (TJs), endosomes and microvilli. In polarized endocervical epithelial cells, TepP recruits EPS8 to early *Chlamydia*-containing vacuoles (“inclusions”), and its recruitment coincides with the disassembly of cell-cell junctions, cell dispersion, and loss of transepithelial electrical resistance (TEER). Epithelial cells in which EPS8 was genetically ablated form functional TJs and establish normal TEER levels yet fail to disperse during infection with *Chlamydia*, indicating that the function of EPS8 is repurposed to specifically disassemble cell-cell junctions. Moreover, we show that the TJ protein zona occludens-1 (ZO-1) is recruited to the nascent inclusion in a TepP- and EPS8-dependent manner, indicating that TJ organization is a major target of TepP activities. Indeed, ectopically expressed TepP co-localizes with EPS8 at sites of TJ remodeling, suggesting that TepP is sufficient to drive TJ rearrangements. We validated these results in a primary endometrial organoid infection model where wild-type, but not TepP-deficient, *Chlamydia* recruit EPS8, disrupt TJ organization, and reduce epithelial barrier function as assessed by the leakage of organoid lumenal content. Furthermore, organoids infected with TepP mutants displayed reduced recruitment of neutrophils. Collectively, these results demonstrate that the effector TepP reprograms a major F-actin regulator to disrupt epithelial architecture and modulate the influx of innate immune cells to infection sites.

MS14

**Disruption of TFAM in T Lymphocytes Leads to Impaired Mtdna Copy Number Regulation, Altered CD8+ T Cell Effector Function and Metabolism**

**S. Kapnick**, R. Genner, P. McGuire; National Institutes of Health, Bethesda, MD.

Mitochondria contain multiple copies of maternally-inherited DNA (mtDNA) that encodes for 13 proteins all important for energy production via the respiratory chain. Loss of mtDNA copy number or integrity is associated with mitochondrial depletion syndromes - clinically heterogenous disorders caused by mutations in genes important for mitochondrial maintenance. Mitochondrial transcription factor A

(TFAM) is an essential protein that binds mtDNA regulating packaging, replication, mitochondrial transcription, and mtDNA copy number and mitochondrial biogenesis. Total disruption of TFAM by gene targeting in mice results in embryonic lethality, while heterozygous mice exhibit reduced mtDNA copy number and respiratory chain deficiency in cardiac tissue. Although TFAM has been shown to also play a role in neurodegeneration, its contribution to mitochondrial maintenance in other tissues with high energetic demands remains poorly understood. To mount effective immune responses, T cells migrate between tissues, clonally expand, and secrete effector molecules; highly energetic processes known to be dependent on changes in metabolism. To better understand the role of mtDNA copy number regulation in T cell function, we disrupted TFAM expression in T cells using a loxP-flanked *Tfam* allele in combination with a Cre-recombinase transgene under the control of the CD4 promoter. TFAM-deficient T cells undergo normal thymic development and exhibit expected total numbers in the periphery. While T cell receptor-mediated activation of naïve CD8+ T cells results in reduced proliferative capacity, stimulated CD8+ T cells display increased expression of activation markers and enhanced cytotoxic function, on a per cell basis. Although mtDNA content is significantly reduced in TFAM-deficient T cells, MitoTracker staining suggests increased mitochondrial mass. Studies are currently underway investigating the bioenergetics and expanded immune responses in TFAM-deficient T cells. By understanding how loss of TFAM alters T cell metabolism, we expect to contribute to our knowledge of the link between mtDNA copy number regulation and immune cell function. This work has the potential to aid in the development of improved strategies for evaluating and treating immune dysfunction in patients with mitochondrial disease associated with mtDNA depletion.

MS15

**Axon-glial Interactions: Oligodendrocyte-mediated Enhancement of Axonal Energy Metabolism Via Post-translational Acetylation of Neuronal Mitochondria**

**K. A. Chamberlain**, F. LiCausi, Z. H. Sheng; National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD.

Oligodendrocytes (OLs) are widely known for their role in central nervous system (CNS) myelination. Recent emerging evidence demonstrates that these specialized glial cells dynamically support axonal integrity in addition to passively maintaining saltatory conduction. Mouse models of OL dysfunction present with axonal transport deficits, mitochondrial abnormalities, and axonal degeneration, even in the presence of intact myelin. However, how these cells convey myelin-independent support of neuronal axons remains largely unknown. One attractive hypothesis is that OLs supply energy metabolites in order to meet local, axonal energy demand. Here, we measured the direct effect of OLs on distal axonal ATP levels. Cortical neurons expressing the FRET-based intracellular ATP probe, GO-ATeam2, were cultured in microfluidic chamber devices, allowing for physically isolated axons to be co-cultured with OLs purified via magnetic activated cell sorting. OLs increased axonal [ATP] levels across several *in vitro* time points, a phenotype that was recapitulated by addition of OL conditioned media (OLcm), demonstrating that OL-derived secreted factors directly enhance axonal energy level. We observed that mitochondrial proteins isolated from neurons treated with OLcm exhibited reduced lysine acetylation, a post-translational modification associated with increased mitochondrial energy production. Interestingly, the NAD-dependent deacetylase sirtuin 2 (SIRT2) is robustly enriched in OLs and expressed at much lower levels in neurons. We hypothesized that SIRT2 mediates the effect of OLs on axonal energy production. To test this, axonal [ATP] levels were measured in axons co-cultured with OLs transfected with control or SIRT2 specific siRNA. While control OLs increased axonal [ATP], SIRT2-

depleted OLs had no effect on axonal [ATP]. Overall, our study suggests that OLs directly affect local, axonal bioenergetics via extracellular transport of SIRT2 from OLs to distal axons. Disrupted axon:OL coupling likely serves as a common pathological mechanism in neurological disorders associated with white matter pathology, including AD, PD, HD, MS, and ALS. Thus, revealing this new contribution of OLs in maintaining axonal energy metabolism advances our knowledge of fundamental processes affecting human neurological disorders (Supported by the Intramural Research Program of NINDS, NIH).

MS16

**Analysis of Early Cellular and Sub-cellular Changes During Tumor Initiation and Progression in Live Animals.**

**W. Wang**, K. Rechache, R. Weigert; National Institutes of Health, Bethesda, MD.

Head and neck squamous cell carcinoma (HNSCC), most of which arise in the oral cavity, is one of the most common cancers with a 65 % 5-year survival rate. It accounts for more than 650,000 cases and 330,000 deaths worldwide annually. In 2019, it is estimated to have more than 53,000 new cases of HNSCC in the United States, which consists of 3% of all new cancer cases. Understanding the mechanisms that control initiation, progression, and spreading of the tumor to distal sites is of paramount importance to develop effective therapies. To this end, we have used a well-established carcinogen model in live mice that mimics the progression of HNSCC observed in patients. Here, we show, for the first time, the visualization within the same animal of the onset and progression of cancerous and pre-cancerous lesions that were followed for 22 weeks. By using intravital subcellular microscopy (ISMic), we acquired information on cellular and subcellular changes in the tumors cell and the microenvironment (blood vessels, ECM, an immune cells), and specifically, we focused on changes in the architecture of the actomyosin cytoskeleton, mitochondrial metabolism, and autophagy. This powerful approach allows for the first time to investigate the cell biology of tumor initiation and progression in a live animal.

MS17

**CARD19 Interacts with MICOS Complex Proteins and Protects Against Mitochondrial Dysfunction**

**K. Rios**[1,2], C. Beauregard[1,2], M. Zhou[3], T. P. Conrads[3], B. Schaefer[1]; [1]USUHS, Bethesda, MD, [2]HJF, Rockville, MD, [3]Inova Schar Cancer Institute, Annandale, VA.

CARD19 is a mitochondrial protein of unknown function; gene expression databases indicate that CARD19 is highly expressed in myeloid cells. We have observed that *Card19 -/-* mice injected with LPS display elevated levels of macrophage-specific pro-inflammatory cytokines relative to *Card19 +/+* mice. Because CARD19 is a mitochondrial protein and mitochondrial dysfunction is frequently associated with immune dysregulation and chronic inflammation, we sought to identify the molecular function of CARD19 in macrophages and fibroblasts. We confirmed via confocal and super resolution microscopy that CARD19 colocalizes with mitochondrial markers ATP Synthase Beta and TOMM20 in bone marrow derived macrophages (BMDMs), primary fibroblasts, and a macrophage cell line. Through immunoprecipitation and mass spectrometry analyses, we identified proteins that interact with CARD19, including MIC19 and MIC60. Both proteins are components of the mitochondrial contact site and cristae organizing system (MICOS) complex. We further demonstrated that CARD19 colocalizes with MIC19 in mitochondrial sub-domains via super resolution microscopy in primary fibroblasts and a macrophage cell line. Because deficiencies in the MICOS complex can cause dysfunction in mitochondria, we

measured outcomes of mitochondrial stress in BMDMs. We found that *Card19 -/-* BMDMs display elevated levels of mitochondrial reaction oxygen species (mROS) and a population of mitochondria with a decreased mitochondrial membrane potential, as measured by flow cytometry. Furthermore, *Card19 -/-* BMDMs have a modestly decreased oxygen consumption rate (OCR) measured by a Seahorse extracellular flux analyzer relative to *Card19 +/+* BMDMs. Based on these data, we propose that CARD19 may be a previously unknown regulator of MICOS function which potentially links this complex to other mitochondrial and/or non-mitochondrial CARD proteins.Funding for this research was provided by an NIH/NIAID grant to B.C.S. and T.P.C. (R01AI125552).

MS18

**Septin Recruitment of Contractile Acto-myosin Cages to Vesicle Membranes Drives Regulated Exocytosis in Exocrine Glands of Live Mice**

**S. Ebrahim, 20892**, M. Weiss, D. Chen, R. Weigert, 20892; NCI/NIH, Bethesda, MD.

The constant remodeling of cellular membranes into various curved configurations is a requisite for myriad biological processes ranging from cytokinesis to intracellular trafficking. To elucidate the molecular machines and processes driving membrane remodeling under physiological conditions, we use the process of regulated exocytosis in the salivary gland of live mice as a model system. In this model, we recently showed that 1) F-actin and non-muscle myosin (NMII) form polyhedral-like lattices around large membrane-bound secretory granules (SGs) undergoing exocytosis, and 2) use contractility and polymerization-forces to push the SG membrane into the plasma membrane (integration), allowing content release into the extracellular space.This study raised new questions regarding how this unique acto-myosin network is both recruited and organized to perform its key function. To address this, we explored the role of septins, which we identified in a screen of purified SGs, as potential candidates, based on their known abilities to sense/generate micron-scale membrane curvature, interact with the actin cytoskeleton and recruit NMII.We found, strikingly, via immunofluorescence and super-resolution microscopy that septins 2, 6 and 7 (SEPT2, SEPT6 and SEPT7) are in fact present on the surface of fused SGs, where they each organize into cage-like lattices. Using transgenic mice expressing GFP-NMIIA, we show that NMIIA colocalizes with the SEPT2 and that pharmacological inhibition of SEPT2 results in a significant decrease in the presence of activated NMII and of myosin light chain kinase (MLCK) on actin-coated fused SGs. Conversely, disruption of F-actin assembly on the SG surface leads to an expansion in SG size without impairing NMII or septin recruitment. Finally, we find that the dynamics of integration and the levels of acto-myosin on the SG surface are both compromised in mice lacking SEPT7 expression in salivary gland secretory cells.Based on our data, we propose that the newly observed septin-lattices: 1) provide a molecular scaffold to recruit and curve acto-myosin filaments populating the surface of SGs, and 2) are needed for the activation of NMII, likely through MLCK-mediated phosphorylation, which ultimately drives SG exocytosis. Finally, the pattern of organization of both septins and NMII on the SG surface provide new structure-function insights into the molecular mechanisms driving membrane remodeling *in vivo*.

## Microsymposium 4: Intracellular Organization & Phase Transitions

MS19

**Designer Membraneless Organelles Equip Eukaryotic Cells with a Second Genetic Code to Enable Orthogonal Translation of Selected Messenger RNAs**

**C. D. Reinkemeier**, G. Estrada Girona, E. A. Lemke; EMBL, Heidelberg, GERMANY.

Genetic code expansion (GCE) is a powerful tool to study and control protein function with single-residue precision in a variety of organisms ranging from *E. coli* to mice. It is widely used to perform labeling for super-resolution microscopy, photocontrol cellular function, or to introduce site-specific posttranslational modifications. This is achieved by introducing an orthogonal tRNA/synthetase suppressor pair into the living host, to recode a stop codon to incorporate a noncanonical amino acid (ncAA) into the nascent chain. This technique is codon-specific, but it cannot select specific mRNAs, so other naturally occurring stop codons could be suppressed leading to potential interference with housekeeping translation.Nature avoids unwanted cross-talk between cellular processes by confining specific functions into organelles. We aimed to design an organelle dedicated to protein engineering. Inspired by the concept of phase separation we hypothesized that such an organelle could be designed membraneless. Phase separation can generate high local concentrations of proteins and RNAs in cells and has recently gained attention owing to its role in the formation of specialized organelles such as nucleoli or stress granules that perform complex and specific functions inside the cell.We combined phase separating proteins (FUS/EWSR1 or SPD-5) with microtubule motor proteins (KIF13A or KIF16B) to generate orthogonally translating organelles in living cells that contain an RNA-targeting system, the stop codon suppression machinery and ribosomes. These large organelles enable site- and mRNA-specific ncAA incorporation, decoding one specific codon exclusively in the mRNA of choice.Our results demonstrate a simple yet effective approach to the generation of artificial organelles in eukaryotic cells. These semi-synthetic cells are capable of simultaneously executing two genetic codes, one canonical one for untargeted proteins in the cytoplasm and one expanded genetic code for chosen mRNAs inside of the synthetic organelles, thereby providing a route towards customized orthogonal translation and protein engineering.

MS20

**Viral Assembly Site Phase-separation Mediated by Transbilayer-coupling and Membrane Curvature Drives Selective Protein Incorporation Into HIV Membranes**

**P. Sengupta**[1], A. Seo[1], A. Pasolli[1], M. Johnson[2], J. Lippincott-Schwartz[3]; [1]HHMI, Ashburn, VA, [2]University of Missouri, Columbia, MO, [3]HHMI, Janelia Research Campus, Ashburn, VA.

Particles that bud off from the cell surface, including enveloped viruses and microvesicles, typically have a unique protein composition distinct from that of the originating plasma membrane (PM). This selective protein composition is particularly important for viral particles such as HIV, since it enables the virus to evade immune response and infect other cells. But how proteins sort into budding viruses remains unclear. We used high resolution time-lapse imaging and molecular dynamics simulations to dissect the mechanism(s) underlying protein sorting into membranes of budding HIV particles. An existing view is that protein sorting into HIV membrane is a passive process, reflecting the pre-existing protein distribution at viral assembly site on the PM. We discovered that, contrary to this view, oligomerizing HIV Gag actively remodels the viral assembly site membrane by generating a phase-separated ordered

lipid domain. The remodeling involves protein redistribution driven by lipid-based partitioning between the ordered assembly site and the surrounding bulk PM. Protein redistribution spans the entire duration of viral assembly and continuously remodels the assembly site membrane to transform it into the final viral membrane. We further find that the phase separation of the assembly site is driven by the combination of a novel *transbilayer coupling mechanism* and *increasing membrane curvature of the assembly site*. We show that immobilization of PIP2 at viral assembly sites via its high affinity interactions with HIV Gag leads to ordering of inner-leaflet lipids. The ordering of inner-leaflet lipids is transmitted to outer-leaflet by *transbilayer interactions* between the acyl chains of PIP2 and long, saturated acyl chains of outer-leaflet molecules. The registration of lipid-ordering in two leaflets at the assembly site generates an ordered lipid domain spanning the two leaflets. Experiments with curvature deficient Gag mutants further revealed that *curving of the assembly site* by Gag lattice is also necessary for the progressive ordering of the assembly site and redistribution of proteins. The increased curvature reduces line-tension at boundary between assembly site and surrounding PM and helps to sustain the phase separation. Based on these data, we propose a model where the combination of *Gag-induced transbilayer coupling of lipids at the viral assembly site* and *increasing assembly site membrane curvature* drive phase separation of viral assembly site, thereby providing a physical mechanism for selective protein inclusion in HIV membrane.

MS21

**Domain Characterization of Pericentrin Uncovers the Role of Its Intrinsically Disordered Regions in Mediating Phase Separation and Microtubule Nucleation in Human Cells**

**X. Jiang**, K. Mahe, D. Ho, J. Mia, S. Yamada, L. Jao; University of California, Davis, Davis, CA.

The centrosome is a membraneless organelle that serves as the main microtubule-organizing center (MTOC) in metazoan cells. It orchestrates critical cellular processes such as cell signaling and cell division. The centrosome consists of a pair of centrioles surrounded by a proteinaceous network of pericentriolar material (PCM). It is the PCM that nucleates microtubules (MTs) and thus dictates the MTOC activity of the centrosome. The PCM is a dynamic ensemble of proteins, many of which contain extensive intrinsically disordered regions (IDRs) that do not fold into stable secondary or tertiary structures. How the PCM encompasses the centrioles to form a membraneless, micron-sized centrosome is not well understood, in part because the roles of the IDRs in many of the PCM proteins are poorly defined. IDRs are widespread in eukaryotic proteomes and are associated with a diverse array of functions, including the formation of membraneless organelles through phase separation. Here we use an inducible gene expression system in mammalian cells to dissect the roles of IDRs in pericentrin (PCNT), a conserved PCM component critical for PCM assembly and mitotic spindle organization in vertebrates. In silico predictions suggested that PCNT and its orthologous proteins have extensive IDRs at the N-terminus and a structured centrosome targeting PACT domain at the C-terminus. Through live cell imaging analyses, we found that multiple N-terminal, IDR-containing domains of human PCNT condense into liquid-like assembly in a concentration-dependent manner in cultured cells. In particular, a region containing the putative dynein-interacting domain forms spherical “condensates” that grow in size, coalesce, and move toward the centrosome in a MT-dependent manner. Fluorescence recovery after photobleaching analyses showed that the phase of PCNT condensates transitions from liquid- to gel-like states over time. We further found that PCNT condensates selectively recruit endogenous PCM components and are capable of nucleating MTs in cultured cells. We propose that anchoring PCNT molecules around the centriole through the C-terminal PACT domains drives their N-terminal IDRs to

undergo crowding-induced phase separation at the centrosome; the resulting condensates selectively recruit additional PCM components to promote the assembly of PCM and the maintenance of centrosome integrity. We further speculate that the IDR, a prevalent sequence feature found in centrosomal proteins, plays critical roles in centrosome assembly, likely in part through mediating phase transition of PCM components.

MS22

**Presynapse Active Zones Assemble through Phase Separation of Scaffolding Molecules**

**N. McDonald**, K. Shen; Stanford University, Stanford, CA.

The presynaptic active zone is the site of release of vesicles containing neurotransmitters. Components of the active zone are responsible for tethering and priming synaptic vesicles, recruiting and clustering ion channels to respond to action potentials, and structurally stabilizing the synapse through transmembrane connections to the postsynaptic cell. Decades of study have revealed the protein components of active zones, as well as a number of protein-protein interactions between them. Yet how these components assemble into functional active zones down a distant axon is not clear. Here, we show that two widely conserved active zone components in C. elegans, SYD-2/Liprin-α and ELKS-1, undergo a liquid-liquid phase separation to assemble the active zone. To probe active zone protein behavior *in vivo*, we measured protein mobility in newly formed *C. elegans* synapses. We found that young active zones are dynamic and recover quickly after photobleaching, consistent with a possible phase separation. In contrast, established active zones are highly static with nearly zero cytoplasmic exchange. We find that SYD-2/Liprin-α and ELKS-1, two core active zone scaffolding proteins, are capable of liquid-liquid phase separation *in vitro* and rely upon multiple unstructured motifs for this activity. By selectively deleting short regions in these motifs, we specifically blocked phase separation activity without affecting other known functions of the proteins. When introduced endogenously *in vivo*, these mutations inhibit the early dynamics of developing synapses, supporting a phase separation during normal active zone assembly. We find that SYD-2/Liprin-α phase separation-blocking mutants localize normally to synapses; however, by imaging synapses of the Hermaphrodite Specific Neuron (HSN), we find additional active zone components fail to localize properly and synaptic vesicles fail to appropriately cluster. These defects in HSN active zone assembly lead to defects in its functional output of egg-laying, underscoring the importance of a phase separation in building the presynaptic active zone. Together these data show presynaptic active zones are assembled through a phase separation of core scaffold molecules. This mechanism may aid in tightly concentrating scaffolding proteins at nascent synapses, forming a potent sink to recruit binding partners and downstream active zone components.

MS23

**Sequence Determinants of Laf-1 Phase Separation in P Granule Assembly**

B. Schuster[1], G. Dignon[2], C. Jahnke[3], D. Hammer[3], **M. Good**[3], J. Mittal[2]; [1]Rutgers University, New Brunswick, NJ, [2]Lehigh University, Bethlehem, PA, [3]University of Pennsylvania, Philadelphia, PA.

Phase separation of intrinsically disordered proteins (IDPs) commonly underlies formation of membraneless organelles, which compartmentalize molecules intracellularly in the absence of a lipid membrane. Identifying the protein sequence features responsible for IDP phase separation is important for understanding physiological roles and pathological consequences of biomolecular condensation. To characterize the chemical determinants of IDP phase separation, we designed and investigated variants

of the intrinsically disordered RGG domain from LAF-1, a model protein sequence involved in phase separation and a key component of P granules. Based on a predictive coarse-grained (CG) IDP model, we identified a region of the RGG domain that has high contact probability and is highly conserved between species; deletion of this region significantly disrupts phase separation in vitro and in vivo. We then assessed the effect of charge patterning on phase behavior, finding that the wild-type sequence has well-mixed charged residues. Shuffling the sequence to generate variants with greater charge segregation dramatically enhances propensity to phase separate, suggesting the natural sequence was under negative selection to moderate this mode of interaction. Finally, we determined that mutating tyrosine to phenylalanine, or arginine to lysine, dramatically perturbs RGG phase separation, which parallels recent findings on other IDPs. Together, these studies identify key biophysical principles of RGG phase separation, including conserved residues and charge patterning, while also advancing a predictive framework for identifying sequence features important to phase separation.

MS24

**P-bodies Mediate the Post-transcriptional Regulation of *Draxin*During Neural Crest EMT**

**E. J. Hutchins**, M. L. Piacentino, R. Galton, G. da Silva Pescador, M. E. Bronner; California Institute of Technology, Pasadena, CA.

Neural crest cells undergo a tightly regulated epithelial-to-mesenchymal transition (EMT) to delaminate from the neural tube. In the cranial neural crest, we have shown that this developmental EMT program is controlled by temporally restricted expression of the Wnt antagonist, Draxin. A hallmark of Draxin's function during EMT is its transient expression. However, precisely how *Draxin* expression is regulated has been unclear. Here, we show with an *in vivo* reporter construct that the rapid degradation of *Draxin* mRNA is mediated post-transcriptionally via its 3'-untranslated region (3'-UTR). Using an MS2-MCP reporter system and time lapse imaging of neural crest explants, we further demonstrate that the MS2 construct containing the 3'-UTR of *Draxin* (MS2-*Draxin* 3'-UTR) localizes to small cytoplasmic granules resembling P-bodies in migrating neural crest cells. To further characterize these granules, we performed *in situ* hybridization for known P-body components, and found that *CNOT1*, *EDC3/4*, and *TNRC6A* (GW182) mRNAs are expressed in migratory neural crest. Using co-localization of MS2-*Draxin* 3'-UTR with RFP-tagged P-body components, and perturbation of P-bodies with both cycloheximide, and mutant forms of GW182 and DDX6, we describe the role of P-bodies during neural crest migration and in the degradation of *Draxin* during neural crest EMT. Together, our data highlight a novel and important role for P-bodies in an intact organismal system— playing an essential role in neural crest EMT via post-transcriptional target degradation. *This work was funded by the US National Institutes of Health K99 DE028592 (EJH) and R01 DE024157 (MEB).*

## Microsymposium 5: Microtubule Motors & Transport

MS25

**Systems Biology Identifies Gleevec as a Specific Inhibitor of CLIP-170S, a Novel +Tip Isoform, Which Causes Taxane Resistance in Cancer Cells and Patients by Obstructing the Microtubule Pore**

**P. V. Thakkar**[1], K. Kita[1], G. Galletti[1], N. S. Madhukar[1], E. V. Navarro[1], K. Cleveland[1], I. Barasoain[2], H. V. Goodson[3], D. L. Sackett[4], J. F. Diaz[2], O. Elemento[1], M. A. Shah[1], P. Giannakakou[1]; [1]Weill Med College/Cornell University, New York, NY, [2]Centro de Investigaciones Biológicas, Madrid, SPAIN,

[3]University of Notre Dame, Notre Dame, IN, [4]Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), NIH, Bethesda, MD.

Taxanes are widely used in the treatment of solid tumor patients including gastric cancer (GC). Post-hoc analysis of the clinical trial that led to docetaxel approval in GC, revealed that patients with diffuse histological subtype were intrinsically resistant to taxanes. As yet, the molecular basis of clinical drug resistance remains poorly elucidated. Using a panel of GC cell lines, we identified a subset with intrinsic taxane resistance due to impaired drug-target engagement, in the absence of tubulin mutations or decreased drug accumulation. We discovered a novel, short variant of the microtubule (MT) +TIP binding protein CLIP-170, hereafter CLIP-170S, which was preferentially expressed in resistant cells. Mass-spec proteomics and 5'RACE showed that CLIP-170S lacked the first 150 amino acids, thus, missing the Cap-Gly domain required for +TIP localization. Microscopy of endogenous or exogenous proteins revealed that CLIP-170S was mislocalized from +TIP to the MT lattice in contrast to the canonical CLIP-170. Stable CLIP-170S knock down (KD) entirely reversed taxane-resistance (~300 fold), directly establishing CLIP-170S as the cause of taxane resistance.Quantitation of Flutax-2 (fluorescently labeled taxane) binding kinetics by live-cell imaging of native cytoskeletons in sensitive and resistant cells, showed that Flutax-2 dissociated faster from MTs in CLIP-170S-expressing resistant cells due to slower association rate. CLIP-170S-KD fully restored Flutax-2 binding to MTs, indicating that CLIP-170S impedes taxane-MT interaction. As taxane binding to MT lumen requires entry via the MT pore, we used chemical probes binding at the outer-only (hexaflutax) or luminal (cyclostreptin) pore sites and showed reduced binding of both compounds to resistant cell cytoskeletons. In contrast, CLIP-170S had no effect on peluroside whose MT binding does not require access through the pore. Together, these data indicate that CLIP-170S obstructs the MT pore, preventing drug access to the MT lumen and causing taxane resistance. Clinically, we found CLIP-170S to be expressed in ~60% of GC patient tumors and that its expression was significantly associated with resistance to cabazitaxel monotherapy. Computational analyses of RNAseq data from sensitive and resistant cells predicted Gleevec (Imatinib) as a drug that could overcome taxane resistance. Indeed, we showed that Gleevec reversed taxane resistance by specific depletion of CLIP-170S protein.Taken together, these data reveal an entirely novel mechanism of taxane resistance via obstruction of the MT pore by the previously unrecognized CLIP-170S. We further found CLIP-170S to be highly prevalent in patient tumors and identified Gleevec as the first specific inhibitor of CLIP-170S.

MS26

**Kinesin-based Transport Is Controlled by Cholesterol in the Cargo Membrane**

Q. Li[1], J. Wilson[1], K. Tseng[2], W. Qiu[2], S. King[3], M. Vershinin[4], **J. Xu**[1]; [1]University of California, merced, Merced, CA, [2]Oregon State University, Corvallis, OR, [3]University of Central Florida, Orlando, FL, [4]University of Utah, Salt lake city, UT.

Motor protein-based transport in cells underlies all eukaryotic cell function and survival; dysfunctions in this transport are implicated in many diseases, including neurodegeneration. While the properties of motor proteins have been extensively studied both in vivo and in vitro, many important questions remain, including how the properties of the cargo itself impact motor function. In cells, cargos are often membrane-bound; the composition of the cargo membrane has long been hypothesized to impact motor protein-based transport. However, quantitative investigation of this hypothesis is limited; most cargos in current in vitro assays lack a physiological membrane. To address this technical challenge, here

we combined advances in membrane biophysics with established single-molecule optical-trapping experiments to characterize the transport of membrane-enclosed cargos in vitro. Our study focused on the major microtubule-based motor kinesin-1 and employed the microtubule-associated protein tau to mimic the crowded surface of the cytoskeletal tracks in cells. In contrast to the traditional, membrane-free approach, we found that coupling motors via a biomimetic membrane significantly enhanced the transport of cargos along tau-decorated microtubules. This effect diminished when we added cholesterol to our model membrane. Stochastic simulations indicated that the observed membrane effects reflect a change in the number of motors available to drive cargo transport. To our knowledge, our study uncovers the first direct link between cargo-membrane composition and kinesin function. The experimental approach employed here is generally applicable as a controlled experimental platform for interrogating the control of motor proteins in a context directly relevant to in vivo scenarios.

MS27

**Structural Insights Into the Chemical Inhibition of Dynein**

**C. C. Santarossa**[1], K. J. Mickolajczyk[1], L. Urnavicious[1], N. Coudray[2], J. B. Steinman[1], D. Ekiert[2], G. Bhabha[2], T. M. Kapoor[1]; [1]The Rockefeller University, New York, NY, [2]New York University, New York, NY.

Cytoplasmic dynein is a AAA (ATPase Associated with various Activities) motor protein that transports cellular cargoes towards the microtubule minus-end. The motor domain of dynein is comprised of six non-equivalent AAA domains, where AAA1, AAA2, AAA3 and AAA4 can bind nucleotide. We have recently designed a chemical inhibitor of human cytoplasmic dynein that is based on the dynapyrazole-A and -B pharmacophore. We termed this inhibitor dynapyrazole-E (E for ether) and here we show that it inhibits the ATPase activity of yeast dynein with single-digit micromolar potency. We report a cryo-electron microscopy structure of yeast dynein in the presence of dynapyrazole-E with an average resolution of 4 Å. In combination with mutagenesis studies, we determine that the compound binds to a AAA site in dynein. We find that dynapyrazole-E locks dynein in a unique conformation, which is associated with weak microtubule binding. Together, our data suggest that targeting the allosteric communications between the AAA domains can be employed as a strategy to inhibit dynein's catalytic activity.

MS28

**A Pushing Mechanism for Microtubule Aster Positioning in a Large Cell Type**

**J. Meaders**, D. R. Burgess; Boston College, Chestnut Hill, MA.

Following fertilization, microtubule (MT) sperm asters accurately position male and female pronuclei allowing for pronuclear fusion, a process essential for dictating the first division plane. Due to the relatively large sizes of zygotes compared to somatic cells, the forces that drive sperm aster migration have been hypothesized to be due to pulling on the leading astral MTs by retrograde motility of dynein anchored either to the cell cortex or to cytoplasmic vesicles. Conversely, pushing forces from rear facing MTs growing against the cortex are have not been documented due to potential MT buckling and slipping as they grow to greater lengths. Here, we re-investigate the forces required for sperm aster centration in the large sea urchin zygote (~100 um diameter) using live confocal microscopy of astral MTs and MT plus-ends, chemical manipulation of aster sizes, inhibition of dynein, and targeted ablation of the sperm aster. We find that leading, front portions of the aster are shorter and less dense than rear, cortical facing portions of the aster. This aster geometry precludes the current cytoplasmic pulling

model, which is dependent on front portions of the aster that are longer and more dense than the rear portions of the aster. Second, we find that aster migration distance and rates are equal to and limited by the length and growth rates of rear portions of the aster, with no influence from front lengths and growth rates. Rear astral MTs are also anchored to the cortex, which would antagonize pulling from dynein along front astral MTs. Ciliobrevin treatment, which was previously reported to inhibit aster migration in this system, causes immediate disruption of sperm aster integrity, complicating interpretations of ciliobrevin as a probe for dynein function. Therefore we inhibit dynein by injecting the CC1 subunit of p150 and follow aster migration. CC1 injection does not abrogate sperm aster migration despite inhibiting normal mitotic spindle formation indicating that dynein is not essential for aster migration in the sea urchin zygote. Finally, using the UV light inducible MT depolymerizing agent, caged-combretastatin, we show that ablation of rear astral MTs halts aster migration, while ablations of front and side astral MTs does not block aster migration. Collectively, our data indicates that a pushing mechanism by rear, cortical facing MTs can drive aster migration in a large cell type.

MS29

**Vasohibins-Mediated Microtubule Detyrosination Regulates Mitotic Spindle Morphology and Orientation through Kinesin13/MCAK**

**G. Rajendraprasad**[1], S. Eibes[1], S. Liao[2], C. Xu[2], M. Barisic[1]; [1]Danish Cancer Society, Copenhagen, DENMARK, [2]Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei, CHINA.

Microtubules (MT) are a dynamic cytoskeletal protein network that plays key roles in various cellular processes such as protein trafficking, migration, signaling and division. They consist of α- and β-tubulin heterodimers which are subjected to diverse posttranslational modifications (PTM's) that have been known to influence their spatiotemporal regulation and dynamics. Cyclic removal and addition of tyrosine residue at the C-terminal of α-tubulin is a highly heterogeneous PTM that creates a distinct sub-cellular distribution pattern which has been suggested to play a critical role during chromosome congression, cellular differentiation, cardiomyocyte contraction and progression of several forms of cancer. Just recently the enzymes responsible for the removal of the tyrosine residue were identified as vasohibins (VASH1 and VASH2) in complex with small vasohibin binding protein (SVBP). Although the importance of the enzyme complex has been studied in neuronal differentiation, its significance in mitosis still remains unclear. In this study, we successfully solved a series of crystal structures of VASH1 in complex with SVBP and its substrate, a peptide mimicking the C-terminal tail of α-tubulin. These structures provide insights into the molecular mechanism of VASH1-SVBP interaction and offer rationale for its substrate binding and enzymatic activity. We complement our structural findings with a series of cell biology and biochemistry experiments and evaluate the role of VASH-SVBP complex during mitosis. Upon knockdown of the vasohibins, the MT detyrosination levels in interphase and on the mitotic spindles were considerably reduced. Furthermore, perturbation of the complex significantly affected mitotic duration, as well as spindle morphology and orientation. In agreement with previously reported *in-vitro* findings, our *in-cellulo* live-cell imaging-based MT dynamics assays show that MT detyrosination levels regulates the depolymerization activity of kinesin-13/MCAK, thereby altering the stability of astral MT. Consequently, the observed spindle positioning and spindle length phenotypes were largely rescued upon co-depletion of MCAK. Thus, our investigation deciphers the critical link between MT detyrosination levels and MT depolymerase activity in mitosis and provides molecular insights into the functionality of tubulin detyrosination cycle.

MS30

**RETROGRADE Transport Is Required for Mitochondrial Health and Function in Neurons**

**A. Mandal**, K. Pinter, C. M. Drerup; NIH/NICHD, Bethesda, MD.

Maintenance of functional neural circuits is critical for organism survival. To form and maintain the elaborate structure of a neuron, proteins and organelles must be transported to the correct location throughout the cell. One organelle of particular importance is mitochondria. Mitochondria produce ATP and regulate cytosolic calcium, among perhaps lesser known functions, critical for neuronal maintenance. Abnormalities in mitochondrial transport are associated with neurodegenerative diseases but the mechanisms leading to pathology are largely unknown. While we have a basic understanding of the molecular regulators of anterograde transport of mitochondria (axon terminal directed), the mechanisms regulating retrograde (cell body directed) transport and the actual function of this process were not clear. Furthermore, the dynamics of mitochondrial movement in neurons on timescales longer than minutes were completely unknown. To begin to address these long-standing questions, we used photoconversion and long-term tracking of mitochondria in vivo to assess the frequency of retrograde mitochondrial transport. Our results indicate that mitochondria in mature axons are largely stationary on the order of minutes but move long distances over the course of hours. Strikingly, mitochondria that originate in axon terminals utilize retrograde transport to return to the cell body from the distal axon with complete population turnover from axon terminals within three hours. Our data also illustrates that this process is not just for mitochondrial degradation: inhibition of retrograde mitochondrial movement leads to loss of cell body mitochondrial load. Using various fluorescent reporters, we found that organelles which cannot move back to the cell body show an imbalance of the oxidation/redox system, loss of matrix potential and impaired calcium buffering capacity in neurons. Finally, we provide evidence that retrograde mitochondrial transport back to the cell body is important for maintaining the mitochondrial proteome, likely by bringing the organelle back to the center of protein synthesis for the cell. Altogether, our work has shown the first direct evidence that retrograde transport is essential to maintain mitochondrial homeostasis in neurons.

## Microsymposium 6: Mitosis and Meiosis

MS31

**The DNA Repair Protein Nopo Has a Mitotic Function that Suppresses Neuronal Stress Response to Prevent Microcephaly**

**R. S. O'Neill**, C. J. Fagerstrom, N. M. Rusan; National Heart, Lung, and Blood Institute, NIH, Bethesda, MD.

Microcephaly is a reduction in brain size caused by a loss of neuronal proliferation and survival. Most genes linked to microcephaly primarily function at either the mitotic spindle or in DNA replication and repair (DRR) pathways, and thus microcephaly is thought to arise via one of two pathways. However, in many cases the precise roles of microcephaly-related genes in brain development have not been studied directly. One such gene is *TRAIP*, which is mutated in a severe form of microcephaly and is well studied for its roles in DRR. Accordingly, *TRAIP* microcephaly is thought to arise via delays in S-phase and accumulation of DNA damage. We characterized *nopo*, the *Drosophila* homolog of *TRAIP*, and found a novel mitotic function, thereby linking the two microcephaly pathways in a single gene. *nopo* mutants have microcephaly-like defects in a pair of brain structures called the mushroom bodies (MBs). MBs are

normally made of ~2000 neurons that arise from four neuroblasts (NBs); *nopo* mutant MBs have reduced size and neuron numbers. Contrary to its canonical role in DRR, we did not find evidence for increased DNA damage in *nopo* mutant MB neurons or NBs. Instead, the MB NBs of *nopo* mutants are often multi-nucleate, suggesting mitotic failure, and are progressively lost during development via caspase-dependent cell death. Consistent with its established DRR functions, Nopo has nuclear localization during interphase; however, it has dynamic localization in mitosis, streaming along mitotic spindles and concentrating at the cytokinetic furrow. Deleting the nuclear localization sequence (NLS) evicts Nopo from the nucleus during interphase, presumably abrogating any DRR functions; nonetheless, expressing Nopo lacking the NLS fully rescues the *nopo* mutant microcephaly-like phenotype. Together, these results reveal a novel mitotic function for *nopo* that is critical for neurogenesis, thus raising questions about the current model of *TRAIP* microcephaly and whether other microcephaly-related genes also play dual roles. Finally, by screening for downstream pathways in *nopo* mutants, we discovered that inhibiting the neuronal stress pathway MAP3K *wnd*/*DLK* suppresses microcephaly-like phenotypes. Thus, the *wnd*/*DLK* pathway is identified as a novel key factor and is a potential therapeutic target for minimizing neuron loss in microcephaly.

MS32

**The Mechanical Integrity of the Mammalian K-fiber and Its Molecular Origin.**

**M. A. Begley**[1], E. M. Davis[1], R. Ohi[2], M. W. Elting[1]; [1]North Carolina State University, Raleigh, NC, [2]University of Michigan, Ann Arbor, MI.

During mitosis, the mitotic spindle, a self-constructed microtubule-based machine, segregates chromosomes into two eventual daughter nuclei. Microtubule bundles that align and anchor chromosomes, called kinetochore-fibers (k-fibers), are vital for the structural and functional integrity of this spindle, yet the composition and dynamics of k-fibers are still not well-understood. To probe k-fibers in mammalian PtK2 cells, we perform both high-resolution single-molecule speckle fluorescence imaging and mechanical perturbation by targeted laser ablation. In searching for the identities of microtubule crosslinking proteins that mechanically support k-fiber integrity, we biochemically and genetically perturb candidate molecules in spindles, ablate k-fibers, and subsequently quantify the physical response of the k-fiber. While speckle microscopy experiments in unperturbed spindles demonstrate that k-fibers act as mechanically cohesive units that are held together along their lengths, laser ablation of k-fibers often results in the splaying of the remaining stubs. Together, these results suggest that forces holding k-fibers together are tuned to be of similar magnitude as other cellular forces. Following ablation, it has previously been shown that stub minus-ends are typically detected and pulled poleward, repairing the spindle. In spindles with reduced NuMA, a molecule that recruits dynein to microtubule minus-ends and mediates the post-ablation poleward transport, splaying is reduced, suggesting that when splaying occurs, it is may be because k-fibers are torn apart during poleward transport. Additionally, pharmacological inhibition of kinesin-12 Kif15 microtubule binding increases the frequency of post-ablation splaying, while inhibition of its motor activity but not its microtubule binding does not affect splaying, suggesting that Kif15 may fortify and stabilize k-fibers. Interestingly, inhibition of Kif15 also results in dramatically delayed reincorporation of ablated k-fibers, although the process by which Kif15 impacts spindle repair remains unclear.

MS33

**Anti-tropomyosin Drugs Prevent the Rescue of Vincristine-induced Mitotic Spindle Defects**

**P. W. Gunning**[1], Y. Wang[1], J. H. Stear[1], A. Swain[1], X. Xu[1], N. Bryce[1], I. B. Alieva[2], V. B. Dugina[2], T. Cripe[3], J. Stehn[1], E. C. Hardeman[1]; [1]University New South Wales, Sydney, AUSTRALIA, [2]Moscow State University, Moscow, RUSSIAN FEDERATION, [3]Nationwide Children's Hospital, Columbus, OH.

Drugs targeting a major component of the actin filaments of cancer cells, tropomyosin Tpm3.1, synergize with anti-microtubule drugs in neuroblastoma and lung cancer models both *in vitro* and *in vivo* and a wide range of other cancer types *in vitro*. We have determined the mechanism of synergy in HeLa cells to gain insight into the potential interaction of actin filaments and microtubules in the survival and proliferation of cancer cells. HeLa cells exhibit a strong synergistic response to the combined treatment of vincristine (VCR) and anti-Tpm3.1 compounds, marked by an enhanced reduction in cell viability, apoptosis induction and mitotic cell cycle arrest. Tpm3.1 localizes to the cell cortex during mitosis, potentially associating with the microtubule network, particularly the dynein/dynactin complexes responsible for mediating cortical pulling forces during spindle assembly. VCR alone causes supernumerary NuMA organized acentrosomal microtubule organizing centers upon nuclear envelope breakdown, which can be resolved via a clustering mechanism to achieve bipolar cell division. The addition of anti-Tpm3.1 compounds inhibits NuMA-associated clustering in VCR-treated cells, leading to irreparable defects during spindle assembly and thus a largely increased number of cells with multi-polar spindles undergoing mitotic delay and catastrophe. We conclude that actin/Tpm3.1 filaments contribute to the formation of the bipolar spindle and play a critical role in the clustering of acentrosomal microtubule asters.

MS34

**A Minimal Spindle-Midzone Protein Module Differentially Regulates Single Microtubules and Crosslinked Microtubule Arrays**

**N. Mani**, S. Jiang, R. Subramanian; Harvard Medical School / Massachusetts General Hospital, Boston, MA.

A remarkable feature of the microtubule cytoskeleton is the co-existence of distinct sub-populations with different stabilities in the cellular cytoplasm. A striking example is the spindle midzone, an array of antiparallel microtubule bundles that encodes positional cues for accurate cytokinesis in dividing cells. At the cell center, these stable bundles exist alongside dynamic microtubules that are thought to contribute to midzone organization. How do stable bundles and dynamic single microtubules coexist at the center of the dividing cell? Here we reconstitute a minimal system composed of the antiparallel microtubule crosslinker PRC1, and its binding partners Kif4A and CLASP1, midzone-associated proteins that suppress and promote microtubule growth respectively. We find that the activity of these proteins on dynamic microtubules results in a system with stable bundles and elongating single microtubules. Analysis of the protein-protein interactions reveals that two parameters are sufficient to explain the differential regulation of single and bundled microtubules: (i) higher microtubule affinity of CLASP1 over Kif4A and (ii) higher PRC1 affinity of Kif4A over CLASP1. Together with cell biological analyses, our findings provide new insights into how antagonistic regulators of microtubule dynamics can differentially regulate proximal subsets of microtubules for midzone assembly. More broadly, our findings illuminate the design principles that can confer different stabilities to microtubule sub-populations that coexist at the same subcellular site.

MS35

**Branching Microtubule Nucleation Is the Main Source of Microtubules Generated At Chromosomes in Meiotic *Xenopus* Egg Extracts**

**S. U. Setru**, J. W. Shaevitz, S. Petry; Princeton University, Princeton, NJ.

Microtubules are made in several places in the dividing eukaryotic cell to form the spindle. Though centrosomes are the most visually prominent hubs of microtubule nucleation in most cell types, microtubules also form at chromosomes during cell division. Indeed, chromosomal microtubules are necessary for assembling the entire spindle in cells that lack centrosomes, such as egg cells. Alongside centrosomes and chromosomes, microtubules themselves act as sites for generating new microtubules, a process referred to as branching microtubule nucleation. Chromosomal and branched microtubules are crucial for the proper alignment and segregation of chromosomes, and both rely on the GTPase RanGTP. RanGTP exists as a gradient centered around chromosomes and spurs the release of spindle assembly factors (SAFs). One of these SAFs is the protein TPX2, which stimulates branching microtubule nucleation together with the protein complex augmin and the γ-tubulin ring complex. It is currently not understood how microtubule nucleation around chromosomes is related to branching microtubule nucleation. In this study, we investigated the relationship between chromosomal and branching microtubule nucleation. To do so, we developed an assay to watch microtubule nucleation and growth at chromosomes *ex vivo*. We attached chromosomes purified from mitotic cells to the coverslip of a microscope flow chamber and then introduced *Xenopus* egg extract. Using TIRF microscopy, we visualized that chromosomes generate branched microtubule networks. This can even occur from a single microtubule whose lattice directly overlaps or touches chromatin. These experiments define the distances and times at which branching microtubule nucleation is stimulated around chromosomes. Notably, we saw that a single microtubule captured at the kinetochores can also serve as the mother microtubule upon which daughter microtubules will nucleate. This finding helps explain how a kinetochore fiber can be made efficiently. Finally, by depleting TPX2 and augmin from the extract, we demonstrated that branched microtubules are the chief source of the microtubules that grow from chromosomes. Taken together, our experiments show that branching microtubule nucleation mediated by TPX2 and augmin is the main source of microtubules generated at chromosomes and kinetochores in *Xenopus* egg extracts. We propose that branching microtubule nucleation is the main pathway initiated at chromosomes to assemble spindles and capture kinetochores.

MS36

**Identification of Novel Synaptonemal Complex Components in *C. Elegans***

**M. E. Hurlock**[1], I. Čavka[2], J. M. Haversat[1], M. Wooten[1], Z. Nizami[3], R. Turniansky[1], P. Hoess[2], J. Ries[2], J. G. Gall[3], S. Köhler[2], Y. Kim[1]; [1]Johns Hopkins University, Baltimore, MD, [2]The European Molecular Biology Laboratory, Heidelberg, GERMANY, [3]Department of Embryology, Carnegie Institution for Science, Baltimore, MD.

Proper chromosome segregation during meiosis requires that chromosomes pair and undergo crossover recombination with their homologs. In most eukaryotes, crossover formation depends on the assembly of the synaptonemal complex (SC), a zipper-like protein scaffold that forms between paired homologs. The SC is a tripartite protein structure consisting of two parallel stretches of lateral elements that are held together by a central element. The SC central region in *C. elegans* is known to be comprised of four coiled-coil domain proteins, SYP-1, SYP-2, SYP-3 and SYP-4. The four SYP proteins were identified more

than a decade ago, and it has been widely believed that they represent the entire set of SC components. Here we report the identification of two new SC components, SYP-5 and SYP-6. SYP-5 and SYP-6 localize along the SC between the two chromosome axes, and their recruitment depends on chromosome axes and other SC components. Using single-molecule localization microscopy, we demonstrate that SYP-5 and SYP-6 transverse the width of the SC in a head-to-head manner with the two N-termini at the center and the C-termini facing the chromosome axes. Interestingly, SYP-5 and SYP-6 are paralogous to each other and play redundant roles in synapsis, providing an explanation for why these proteins have not yet been identified by previous genetic screens. We further find that SYP-5 and SYP-6 contain stretches of low complexity amino acid sequences in their C-termini and that truncating these regions leads to aberrant synapsis and disruption of crossover control. Together, our findings establish SYP-5 and SYP-6 as *bona fide* components of the SC and will provide crucial insights into the mechanisms of SC assembly and its role in crossover regulation.

## Microsymposium 7: Cell Biology of the Nucleus

MS37

**Linc-ing the Nucleus and the Cytoskeleton**

**P. M. Davidson**[1], A. Battistella[1], T. Déjardin[1], T. Betz[2], J. Plastino[1], B. Cadot[3], N. Borghi[4], C. Sykes[1]; [1]Institut Curie, Paris, FRANCE, [2]Institute of Cell Biology, ZMBE, Munster, GERMANY, [3]Center for research in Myology, Paris, FRANCE, [4]Institut Jacques Monod, Paris, FRANCE.

Mammalian cells migrate through narrow pores in tissues to perform essential duties, including wide-scale migration throughout development, immune cell migration to sites of inflammation and fibroblast migration to repair wounds. The mechanisms by which cells exert forces on their nuclei to migrate through openings smaller than the nuclear diameter remain unclear. In microfluidic devices, the hourglass shape of the nucleus and its strain patterns as it translocates through narrow constrictions suggest pulling forces. Here we use CRISPR/Cas9 to label nesprin-2 giant, a protein that links the cytoskeleton to the interior of the nucleus. We demonstrate that nesprin-2 giant accumulates at the front of the nucleus during nuclear deformation through narrow constrictions, independently of the nuclear lamina. We show that nesprins are more mobile than lamin A/C, and hypothesize that the accumulation is due to nesprins that are pulled forward by the cytoskeleton. Using artificial constructs, we show that the actin-binding domain of nesprin-2 is necessary and sufficient to generate this accumulation, and that microtubules are not involved. We demonstrate a barrel shape of filamentous actin around the nucleus, which colocalizes with nesprin-2, but strikingly no actin at the front surface of the nucleus. Our working hypothesis is that this barrel structure pulls the nucleus forward via nesprin-2 and is responsible for pulling the nucleus forward in these fibroblasts. These results indicate important roles for nuclear envelope proteins in force transmission during cell migration through tissues.

MS38

**Increased Lamin B1 Levels Promote Cancer Cell Migration by Altering Perinuclear Actin Organization**

A. Fracchia, **G. Gerlitz**; Ariel University, Ariel, ISRAEL.

Cell migration requires active reposition and/or reshaping of the cell nucleus. The metazoan nucleus is engulfed by the nuclear envelope that is composed of two membranes, nuclear pore complexes that traverse them and an underlying mesh of filaments termed the nuclear lamina. The lamina is mainly

composed of type V intermediate filaments that include A-type and B-type lamins. Lamins are key structural components that affect the mechanical properties of the nucleus. As such, the nuclear lamina is highly important for migration of both primary and cancer cells. B-type lamins are important for proper migration of epicardial cells and neurons and increased lamin B to lamin A ratio was shown to accelerate cancer cell migration through confined spaces. In addition, positive association between lamin B1 expression levels and tumor formation and progression is found in various types of cancer including skin, prostate and breast. Still, the molecular mechanism by which B-type lamins promote tumor cell migration is not understood. To better understand the mechanism by which B-type lamins accelerate tumor cell migration we tested the effects of lamin B1 on perinuclear actin organization. Here we show that induction of melanoma cell migration leads to the formation of a cytosolic perinuclear actin rim, which is composed of actin filaments with slower dynamics than most other cytoplasmic actin filaments. This perinuclear actin rim has not been detected in migrating cells, yet. Interestingly, increasing the levels of lamin B1 but not the levels of lamin A prevents the formation of the perinuclear actin rim while accelerates the cellular migration rate. To verify if the perinuclear actin rim attenuates the cellular migration rate, we generated a chimeric protein that is localized to the outer nuclear membrane and contains an active form of the actin severing protein gelsolin. This tool enabled us to cleave perinuclear actin filaments in a specific manner without disrupting other cytosolic actin filaments. Using this tool we found that disruption of the perinuclear actin rim accelerates the cellular migration rate in a similar manner to lamin B1 over-expression. Taken together, our results suggest that increased lamin B1 levels accelerates cancer cell migration by inhibiting the association of the nuclear envelope with actin filaments that may reduce nuclear movement and deformability.

MS39

**Climp-63 Regulates Nuclear Movement During Polarization of Migrating Cells**

**C. S. Janota**, J. Costa, A. Pezzarossa, E. R. Gomes; Instituto de Medicina Molecular, Lisbon, PORTUGAL.

In our lab we are interested in understanding how cells position their nuclei in different biological contexts and the role for nuclear positioning. The connection between the cytoskeleton and the nucleuskeleton are crucial for most of these events, however how the endoplasmic reticulum (ER) is organized during cellular polarization remains to be elucidated. The ER is a dynamic organelle with three functional and morphological domains, the nuclear envelope, flat sheets and reticular tubules. Being the largest cell organelle, its morphology is also the most complex. Using migrating NIH 3T3 fibroblasts in a wounding assay where nuclei are repositioned away from the leading edge, we found that ER sheets accumulate at the perinuclear region, while ER tubules distribute along the leading edge upon nuclear movement. Moreover, by performing FIB-SEM we observed that nuclear positioning was correlated with bigger and more continuous ER sheets at the perinuclear region. Therefore we tested if ER morphology could regulate nuclear positioning in the wound assay. To this end, we depleted different ER proteins previously reported to be involved in ER morphology and found that depletion of Climp-63, a sheet-enriched ER transmembrane protein thought to be responsible for ER luminal spacing, prevents nuclear movement away from the leading edge. In addition, cells without Climp-63 failed to increase perinuclear ER accumulation during nuclear movement. Nuclear movement away from the leading edge is mediated by actin retrograde flow and actin dorsal cables attached to the dorsal side of the nucleus via TAN lines. We tested if depletion of Climp-63 had any effect on actin cytoskeleton, either on dorsal or ventral actin, and on TAN lines. We found that the speed of actin retrograde flow and the number of TAN lines upon Climp-63 depletion were not different from the control. Surprisingly, we found that the number of

ventral actin stress fibers under the nucleus increased. In the ventral side of the nucleus we observed that ER was wrapped around ventral actin cables. In the absence of Climp-63, the ER area and thickness in the ventral side of the nucleus was decreased and we observed less wrapping of the ER around the actin cables. Given these results, we propose that ER membrane might be necessary to wrap around actin cables under the nucleus to prevent the connection of ventral static actin cables to the nuclear envelope proteins and allow the nucleus to slide on top of immobile ventral actin cables.

MS40

**Macronuclear Shape Change in the Giant Ciliate, *Stentor Coeruleus***

**R. M. McGillivary**, P. Sood, W. F. Marshall; University of California, San Francisco, San Francisco, CA.

A fundamental question relating to cellular spatial patterning is how nuclei are shaped within cells. The giant ciliate *Stentor coeruleus* provides a unique opportunity to investigate the mechanisms behind nuclear shape, because *Stentor* takes this aspect of the nucleus to the extreme: *Stentor* can reach 1 mm in length, and it possesses a correspondingly gigantic macronucleus shaped like a string of spherical beads. During cell division, the macronucleus dramatically changes shape before dividing amitotically into the two daughter cells. The macronucleus condenses into single sphere, extends, and renodulates in 2-3 hours near the end of cell division. We can experimentally induce this macronuclear shape change by causing *Stentor* to regenerate its oral apparatus. The morphological events during regeneration are similar to cell division, including the cycle of shape changes performed by the macronucleus. It is unclear how this extreme macronuclear shape change is regulated. While microsurgical and electron microscopy studies addressed this question in the past, we have had virtually no molecular insight into this feat of subcellular morphogenesis.Our first molecular foothold into this question comes from an RNAi screen of genes differentially expressed during regeneration in *Stentor*. We found that a homolog of the nuclear exportin gene CSE1 was among the top differentially expressed genes during this process. In yeast, CSE1 is required to export importin-alpha from the nucleus. In humans it is reported that CSE1 also co-localizes with microtubules. We tested the function of this gene in *Stentor* using RNAi. Knockdown of CSE1 results in *Stentor* with clumped and irregularly shaped macronuclear nodes, suggesting CSE1 plays a role in elongation of the macronucleus. Immunofluorescence of CSE1 in *Stentor* results in punctate staining in the cytoplasm with no staining in the macronucleus during the early stages of cell division and regeneration. When the macronucleus is condensed, CSE1 puncta appear in the interior of the macronucleus. Previous electron microscopy studies have shown that the elongating macronucleus is surrounded by a transient microtubule mesh, and contains nuclear envelope-bound channels full of microtubules. Given CSE1's known roles in other systems, we are currently investigating whether CSE1 facilitates macronuclear elongation by interacting with these transient microtubule structures or by another mechanism involving nuclear transport.

MS41

**Nuclear Pore Complex Scaffold Experiences Conformational Changes in *Vivo in* Response to Transport State**

**J. Pulupa**, H. Prior, S. M. Simon; Rockefeller University, New York, NY.

Recent breakthroughs in structural studies have provided a wealth of information about the architecture of the nuclear pore complex (NPC). However, measuring the dynamics of nucleoporins (Nups) *in vivo* remains highly challenging. By using polarized-total internal reflection fluorescence microscopy (pol-

TIRFM), we have measured the orientations and dynamics of Nup-mEGFP fusion proteins *in vivo.* Our measurements have shown that components of the NPC scaffold assume distinct conformational states under various transport conditions.We developed imaging tools that allow us to visualize protein dynamics within diffraction-limited puncta *in vivo.* By rapidly switching the polarization of our excitation light between two orthogonal polarizations, we can measure the orientation of mEGFP. We conjugated mEGFP to several Nups via rigid alpha helical linkers and used the orientation of the mEGFP to report on the orientation of the Nups of interest.Using this technique, we measured the orientations of domains within NUP133, NUP54, NUP93, and NUP58. After confirming that the mEGFP reports on the orientation of our Nup of interest, we then used these conformational sensors to measure the orientations and dynamics of Nups within different parts of the NPC.Using CRISPR/Cas9, we replaced all endogenous copies of the Nups with our mEGFP-Nup fusion proteins and were able to confirm that the NPCs were functional *in vivo*. Under conditions of cargo depletion, the NPC assumed one distinct conformation. Under conditions of excess cargo, the NPC was driven into an alternate state. We modulated the transport state in a number of ways: starving cells, using drugs to block export, using dominant negative proteins, and finally by introducing nuclear transport factors directly to the nuclear periphery by permeabilizing the plasma membrane while leaving the nucleus intact. Under each of these perturbations, we found that most regions of the NPC do not shift their orientation in response to cargo, but specific domains of the inner ring complex are reorganized with altered *in vivo* transport state.Our results suggest that the NPC experiences a large-scale conformational change in response to cargo translocation. In addition, our results demonstrate how fluorescence polarization microscopy is a powerful tool for monitoring the organization and dynamics of proteins *in vivo* and *in vitro*.

MS42

**Mechanosensitive Phospho-dependent Recruitment of Baf to the Nuclear Membrane Inhibits Nuclear Accumulation of E2f1 and Yorkie**

**T. Volk, 76100**, U. CP, A. Reuveny, D. Grunbaum; Weizmann Institute of Science, Rehovot, ISRAEL.

Mechanotransduction has been implicated as an important factor in regulating cell cycle progression, however the underlying mechanism has not been fully elucidated. Here we describe a novel mechano-sensitive component, namely “Barrier to Autointegration Factor” (BAF), which regulates DNA replication (endoreplication) in *Drosophila* muscle fibers. BAF is a small protein shown previously to regulate dsDNA assembly post mitotically, and nuclear membrane ruptures. We show that BAF negatively regulates DNA endoreplication in fully differentiated muscle fibers through inhibition of the nuclear accumulation of E2F1 and Yap/Yorkie, two key components in cell cycle control. Furthermore, BAF localization at the nuclear membrane was found to be mechanosensitive, as it is downregulated in LINC mutants, as well as, following disruption of nucleo-sarcomeres connections. Knockdown of BAF in muscles fibers led to increased levels of DNA in myonuclei, and elevated nuclear levels of E2F1 and Yap/Yorkie. BAF forms a protein complex with E2F1, which depends on BAF phosphorylation. Knockdown of BAF kinase VRK1/Ball disrupts localization of BAF at the nuclear membrane. Taken together, our results reveal a novel mechanosensitive pathway controlling BAF phosphorylation and localization at the nuclear membrane, which in turn, represses nuclear accumulation of positive cell cycle regulators.

## Microsymposium 8: Cell Migration in Development and Cancer

MS43

**Single Cell Dynamics Driving Vertebrate Heart Valve Interstitial Layer Morphogenesis**

**F. Gunawan**, A. Gentile, S. Gauvrit, D. Stainier, A. Bensimon-Brito; Max Planck Institute for Heart and Lung Research, Bad Nauheim, GERMANY.

Heart valves are critical structures that close the cardiac lumen to prevent retrograde blood flow. Within the valve, specialized cells called valve interstitial cells (VICs) secrete extracellular matrix (ECM) that confers biomechanical strength to withstand blood flow. Considering that many hereditary congenital heart valve defects in humans arise due to aberrant VIC formation, it is crucial to better understand the mechanisms driving this process.As VICs arise later in development, the *in vivo* dynamics that drive their morphogenesis have not been studied in live animals or at single cell resolution. Using the zebrafish atrioventricular (AV) heart valve, we provide the first detailed analysis of the cellular processes that lead to the establishment of the VIC layer. Initially, a group of endocardial cells (ECs) at the AV canal collectively migrate to establish pre-valvular leaflets at the embryonic stage. Some of these cells then undergo endothelial-to-mesenchymal transition (EMT) and invade the space between two EC monolayers to become the first VICs. Lineage tracing shows that VIC establishment also comprises a smaller contribution from neural crest cells, which persist throughout development. Mature VIC establishment further includes a peak of cell proliferation at the juvenile stage, and a progression of ECM components from a pro-migratory to an elastic profile.Furthermore, the molecular regulation of VIC establishment has been poorly understood. We establish and analyze the first zebrafish model with defective VIC development: mutations in the gene encoding the NFκB-related transcription factor Nfatc1 lead to significantly fewer VICs, causing valve malformations and retrograde blood flow. *nfatc1* mutants exhibit reduced VIC invasion and proliferation, as well as defects in ECM deposition. Interestingly, similar to symptoms in patients with heart valve defects, *nfatc1* mutants can survive to adulthood, but display severe morphological and functional cardiac defects. We found that Nfatc1 functions at least in part by regulating the expression of one of the EMT-promoting factors, Twist1, suggesting a complex transcriptional network that drives VIC invasion.In summary, our study uncovers the single cell dynamics and molecular signatures underlying VIC morphogenesis. With this unique animal model, we propose to determine the gene regulatory networks that mediate the invasive and proliferative behaviours of VICs.

MS44

**Anisotropic Cues Promote Symmetry Breaking to Initiate Migration of Adherent Cells**

**D. Li**, Y. Wang; Carnegie Mellon University, Pittsburgh, PA.

Symmetry breaking is the transition of cells from a symmetric to an asymmetric state. After symmetric spreading of adherent cells, symmetry breaking generates regions of protrusion and retraction, enabling cells to develop polarity and initiate migration. It is unclear if the timing of symmetry breaking is predominantly controlled by an internal timing mechanism, or whether it is responsive to external anisotropic cues such as confinement, substrate adhesiveness, and cell-cell contact. While these cues are known to affect the behaviors of post-symmetry breaking cells such as migration, their effects on symmetry breaking are less explored. By manipulating culture conditions or micropatterning the substrate, we show that NRK-52E epithelial cells and NIH 3T3 fibroblasts break symmetry at faster rates in response to anisotropic cues including cell-cell contacts, cell-substrate adhesions, and substrate

stiffness. These cues may be located tens of microns away from the cell border. Furthermore, symmetry breaking may be promoted by asymmetric stimulation of either protrusion or retraction, with the former being more stimulative than the latter. Using pharmacological agents, we show that the promotion of symmetry breaking by anisotropic cues is dependent on the formin pathway, suggesting involvement of formin-dependent filopodia in probing the environment. While it is difficult to rule out an internal timing mechanism for symmetry breaking, our results suggest that mechanotransductive cues that regulate directed cell migration also provide effective stimulations for symmetry breaking, and that filopodia may play a central role of sensing for regulating both initial polarization and steady state migration.

MS45

**Mechanically Driven Cellular Competition Promotes the Collective Extrusion of Bacteria-infected Epithelial Cells**

**E. E. Bastounis**[1], P. Rhadhakrishnan[1], P. Engström[2], F. Alcalde[3], M. J. Gómez Benito[3], J. M. García Aznar[3], M. D. Welch[2], J. A. Theriot[1]; [1]University of Washington, Seattle, WA, [2]University of California, Berkeley, Berkeley, CA, [3]University of Zaragoza, Zaragoza, SPAIN.

Intracellular bacterial pathogens often alter the behavior of their host cells, including their mechanics, to promote their own survival and spread. For instance, food-borne *Listeria monocytogenes* secretes effector proteins that loosen the cortical tension of infected epithelial cells facilitating its spread from cell to cell or through basement membranes. What is less clear is whether host cells are capable of changing their biomechanics in response to infection in a way that would benefit the host by limiting bacterial spread. Inspired by this question, we followed Madin-Darby Canine Kidney epithelial cell monolayers infected with low levels of *L. monocytogenes*over the course of 3 days. We found that, as the bacteria replicate and spread intercellularly, the host cells containing bacteria get progressively squeezed by surrounding uninfected cells. Surrounding uninfected cells become highly migratory, elongate perpendicular to the tangent of the focus, and squeeze the infected cells that eventually get extruded. Extruded cells continue adhering to the cellular monolayer giving rise to “mounds” of infected cells. We hypothesized that cellular compression and extrusion of infected cells is driven by changes in the mechanics of uninfected and infected cells. Indeed, when we measured the traction forces that cells exert while residing on deformable matrices mimicking their natural environment, we found that infected cells weaken their cell-substrate adhesion-dependent forces over time as compared to nearby uninfected cells. Concurrently, infected cells get softer than surrounding uninfected cells, and collectively the monolayer behaves more like a fluid with a higher diffusion coefficient as compared to more solid-like cells from an uninfected monolayer, a behavior reminiscent of the jamming-unjamming transition. We are using 3D agent-based modeling to model infection-driven cellular extrusion in order to understand how changes in cellular stiffness, cell-matrix and cell-cell forces alone and synergistically can drive the observed cellular competition between infected and uninfected cells. The goal is to uncover how the mechanical forcesthat lead tothe creation of extrusion domains of infected cells are coordinated with the underlying biochemical changes associated with infection, and in particular with innate immune defense pathways and NF-kB activation. Overall, our findings underline the dynamic remodeling capability of epithelial tissue and might hint at a potential mechanism employed by host epithelial monolayers to contribute to elimination of infected host cells.

MS46

**Tissue Stiffness Modulates Extracellular Vesicle Function in Metastatic Breast Cancer**

**A. Sneider**[1], Y. Liu[2], C. Marar[1], N. Faqih[1], G. Ciotti[2], J. Kim[1], M. Igboko[1], S. Ibrahim[3], S. Krishnan[4], A. Locke[5], B. Starich[1], G. Russo[1], M. Karl[1], P. Nair[1], R. Vij[1], E. Gomez-de-Mariscal[6], D. Lewis[1], A. Munoz-Barrutia[6], L. Gu[1], T. Eisinger[2], D. Wirtz[1]; [1]Johns Hopkins University, Baltimore, MD, [2]University of Pennsylvania, Philadelphia, PA, [3]Pacific Lutheran University, Tacoma, WA, [4]Harker School, San Jose, CA, [5]Lamar University, Beaumont, TX, [6]Universidad Carlos III de Madrid, Madrid, SPAIN.

**Introduction:** Metastasis accounts for 90% of cancer related deaths; however, few clinical therapies exist to target the metastatic cascade. Transformation of the tumor microenvironment is a critical step in this process. Enhanced extracellular matrix (ECM) stiffness in collagen-rich environments correlates with poor prognosis in solid-tumor cancers by promoting cell polarization, migration, and invasion. Recently, we learned that tumor-derived extracellular vesicles (EVs) transform the local ECM and promote tumorigenesis. Our current work has revealed that investigating the role of EVs using traditional tissue culture approaches is insufficient. Collecting EVs from cells cultured on plastic removes aspects of the *in vivo* tumor environment that influence cell behavior. Our research explores how physiological tissue stiffness affects the generation, molecular cargo, and functionality of EVs. We also show that EVs produced by tumor cells at physiological stiffnesses modulate migration and tropism of disseminating tumor cells in mouse and zebrafish models. EVs from primary patient tissue reveal the translational capacity of our work. **Methods:** EVs are isolated by centrifugation and filtration. Nanoparticle tracking, electron microscopy, western blots and proteomics confirm size, concentration, morphology, and protein expression. Murine studies show vesicle biodistribution and tissue tropism. We developed a novel tri-color zebrafish xenograft model to simultaneously visualize EVs and cancer cells as they navigate zebrafish vasculature. **Results:** We observed that EV secretion number and protein expression depends on ECM stiffness. Moreover, breast cancer cell line-derived EVs, produced on ECM at the physiologically relevant stiffness in breast tumors, are able to modulate vesicle biodistribution and tissue tropism in mice. These EVs are retained in the lung, liver, and spleen longer than those generated on soft ECM. Using our zebrafish model, we observed that stiff ECM vesicles stimulate tumor cell migration more effectively than those from soft ECM. Primary patient samples show that our model of soft and stiff ECM approximates normal and tumor tissue respectively, and that protein expression from isolated vesicles supports observed trends. **Conclusions:** ECM stiffness significantly affects the quantity and quality of vesicles produced by breast cancer cells, which contributes to metastasis through increased cell migration and extravasation.

MS47

**Cellular Herding: Programming Collective Cell Migration through Electrotaxis**

**D. Cohen**; Princeton University, Princeton, NJ.

Collective cell migration, arising from the complex choreography between cell-substrate and cell-cell forces, ultimately underlies key processes in morphogenesis, healing, and even pathogenesis. Given such importance, the ability to interactively 'herd' collective migration would offer exciting new possibilities in the study and control of multicellular systems. Accomplishing such control requires a broadly applicable, programmable, and powerful cue capable of directing migration. Electrotaxis—the directed migration of cells along DC electric field gradients—is just such a cue. In vivo, endogenous electric fields on the order of 1 V/cm arise due to ion imbalances such as occur during wounding, and

simulating such fields in vitro is known to direct cell migration. A major thrust in our lab is to better understand and harness electrotaxis to control collective migration. Recently, we have developed a new style of electro-bioreactor incorporating a multielectrode array that allows us to dynamically program patterns of collective migration within living tissue samples. Here, we present data on how electrotaxis can be used not only to precisely set the speed of large-scale collective migration in monolayers (up to a 4X increase), but also to literally program sequences of collective motion such as 'move forward; move right; move left." Intriguingly, electrotaxis and 'driven migration' carry pronounced mechanical consequences due to heterogeneities in cellular responses that result in pronounced gradients of cell density and cell area. We hypothesize these gradients reflect large-scale shifts in cell-cell tension and traction forces, which is supported by micropatterning assays. Further, the nature of the electrotactic response appears to be modulated by the strength of cell-cell adhesion; a fact we leverage to tune the stimulation conditions to induce millimeter-scale, 'supracellular' migration of large monolayers of primary keratinocytes. These data indicate the exciting potential of electrotaxis both as a phenomenon in its own right and as a powerful approach to steer collective cell migration and dynamically alter mechanics in cellular ensembles.

MS48

**Single-Cell RNA-Sequencing of Lung Cancer Leader and Follower Cells Reveals Distinct Mutational Profiles and Cancer Stem Cell-Like Gene Expression Patterns**

**B. Pedro**[1], J. M. Konen[2], M. Rupji[3], B. Dwivedi[3], J. Kowalski[4], P. M. Vertino[5], A. I. Marcus[1]; [1]Emory University, Atlanta, GA, [2]University of Texas MD Anderson Cancer Center, Houston, TX, [3]Winship Cancer Institute, Atlanta, GA, [4]The University of Texas at Austin, Austin, TX, [5]University of Rochester Medical Center, Rochester, NY.

Previous work has demonstrated heterogeneity within collectively invading packs of lung cancer cells, including leader and follower cells that cooperate to facilitate invasion into the microenvironment. To better characterize the genetic differences between these two distinct cell types, we performed RNA-seq on purified populations of leader and follower cells from the H1299 non-small cell lung cancer cell line. Through this analysis, we identified the first known compilation of leader- and follower-enriched genetic mutations, including 17 point mutations found uniquely in leaders and 18 point mutations found uniquely in followers. To further evaluate whether these mutational profiles correlated with distinct gene expression profiles on the single-cell level, we performed single-cell RNA-seq on H1299 parental, leader and follower cells isolated directly from collectively invading packs in a 3-D matrix. Hierarchical clustering and tSNE analysis of 105 single cells, based upon the most variably expressed 1,155 genes, revealed four distinct cell clusters; two expressing higher levels of leader cell genes including *MYO10*, (clusters 1 and 4), and two expressing higher levels of follower genes including *IL13RA2* (clusters 2 and 3). Interestingly, clusters 1 and 4 are composed exclusively of cells with leader mutational profiles, while nearly all cells in clusters 2 and 3 contain follower mutational profiles, suggesting that these mutations could be involved in driving the differential gene expression, and ultimately the unique biological behaviors, of leaders and followers. In addition, clusters 1 and 2 display higher levels of proliferative markers including Ki-67, suggesting the existence of proliferative and non-proliferative subpopulations within the leader and follower populations. Furthermore, we found cells with leader mutational profiles to have cancer stem cell-like gene expression patterns, including upregulation of *BMP6*, *ID2*, *ID3*, *ID4*, *ALDH1A3*, *SOX9*, and *CTCFL*, and increases in the Wnt, Notch, and angiogenesis signaling pathways. Taken together, these data give us new insight into the layers of heterogeneity that exist within an

invasive tumor cell population, suggest novel mechanisms of leader and follower cell cooperation in collective invasion, and open the door to new potential strategies for targeting and inhibiting metastasis in human lung cancer.

## Microsymposium 9: Cytoskeletal Regulation of Cell Migration

MS49

**Leveraging Population-Level Intercellular Variation of 2D Cell Shape for Mechanistic Insight Into Cell Migration**

**R. D. Labitigan**[1,2], A. L. Sanborn[1], C. V. Hao[1], J. A. Theriot[2]; [1]Stanford University, Stanford, CA, [2]University of Washington, Seattle, WA.

Cell shape is a large-scale manifestation of the overall physical and functional state of a cell's cytoskeleton and other structural elements. Dynamic control of cell shape is essential for diverse cell types ranging from neurons to single-celled amoeba. For some well-characterized motile cell types (such as human immune cells and fish skin cells), it has been shown that control of a cell's shape is inherently linked to control of its motile behavior. Understanding the variation in a cell's shape, both through time and across a population of cells, can thus shed light on the molecular mechanisms involved in cell shape determination and motility.We have developed an approach to identify the most important axes of 2D cell shape variation in a population of thousands of fixed cells from a single imaging sample. In contrast to using simple handpicked summary metrics (such as cell area or circularity), our approach uses principal component analysis (PCA) to provide an unbiased, compact representation of cell shape, while maintaining the rich information embedded in the population's cell-to-cell variation. Furthermore, this allows quantitative, biologically-interpretable comparisons upon pharmacologic or genetic perturbation and sensitive detection of differences that would otherwise be obscured using simple metrics.This approach has enabled us to precisely quantify cell shape changes upon systematic perturbation of the actin cytoskeleton and other structural components like organelles. As a case study, we have mapped the shape space of human osteosarcoma U2OS cells (a motile cell type that forms dynamic cell shapes) and then quantified the effect of Rho kinase (ROCK) inhibitor Y-27632 on cell shape. Although individual Y-27632-treated, fixed cells do not show any obviously aberrant cell shapes relative to control cells, a population analysis reveals a striking change in their shape distribution. Upon Y-27632 treatment, U2OS cells exhibit a dose-dependent shift toward more polarized, crescent-shaped morphologies, and a wider range of left-right asymmetry. Along with live cell imaging, this population-level shape analysis of fixed U2OS cells suggests that ROCK inhibition may enable (1) higher migration speeds via enhanced front-back polarization and (2) more robust whole-cell turning via enhanced left-right asymmetry, pointing toward a role for ROCK signaling in limiting U2OS cells' intrinsic migratory behavior.

MS50

**Osteoporosis Mutations Lead to Impaired Calcium Regulation of Actin Bundling by Plastin-3**

E. Kudryashova[1], C. Schwebach[1], W. Zheng[2], M. Orchard[1], L. Runyan[1], H. Smith[1], E. Egelman[2], **D. Kudryashov**[1]; [1]Ohio State University, Columbus, OH, [2]University of Virginia, Charlottesville, VA.

Mutations in actin-bundling protein plastin 3 (PLS3) emerged as a cause of osteoporosis, but the underlying mechanisms are unknown. Of the five tested in this study osteoporosis PLS3 mutations not associated with gene deletions/truncations, one distorts an actin-binding loop and abolishes F-actin

bundling as revealed by cryo-EM and protein interaction assays. The remaining four mutants retained the bundling ability but showed either decreased or increased sensitivity to $Ca^{2+}$ in reconstituted in vitro assays. Not being located in the regulatory calcium-binding domain, the loops harboring the mutations are protected by the regulatory domain, revealing for the first time its position at the interface of the actin-binding domains and suggesting the mechanism of plastin activity inhibition. In cells, wild-type PLS3 was distributed between lamellipodia and focal adhesions. In contrast, the $Ca^{2+}$-hypersensitive PLS3 mutants were restricted to lamellipodia, albeit their localization at focal adhesions could be partially rescued upon chelation of extracellular $Ca^{2+}$ by EGTA. Accordingly, the $Ca^{2+}$-hyposensitive mutants were not found at the leading edge but localized exclusively at focal adhesions/stress fibers, which displayed reinforced morphology. These findings suggest that a mutational disruption of actin bundling or the $Ca^{2+}$-controlled PLS3's cycling between adhesion complexes and the leading edge leads to severe osteoporosis similar to that caused by the PLS3 deletions. To our knowledge, this study is the first direct evidence that the $Ca^{2+}$-dependent regulation of plastins is essential for their physiological functions.

MS51

**Role of the Actin Severing Factors, ADF/cofilin and Gelsolin, in Regulating the Fast Amoeboid or Leader Bleb-based Migration of Cancer Cells**

**M. F. Ullo**, J. S. Logue; Albany Medical College, Albany, NY.

The cortical actomyosin cytoskeleton lies directly underneath the plasma membrane of all cells. In cancer cells, a high level of cortical actomyosin contractility is correlated with invasiveness. Recently, it was discovered that cancer cells can undergo fast amoeboid or Leader Bleb-Based Migration (LBBM), which requires the formation of a large and stable bleb for migration. Contained within all leader blebs, is a fast cortical actomyosin flow driving the cell forward, however the fundamental mechanisms required to maintain contractility and actin flow in leader blebs is not understood. Here, we hypothesize that actin severing factors, such as the ADF/cofilin family and gelsolin, are essential to maintain both contractility and actin flow for LBBM. Using RNAi in melanoma A375-M2 cells and a flow cytometry-based method for measuring F-actin, we find that co-depleting ADF and cofilin and not gelsolin led to a large increase in the level of F-actin, suggesting that ADF and cofilin together regulate actin in these cells. Moreover, using barbed-end assays and high-resolution imaging, RNAi of ADF and cofilin increased the number of cortical, polymerization competent, barbed-ends. Therefore, severing by these proteins appears to promote cortical actin turnover in melanoma A375-M2 cells. Furthermore, actin severing has been shown to promote contractility through the regulation of actin architecture. In line with this concept, RNAi of ADF and cofilin significantly increased cell deformability, as determined by a gel sandwich approach. As LBBM is stimulated by cell confinement, we next used a PDMS slab-based approach, which uses micron-sized beads for the precise control of cell confinement, to evaluate the role of ADF/cofilin in regulating cortical actin dynamics in blebbing cells. Ratio imaging of EGFP-cofilin and mScarlet revealed cofilin to be enriched within leader blebs, whereas RNAi of ADF and cofilin reduced bleb sizes and the frequency of motile cells. Strikingly, many blebs failed to retract in the absence of ADF and cofilin, these cells had exceptionally long necks separating blebs and the cell body. Therefore, actin severing by these proteins appears to be critical to the function of the contractile bleb neck. Collectively, our data identifies ADF and cofilin as cortical actin remodeling factors required for the amoeboid migration of metastatic cancer cells.

MS52

**Cell Matrix Invasion Requires Non-Muscle Myosin 2A/B Polarity and Nuclear-force Coupling Generated From Adhesion Sites**

**L. E. Young**[1], T. Newman[1], T. Waring[1], E. Madondald[1], P. Caswell[2], L. Machesky[3], M. Morgan[1], T. Zech[1]; [1]University of Liverpool, Liverpool, UNITED KINGDOM, [2]University of Manchester, Manchester, UNITED KINGDOM, [3]Beatson Insitute, Glasgow, UNITED KINGDOM.

Dynamic cell polarisation is observed during cell invasion of 3D matrices. In this study we set out to identify the parameters required for invasive cell migration in 3D environments. Transmembrane-spanning integrin adhesion complexes (IACs) allow the cells to respond to changes in the extracellular environment. IACs are well characterised in cells plated on 2D surfaces, yet much less is known about them in cells embedded in 3D matrices.We establish a BioID-based technique to compare the composition of IACs of invasive breast cancer cells in 2D versus 3D matrices and measure 3D IAC dynamics of endogenous (knock-in) proteins. We identify a novel interaction complex consisting of N-WASP/WIP -> β-PIX -> Myosin-18A (M18A), which is enriched in 3D adhesive sites. Depletion of β-PIX or M18A abolishes cancer cell invasion, without negatively affecting matrix degradation or protrusion formation in collagen matrices. Instead, β-PIX/M18A drive the polarised recruitment of non-muscle Myosin 2A (NM2A) to the tips of protrusions. This recruitment of NM2A is required for the creation of a NM2A/B gradient, which assembles from protrusion to the nucleus. A NM2A/B gradient will provide the actomyosin network with differing catalytic properties of the NM2 isoforms. NM2A is known to have faster motor activity than NM2B, whereas NM2B has a higher affinity for ADP, reflecting longer high affinity attachment to actin filaments. The NM2A/B gradient couples protrusion assembly with nuclear movement, enabling effective invasive migration and polarisation. These findings suggest that actomyosin mediated nuclear-force coupling through a NM2 isoform gradient is a crucial mechanism required for invasive cell migration through 3D matrix.

MS53

**Uncovering Microtubule-driven Mechanisms of Melanoma Invasion**

**R. J. Ju**[1], K. M. Dean[2], R. Fiolka[2,3], Y. Chhabra[1], G. Danuser[2,3], N. K. Haass[1], S. J. Stehbens[1]; [1]University of Queensland, Brisbane, AUSTRALIA, [2]Department of Cell Biology, UT Southwestern Medical Center, Dallas, TX, [3]Lyda Hill Department of Bioinformatics, University of Texas Southwestern Medical Centre, Dallas, TX.

Metastatic melanoma is currently incurable and available therapies, although effective, result in resistance and recurrence. The majority of deaths are due to metastatic disease, highlighting the need for ‘migrastatics’, therapeutics which act to inhibit invasion. A recent paradigm shift positions the extracellular matrix as a key player in the metastatic cascade. Cell navigation of 3D matrix requires adaptive changes in cell and nuclear shape to fit matrix physical attributes in a process termed mechanosensing. This process incorporates dynamic remodelling of cell matrix adhesions and the cytoskeleton, to facilitate movement through confined spaces, via proteolytic matrix degradation or cell squeezing. Microtubules play a pivotal role in both of these processes. Our data show that the microtubule-binding proteins, CLASPs, are highly overexpressed in metastatic melanoma lines where they regulate the resistance of microtubule mechanical compression during melanoma invasion in 3D collagen matrices. Using high-resolution live-cell microscopy coupled to genetic alteration and substrate microfabrication, we have identified that patient-derived Melanoma cells utilise CLASP1 and CLASP2, for

differing functions to drive 3D invasion. We report paralog specific depletion of CLASPs results in strikingly different 3D invasion phenotypes. Crucially, paralog specific depletion of CLASPs ablates the ability to inter-convert between adaptive invasion strategies by interfering with microtubule-dependent functions during 3D-invasion. Furthermore, pan-depletion of CLASPs within 1205Lu melanoma cells results in 3D migration stasis and reduced cell viability following conditions of 3D confinement, which we do not observe in 2D. These findings suggest that CLASPs function in melanoma cells to facilitate biomechanically regulated cellular processes of both invasion and survival in confined environments.

MS54

**Microtubule Acetylation Promotes Rigidity Sensing and Mechanosensitive Migration of Astrocytes**

**S. Seetharaman**[1], B. Vianay[2], V. Roca[1], S. Etienne-Manneville[1]; [1]Institut Pasteur, Paris, FRANCE, [2]Paris University, INSERM, CEA, Hôpital Saint Louis, Institut Universitaire d'Hematologie, Paris, FRANCE.

Cell adhesion to the extracellular matrix occurs through integrin-mediated focal adhesions, which sense the mechanical properties of the substrate and impact cellular functions such as cell migration. Mechanotransduction at focal adhesions affects actin dynamics resulting in migration of cells. Although microtubules are key players in cell adhesion and migration, their role in mechanotransduction still remains unstudied. Using a combination of microfabrication methods, biophysical approaches and imaging techniques such as hydrogel patterning, traction force microscopy and electron microscopy, we show that substrate rigidity affects microtubule acetylation through $\beta_1$ integrin signalling in astrocytes but not in glioblastoma cells. Moreover, αTAT1, the enzyme responsible for microtubule acetylation, is required for the mechanosensitive migration of astrocytes. αTAT1 also reorganizes the actomyosin network, increases traction force generation and promotes cell migration on stiff substrates. Our results suggest a novel feedback mechanism involving a crosstalk between microtubules and actin in mechanotransduction at focal adhesions whereby, cells sense the rigidity of the substrate through integrin-mediated adhesions, modulate their levels of microtubule acetylation, which then controls the actomyosin cytoskeleton, force transmission on the substrate and promotes cell migration.

## Microsymposium 10: Lipid Trafficking, Organelles & Their Interactions

MS55

**Phase-partitioning of Vacuole Membrane Directs Inter-organellar Sterol Trafficking to Coordinate Mitochondrial Development and Energy Metabolism with Macro-autophagy**

**A. Y. Seo**[1], F. Sarkleti[1], I. Budin[2], J. Lippincott-Schwartz[1]; [1]HHMI-Janelia Research Campus, Ashburn, VA, [2]University of California San Diego, San Diego, CA.

Cellular ability to switch from aerobic fermentation to mitochondrial oxidative phosphorylation is the key underlying survival mechanism for many organisms during glucose starvation. However, how cells drive this metabolic transition remains unclear. Using a yeast model system, we demonstrate that to sustain mitochondrial respiration, by which lipid is now utilized as the major energy source, starved cells activate a distinct inter-organellar lipid pipeline to mitochondria from vacuole/lysosome via endoplasmic reticulum (ER). We find that macro-autophagy initiates this new lipid route by rapidly relocating endosomal membrane compartments to the vacuole. This prompted vacuole membrane phase separation, generating ergosterol-enriched liquid-ordered membrane-like (Lo) domains. Through the Lo domain, vacuole-ER contacts formed, and vacuolar ergosterols cycled back to ER for mitochondria and

lipid droplet (LD) developments. With a control of vacuolar ergosterol pools by LD sequestration of excessive sterol lipids, the Lo domains further differentiated, and vacuolar LD recruitment and digestion began to work in play for recycling of LD-stored fat molecules. Our findings that the Lo differentiation occurs only when mitochondrial respiration promotes macro-autophagy suggested a feedback interplay between mitochondria and vacuole during the remobilization of the needed substitute energy source from LDs. Taken together, our results reveal an unknown organellar mechanism that mediates metabolic reprogramming in glucose-starved cells, and highlight the importance of inter-organellar communications for cell and organism's survival under metabolic stress.

MS56

**Mitochondrial Fragmentation as a Mechanism for Localized Signaling**

**A. Horn**, S. Raavicharla, S. Shah, J. K. Jaiswal; Children's National Health System, Washington, DC.

Plasma membrane damage can result in a lethal influx of calcium leading to cell death. To survive, cells must generate a polarized response directed to the injury site that results in successful repair of the wounded plasma membrane. We recently demonstrated that mitochondria are needed for signaling that facilitates plasma membrane repair. This involves rapid uptake of calcium followed by local redox signaling, which subsequently activates the repair response. Mitochondria exist throughout the cell as part of a dynamic network, with constant fission and fusion regulating organelle communication, quality control, and function. However, the mechanism that allows this global network of interconnected mitochondria to generate spatially restricted redox signaling in response to plasma membrane injury is not understood. Here, we show by live cell imaging that rapid, Drp1-mediated fragmentation of mitochondria proximal to the injury site regulates local signaling. Fragmentation modulates the overall calcium level in mitochondria such that calcium is highest and sustained longest in the fragmented mitochondria. This allows injury-proximal mitochondria to produce locally elevated levels of redox signaling required for repair. In contrast, mitochondria further from the injury site do not fragment and are able to distribute calcium throughout the mitochondrial network, reducing their capacity for redox signaling. Complete lack of mitochondrial fragmentation after injury, achieved by genetic knockout of DRP1, compromises the polarized calcium uptake and signaling response, resulting in poor plasma membrane repair. While commonly considered to be an indicator of cell damage and death, our findings identify that mitochondrial fragmentation is a mechanism by which the global mitochondrial network can generate localized signaling. These results identify a novel function of Drp1-mediated mitochondrial fragmentation.

MS57

**Spatial Organization of Mitochondrial Inner Membrane Subdomains**

**K. Subramanian**[1], S. Barger[2], A. Guna[3], J. Nunnari[1]; [1]University of California, Davis, Davis, CA, [2]SUNY Upstate Medical University, Syracuse, NY, [3]MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM.

The mitochondrial inner membrane is an essential platform to perform and integrate key cellular pathways ranging from oxidative phosphorylation to mitochondrial genome replication and expression. As such, the inner membrane is protein-dense and highly organized into at least three morphologically and compositionally distinct domains: cristae, cristae junctions, and boundary regions. Based on an analysis of a small fraction of the inner membrane proteome, it is thought that the electron transport

and respiratory complex assembly machinery are distinctively localized to cristae while in contrast, solute transporters and protein import machinery are enriched in boundary regions. To gain insight into the mechanisms underlying the domain sorting of inner membrane proteins, we systematically determined the subdomain localization of a majority of inner membrane-associated proteins in budding yeast. We resolved the domain localization of fluorescently-tagged versions of inner membrane proteins by light microscopy by expanding the diameter of mitochondria in vivo using a conditional mutation of MDM12, which encodes an essential component of the ER-mitochondria encounter structure. Our results indicate that a majority of GFP-tagged inner membrane proteins localized to cristae, as opposed to boundary regions, and a majority of respiratory and protein import machinery localized to cristae and boundary regions, respectively. However, there were a number of exceptions including a cristae-localized solute transporter, Yea2, and boundary-localized respiratory assembly protein, Rcf2. Our data suggest a simple model in which boundary and cristae regions are generated by retention of a majority of proteins in cristae.

MS58

**ER Membrane Contact Sites Are a Platform for Regulating Mitochondrial Dynamics and Bioenergetics**

**R. G. Abrisch**[1], S. C. Gumbin[2,3], G. K. Voeltz[2,3]; [1]Biochemistry, University of Colorado Boulder, Boulder, CO, [2]Molecular, Cellular, and Developmental Biology, University of Colorado Boulder, Boulder, CO, [3]Howard Hughes Medical Institute, Boulder, CO.

Mitochondria are organelles derived from ancient endosymbiotic organisms and are present in the cytoplasm of all eukaryotes. Despite the fact that they are rapidly fusing and dividing, they are somehow able to maintain a characteristic morphology. Disruption of this steady-state morphology results in either a fragmented or elongated network, both of which are associated with altered metabolic states and disease. How cells spatially organize division and fusion machineries to produce mitochondria of appropriate size is a fundamental question that is still unresolved. We now know that different organelles form membrane contact sites with each other to coordinate their function in the cellular context and mitochondria form conspicuous contact sites with the endoplasmic reticulum (ER MCS). These ER MCS regulate calcium buffering, lipid transfer, and mark the position of division along the length of the mitochondrion. Here we show that ER MCS also mark the position of mitochondrial fusion using a photoconvertible fluorescent protein targeted to mitochondrial compartments allowing the detection of content exchange upon a *bona fide* fusion event. Surprisingly, we found that both division and fusion machinery (Drp1 and Mitofusins) accumulate together in nodes at ER MCS where mitochondria can perform either process. Additionally, depolarization of individual mitochondria revealed that fusion at ER MCS nodes rescues the polarization state of the large majority of depolarized mitochondria. Thus, we propose that division and fusion are spatially coordinated at ER MCS where mitochondria are poised to alter their morphology in response to metabolic needs.

MS59

**Synergistic Role of Two Parkinson's Disease Linked Phosphoinositide Phosphatases in Presynaptic Endocytic Trafficking**

M. Cao[1,2], **D. Park**[1], Y. Wu[1], P. De Camilli[1]; [1]Departments of Neuroscience and Cell Biology, Howard Hughes Medical Institute, Program in Cellular Neuroscience, Neurodegeneration and Repair, Kavli Institute for Neuroscience, School of Medicine, Yale University, New Haven, CT, [2]Present address: Program in Neuroscience and Behavioural Disorders, Duke-NUS Medical School, Singapore, SINGAPORE.

Genetic studies of Parkinson's disease (PD) led to the identification of several proteins implicated in synaptic membrane traffic. Mechanisms of disease, however, remain unknown. A missense mutation (R258Q) in the PI4P-phosphatase domain of synaptojanin 1 (called Sac domain) result in early onset Parkinson's disease. Another Sac domain PI4P phosphatase (Sac2/INPP5F) was linked to Parkinson's disease by genome-wide association studies (GWAS). We previously generated mice carrying the patient mutation of synaptojanin 1 ($SJ1^{RQ}KI$) and shown that they develop neurological manifestations similar to those of human patients. Sac2 knock out (KO) mice do not have an obvious pathological phenotype. Here, we report a strong "synthetic" genetic interaction between these two proteins as revealed by crossing Sac2 KO mice with $SJ1^{RQ}KI$ mice. Nearly all mutant mice homozygous for both mutations die perinatally. In a few double mutant survivors, the occurrence of dystrophic dopaminergic nerve terminals (positive for TH and DAT) in the striatum occurred earlier (2 weeks) than in $SJ1^{RQ}KI$ single mutant mice, where it becomes apparent at 1 month. In addition, the abnormal accumulation of endocytic proteins previously observed in $SJ1^{RQ}KI$ single mutant nerve terminals, most likely reflecting a defect in "uncoating", was more prominent in Sac2 KO/$SJ1^{RQ}KI$ double mutant terminals. We also observed by immunofluorescence an abnormal accumulation of ATG9 (a transmembrane core autophagy protein) in a subset of both $SJ1^{RQ}KI$ presynaptic terminals and Sac2 KO/$SJ1^{RQ}KI$ presynaptic terminals. Accordingly, ATG9 level was increased in total extracts of $SJ1^{RQ}KI$ brain. Together, our data indicate that two PD-linked Sac domain-containing phosphoinositide phosphatases play a synergistic role in membrane traffic in presynaptic nerve terminals, including the traffic of ATG9. These changes have potential implication for neurodegeneration mechanisms.

MS60

**Rafting with Rush: Membrane Rafts Mediate Protein Trafficking through the Biosynthetic Pathway.**

**I. Castello Serrano**, B. Diaz-Rohrer, F. A. Heberle, R. Ippolito, K. R. Levental, I. Levental; UTHealth | The University of Texas Health Science Center at Houston, Houston, TX.

The organelles of eukaryotic cells maintain distinct protein and lipid compositions required for their distinct functions. However, the mechanisms by which components are sorted to their specific locations remain largely mysterious. In particular, how lipids and membrane proteins are coordinately delivered to various membrane-bound organelles is poorly understood. One proposed explanation is that the sorting of membrane components is mediated by membrane microdomains known as lipid rafts. Such domains are small, dynamic clusters of preferentially interacting lipids and protein components. Recently, our lab has defined the structural determinants for protein partitioning to raft domains, identifying three independent aspects of protein transmembrane domains (TMDs) as key for raft affinity: TMD length, TMD surface area, and post-translational palmitoylation. Remarkably, all three were also correlated with protein subcellular localization, strongly suggesting a mechanistic link between raft affinity and protein sorting. To dissect the mechanisms of raft-mediated protein sorting, we have adapted the RUSH (Retention Using Selective Hooks) system to quantify the temporal dynamics of trafficking of transmembrane proteins through the secretory pathway and to the PM. We find that lipid raft affinity is necessary and sufficient for steady-state PM localization of a subset of transmembrane proteins. The TMDs of these proteins, encoding their raft affinity, were fully sufficient for PM sorting.However, raft affinity was not sufficient for rapid exit from the endoplasmic reticulum (ER), which required specific cytosolic sorting motifs. Importantly, we find that Golgi exit rates are highly raft-dependent, with raft preferring proteins exiting ~3-fold faster than mutants with perturbed raft

affinity. We rationalize these observations with a mechanistic, predictive model of trafficking through the secretory pathway, which includes the partitioning of the Golgi into coexisting membrane domains. We identify an isoform of the small GTPase Rab6 as a central regulator for the Golgi-PM trafficking of raft proteins. These observations highlight a central role for lipid rafts in sorting in the secretory pathway and establish the core machinery for raft-mediated cellular trafficking.

## Microsymposium 11: Microtubule Stability & Dynamics

MS61

**The Effect of Tubulin Arginylation on Cellular Microtubules**

**B. MacTaggart**, A. Kashina; University of Pennsylvania, Philadelphia, PA.

Microtubules are involved in a host of cellular processes required for development and health, including cell division, cell morphology, cell motility, and intracellular transport. These functions require morphologically and dynamically different microtubule populations, and this diversity is often conferred by post-translational modifications. Different tubulin modifications are enriched in specific microtubule-based structures and can regulate the dynamics, stability, and protein interactions of the microtubules they decorate. One tubulin modification that has been identified but not well studied is arginylation. Arginylation is the post-translational addition of arginine to glutamate and/or aspartate residues within a protein by arginyl transfer enzyme 1 (ATE1). In mice, *Ate1* deletion causes abnormal cardiac and neurological development, resulting in embryonic lethality starting at E12.5. Many of the observed phenotypes appear to be the result of impaired cytoskeletal function. Accordingly, cellular studies have shown that *Ate1* deletion causes cytoskeletal defects, including reduced cell motility and adhesion. These processes are known to involve microtubules, but the role of tubulin in these defects has not yet been explored. Previously, tubulin arginylation has been identified by mass spectrometry of whole mouse embryos and by *in vitro* arginylation reactions. Here, mass spectrometry of taxol purified microtubules from mouse embryonic fibroblasts (MEFs) has identified arginylation on several residues in both α- and β-tubulin, in regions that have the potential to alter electrostatic interactions and thus to regulate microtubule dynamics and/or stability. MEFs with *Ate1* knockout (*Ate1*$^{-/-}$) show a significant reduction in end binding 1 (EB1) comet velocity compared to wildtype MEFs, suggesting that lack of arginylation results in slower microtubule growth rate. Additionally, *Ate1*$^{-/-}$ MEFs show an increased fraction of depolymerization resistant microtubules compared to wildtype, suggesting that lack of arginylation results in an increased population of stable microtubules. Together these results demonstrate that arginylation plays a role in regulating the dynamics and stability of cellular microtubules.

MS62

**Unexpected Selective Coordination between Axonal Lysosome Transport and Microtubule Dynamics with Neurodegenerative Disease Implications**

**N. Mohd Rafiq**, L. Lyons, S. Gowrishankar, P. De Camilli*, S. Ferguson*; Yale University, New Haven, CT.

* Corresponding Authors

Lysosome filled axonal swellings are a defining feature of Alzheimer's disease pathology. However, the mechanisms that give rise to their formation are not well understood. This reflects significant gaps in our

understanding of the normal mechanisms that coordinate the abundance and motility of axonal endosomes and lysosomes. Building on our previous observations of Alzheimer's-like axonal pathology in mouse JNK-interacting protein 3 (JIP3) KO neurons (*1*), we have now established human induced pluripotent stem cells (iPSCs) and cortical neurons derived from them as a new model system to investigate the mechanisms that support axonal endolysosome transport. This model has been particularly valuable for investigating the relationships between cytoskeletal changes that accompany the axonal swelling and localized disruptions of organelle transport. In contrast to expectations that axonal swellings formed due to a simple loss of interactions between lysosomes and their microtubule-based motors that results in their local accumulation and subsequent expansion of the surrounding axon, we found that focal lysosome accumulations coincided with distinct local disruptions of microtubule organization and dynamics. High-resolution fluorescence microscopy revealed that a subset of microtubules formed abnormal loops at sites of lysosome-filled axonal swellings. Through ongoing experiments involving the imaging of microtubule dynamics combined with genetic and pharmacological manipulations of microtubule stability and post-translational modifications, we are seeking to dissect the causal relationships between JIP3-dependent transport of endo-lysosomes and axonal microtubule organization. Our results establish an unexpected coordination between axonal microtubule organization and the transport of endolysosomal organelles with implications for neuronal cell biology and neurodegenerative disease.1.S. Gowrishankar, Y. Wu, S. M. Ferguson, Impaired JIP3-dependent axonal lysosome transport promotes amyloid plaque pathology. *J Cell Biol* **216**, 3291-3305 (2017).

MS63

**Growing Tip-localized Microtubule Organizing Center Determines Microtubule Orientation in Dendrites**

**M. Kokes**[1,2], X. Liang[1,2], M. A. Pickett[1], M. D. Sallee[1], J. L. Feldman[1], K. Shen[1,2]; [1]Stanford University, Stanford, CA, [2]Howard Hughes Medical Institute, Stanford, CA.

Neuronal subcellular compartmentalization of axon and dendrite provides a directionality that is essential for informational flow through the nervous system, and how it is established remains unclear. By observing the development of a single highly polarized neuron from its birth in *C. elegans*, we find it polarizes and outgrows its axonal and dendritic neurites with highly stereotyped orientation and sequence. The unique behavior of each neurite even as it first emerges suggests that the establishment of subcellular compartmentalization is concomitant with neurite emergence *in vivo* rather than a delayed neurite maturation process as is seen in cultured neurons removed from their *in vivo* environment.As tracks for directional subcellular transport, a polarized arrangement of microtubule arrays lays the foundation for axon versus dendrite compartmentalization. Mature axons are characterized by exclusively "plus-end-out" microtubules while dendrites uniquely contain a large population of "minus-end-out" microtubules. Here we identify a mechanism by which the characteristic minus-end-out microtubule orientation is initially established in dendrites. By observing microtubule plus-end growth during dendrite emergence and outgrowth, we find a microtubule-organizing center (MTOC) that localizes near the distal dendrite tip as it emerges and continues to advance. This MTOC generates numerous minus-end-out microtubules that populate the dendrite. Core components of the gamma tubulin ring complex localize to the dendritic MTOC. Cell-specific degradation of these components indicates they are required for the dendritic MTOC and establishment of the minus-end-out microtubules characteristic of mature dendrites. We continue to characterize the subcellular nature, regulation, and positioning of this growing tip-localized dendritic MTOC.

MS64

**Hierarchical Regulation of Spindle Scaling During Development**

**E. M. Rieckhoff**[1,2,3], F. Berndt[1,2,3], S. Golfier[1,2,3], F. Decker[1,2,3], J. Brugués[1,2,3]; [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.

Cells need to regulate the size and shape of their organelles for proper function. For example, the mitotic spindle adapts its size to changes in cell size over several orders of magnitude, but we lack a mechanistic understanding of how this is achieved. We have recently shown that the upper spindle size is set by the limited activity of microtubule nucleators around chromosomes, even when components are unlimited. For small spindles, however, it has been shown that microtubule dynamics correlate with spindle size during development. Thus, the mechanisms of cell-size dependent spindle scaling are still unclear due to the lack of experimental data on the underlying microtubule processes in a system that covers the full regime of spindle size scaling.Here, we combine quantitative microscopy, laser ablation, and modelling in zebrafish embryos and encapsulated *Xenopus* egg extract to reveal the relative contributions of microtubule length dynamics and nucleation to cell-size dependent spindle scaling. We find a hierarchical regulation of spindle scaling. In large spindles, microtubule dynamics and turnover are independent of spindle size—implying a scale-invariant microtubule length. Below a threshold cell size, microtubule polymerization dynamics and turnover correlate with spindle size. However, the change in microtubule dynamics alone is not sufficient to account for the observed spindle scaling. Instead, we find that microtubule nucleation is the main biophysical process that scales spindle size across all cell sizes, although microtubule dynamics still fine tune spindle size in small cells.We hypothesize that this hierarchical regulation of spindle scaling is an inherent consequence of building spindles from microtubules that are smaller than the size of the spindle. The gradual transition from a microtubule nucleation-based to a microtubule dynamics-based mechanism of spindle assembly implies a change in spindle architecture, and may ensure faithful chromosome segregation by spindles of all sizes.

MS65

**Effect of Cytoplasm Concentration on Cytoskeleton Dynamics**

**A. T. Molines**[1], J. Lemière[1], A. Serra-Marques[1], G. Goshima[2], F. Chang[1]; [1]UCSF, San Francisco, CA, [2]Nagoya University, Nagoya, JAPAN.

The cytoplasm is a very crowded environment harboring most of the metabolic reactions necessary for cellular function. Its biophysical properties are influenced by cellular metabolism. Indeed, changes in cytoplasm mechanical properties have been linked to cellular differentiation and changes in cytoplasm concentration to cell death. However, the reverse relationship has not been extensively studied. So, here we tried to address the effects of the cytoplasm biophysical properties on various dynamic processes happening inside cells from protein diffusion to cytoskeleton dynamics. Our results demonstrate the sensitivity of cellular dynamics, specifically of the cytoskeleton dynamics, to cytoplasm concentration. In fission yeast, changing cytoplasmic concentration reversibly affects all the dynamic processes we looked at from protein diffusion to complex dynamic processes such as actin and microtubule dynamics. We found that microtubule dynamics is inversely correlated to cytoplasmic concentration. Surprisingly, cytoplasmic concentration not only slowed down the microtubule growth rate but also the shrinkage rate. Moreover, the effect of cytoplasmic concentration on cytoskeleton dynamics did not seem to depend on stress pathway. We extended our observation to other eukaryotes,

HeLa cells and moss, and confirmed the effect of cytoplasmic concentration on microtubule dynamics suggesting that we are probing an inherent property of the cytoplasm. Our results highlight how important the biophysical properties of the cytoplasm are to cellular metabolism in eukaryotes. This study reveals the interplay between biology and physics happening in the cytoplasm. Our assay is a first step in understanding how the biophysical properties of the cellular environment affect the biological processes it encloses.

MS66

**A Gelation Transition Enables Long-ranged Active Flows that Organize *Xenopus* Egg Extract Spindles**

D. Oriola, B. Dalton, F. Decker, F. Julicher, **J. Brugues**; Max Planck Institute, Dresden, GERMANY.

During cell division, correct segregation of chromosomes depends on the ability of microtubules to self-organize into a bipolar spindle. Our current understanding of large spindle assembly is based on the interplay between spatial microtubule nucleation and microtubule transport. It has been recently shown that branching nucleation is the main mechanism by which microtubule nucleation occurs away from centrosomes. However, microtubule branching naturally leads to explosive waves of microtubule nucleation that rapidly travel away from initially created microtubules at a speed much faster than the microtubule flux velocity. This behavior should normally result in spindles with outward polarity (or inverted polarity) originating from the chromosomes, where microtubule nucleation is highest. Yet, spindles manage to robustly assemble bipolar spindles despite branching nucleation and slow microtubule flux. Here, we used experiment and theory to study how spindles from Xenopus laevis egg extracts acquire the proper microtubule organization despite the slow microtubule transport and branching nucleation. We found that microtubules in spindles self-organize into two mechanically distinct microtubule networks that undergo a gelation transition. This gelation allows the propagation of long-range extensile stress from the center of the spindle that push the two gels apart. Combining laser ablation and single tubulin molecule microscopy we show that these active stresses are generated by Eg5 that works at the interphase of the two networks whereas dynein creates local contractile stresses throughout the structure. The long-range extensile stresses allow for transporting microtubules independently of their local polarity environment, and thus local sorting. The emergence of two gelated and rigid-like microtubule networks that push against each other explains how microtubules can be sorted out into the proper bipolar structure in the presence of branching nucleation despite the slow microtubule transport.

## Microsymposium 12: Regulation of Cell Division

MS67

**The Ubiquitin Ligase Trim37 Controls Acentrosomal Spindle Formation and Cancer-specific Vulnerability to Plk4 Inhibition**

**F. Meitinger**, M. Ohta, K. Lee, S. Watanabe, R. L. Davis, J. V. Anzola, R. Kabeche, D. Jenkins, A. K. Shiau, A. Desai, K. Oegema; Ludwig Institute for Cancer Research, San Diego, CA.

Centrosomes are microtubule organizing centers (MTOCs) that catalyze microtubule formation for assembly of the mitotic spindle. Centrosomes are duplicated once per cell cycle in a process regulated by the kinase PLK4. Following chemical PLK4 inhibition, continued division in the absence of centrosome duplication generates centrosome-less cells that exhibit delayed, acentrosomal spindle assembly.

Whether PLK4 inhibitors can be leveraged in the treatment of cancer is not yet clear. Here, we show that the centrosomal ubiquitin ligase TRIM37 controls the response of cells to centrosome loss resulting from PLK4 inhibition in a bi-directional fashion. TRIM37 inactivation accelerates acentrosomal spindle assembly and improves proliferation following PLK4 inhibition, whereas elevated TRIM37 expression inhibits acentrosomal spindle assembly, leading to mitotic failure and cessation of proliferation. The region containing the *TRIM37* gene on Chr. *17q* is frequently amplified in neuroblastoma and in breast cancer, which makes mitosis and proliferation of these cancer types highly sensitive to PLK4 inhibition. TRIM37 inactivation improves acentrosomal spindle assembly because TRIM37 inhibits self-assembly of PLK4 into centrosome-independent condensates, which scaffold the formation of ectopic microtubule-organizing centers that improve acentrosomal mitosis. By contrast, elevated TRIM37 expression inhibits acentrosomal spindle assembly via a PLK4-independent mechanism, by preventing coalescence of pericentriolar material proteins associated with acentrosomal spindle assembly. Thus, TRIM37 is a critical determinant of mitotic vulnerability to PLK4 inhibition. Linkage of *TRIM37* to prevalent cancer-associated genomic changes, including *17q* gain in neuroblastoma and *17q23* amplification in breast cancer, may offer an opportunity to use PLK4 inhibition to trigger selective mitotic failure in these cancer contexts.

MS68

**Exploiting Trim37-driven Centrosome Dysfunction to Eliminate 17q23-amplified Breast Cancer Cells**

Z. Yeow[1], B. Lambrus[2], M. Durin[1], D. Moralli[1], C. Green[1], R. Chapman[1], **A. Holland**[2]; [1]Wellcome Centre for Human Genetics, University of Oxford, Oxford, UNITED KINGDOM, [2]Johns Hopkins University School of Medicine, Baltimore, MD.

Genomic instability (GI) is a hallmark of cancer and plays a central role in breast cancer initiation and development[1,2]. The success of Poly-ADP ribose polymerase inhibitors in the treatment of homologous recombination (HR)-deficient breast cancers exemplifies the utility of synthetic lethal drug-gene interactions in the treatment of GI-driven breast cancer[3]. Given that HR-defects are present in only a subset of breast cancers, there is a need to identify additional GI-driver mechanisms, and targeted strategies to exploit these defects in cancer treatment. Here, we identify that centrosome-depletion induces synthetic lethality in cancer cells harbouring the 17q23 amplicon, a recurrent copy number aberration (CNA) that defines ~9% of all breast tumours and is associated with high GI[4-6]. Specifically, small-molecule inhibition of Polo-like kinase 4 (PLK4) leads to centrosome depletion that triggers mitotic catastrophe in cells harbouring amplicon-directed overexpression of *TRIM37*. To explain this effect, we identify TRIM37 as a negative regulator of centrosomal pericentriolar material (PCM). In 17q23-amplified cells elevated TRIM37 blocks the formation of non-centrosomal PCM foci, structures we show to be required for successful cell division in the absence of centrosomes. Lastly, we find TRIM37 overexpression causes GI by inducing cohesion fatigue, mitotic failure, and subsequent whole genome duplications. Collectively, these findings highlight *TRIM37*-dependent GI as a putative driver event in 17q23-amplified breast cancer and provide a rationale for centrosome-targeting therapeutics in their treatment.

MS69

**Cyclin B1 Scaffolds MAD1 At the Corona to Activate the Spindle Assembly Checkpoint**

L. A. Allan[1], M. Reis[1], Y. Liu[2], P. Huis in 't Veld[2], G. J. Kops[3], A. Musacchio[2], **A. T. Saurin**[1]; [1]University of Dundee, Dundee, UNITED KINGDOM, [2]Max Planck Institute of Molecular Physiology, Dortmund, GERMANY, [3]Hubrecht Institute, Utrecht, NETHERLANDS.

Cyclin B/CDK1 is the chief mitotic kinase that phosphorylates hundreds of proteins to regulate mitotic progression. We show here that, in addition to these kinase functions, Cyclin B also scaffolds a localised signalling pathway to help preserve genome stability. Specifically, we demonstrate that Cyclin B1 localises to an expanded region of the outer kinetochore, known as the corona, where it scaffolds the spindle assembly checkpoint (SAC) machinery by binding directly to MAD1. In vitro reconstitutions and size exclusion chromatography map the key binding interface to a few acidic residues in the N-terminus of MAD1. Mutation of three key residues in this region prevents Cyclin B interaction in vitro, removes MAD1 from the corona and weakens the SAC response. Therefore, Cyclin B is the long sought after scaffold that links MAD1 to the corona and this specific pool of MAD1 is important for SAC signalling. We next moved on to investigate how corona MAD1 helps to support the SAC.A key difference of Cyclin B1-bound MAD1, when compared to the canonical MAD1 pool localised at KNL1/Bub1, is that its localisation becomes MPS1-independent after the corona has been established. We demonstrate that this preserves MAD1 at kinetochores when MPS1 activity is reduced, which helps to ensure that MAD1 can still be phosphorylated on a key C-terminal MPS1 phosphorylation site needed for SAC signalling (MAD1-pThr716). Immunofluorescence staining for MAD1-Thr716 demonstrates that the signal is only observed at the outer kinetochore, near the zone of MPS1 activity on NDC80, which begs the question of how MAD1 can reach this region when it is anchored by Cyclin B at the corona. Visualisation of Cyclin B in complex with full length MAD1 by electron microscopy after low-angle metal shadowing, demonstrated that MAD1 has a reach of approximately 66nm from Cyclin B at its N-terminus to the key C-terminal catalytic residues that respond to MPS1 activity. We speculate this this is sufficient to allow a pool of corona-tethered MAD1 to reach MPS1 at the KMN network and catalyse MCC assembly.In summary, this study explains how corona MAD1 generates a robust SAC signal and why stripping of this pool by dynein is essential for SAC silencing. It also reveals that the key mitotic kinase, Cyclin B1-Cdk1, scaffolds the pathway that inhibits its own degradation.

MS70

**Intramolecular Regulation of Anillin During Cytokinesis**

**D. Beaudet**, N. Pham, N. Skaik, A. Piekny; Concordia University, Montreal, QC, CANADA.

Cytokinesis describes the separation of a cell into two daughters, which occurs due to the ingression of an actomyosin ring. Cytokinesis must occur with high fidelity to avoid fate changes and aneuploidy. Recently, we uncovered a novel mechanism whereby the ring protein anillin is regulated by importins during cytokinesis. Importins bind to cargo proteins that contain nuclear localization signals (NLS) and mediate their transport into the nucleus. Active Ran is enriched in the nucleus and competes with importins to release the cargo. During mitosis, the Ran gradient persists around chromatin, where it regulates spindle assembly. The dogma is that importins bind to the NLS of spindle regulators to block interactions with partners required for their function. Thus, active complexes assemble the spindle near chromatin, where active Ran is high. Our data supports a very different function for the regulation of cortical proteins by importin-binding. We found that importins function as a ruler during cytokinesis,

where optimal levels facilitate anillin's cortical recruitment. This mechanism permits precise sensing of chromatin position to ensure that the contractile ring is properly positioned. Here, we determined how importin-binding regulates anillin's cortical enrichment. Anillin has a RhoA-binding domain (RBD) required for recruitment to the equatorial cortex, and we found that the RBD autoinhibits the adjacent NLS-containing C2 domain. Our model is that binding to active RhoA causes a conformational change in anillin that increases accessibility to the C2 domain, and importin-binding stabilizes this change for cortical recruitment. Our data supports this model. We found that active RhoA facilitates importin-binding, while inactive RhoA or mutating the RBD decreases importin-binding. Through live-imaging and FRAP experiments we found that mutating the NLS alters anillin's cortical properties. Anillin's localization and function are abolished when NLS mutations are combined with mutations that weaken the interface between the RBD and C2 domain. Stronger interface mutations similarly fail to localize to the furrow and rescue loss of endogenous anillin. This data shows that the interface is crucial to drive feedback between the C2 domain and the RBD for recruitment to the equatorial cortex. We propose that other cortical proteins could be regulated by importin-binding during mitotic exit, and to reconsider the dogma of how importin-binding could impact protein function.

MS71

**Actin-independent, Microtubule-dependent Cytokinesis In*Chlamydomonas***

**M. Onishi**[1], J. E. Little[2], T. Frej[3], J. R. Pringle[1]; [1]Stanford University, Stanford, CA, [2]Gunn High School, Palo Alto, CA, [3]Carnegie Institution for Science, Stanford, CA.

Cytokinesis in unikonts (animals, fungi, slime molds, and their close relatives) involves the "contractile ring", which contains F-actin, myosin II, and other proteins. The force generated by actin-myosin II interaction is widely viewed as the primary driver of cleavage-furrow ingression. However, many types of unikont cells can form furrows even in the absence of normal myosin-II activity. Furthermore, the wide diversity of non-unikont eukaryotes do not possess myosin II, yet most of them divide by furrowing, using mechanisms that are very poorly understood. Thus, we lack understanding of the division mechanism of the last eukaryotic common ancestor and thus of the true role and evolutionary advantage of the contractile ring in the unikonts.To address these questions, we are studying the mechanisms of cytokinesis in *Chlamydomonas*, an alga that has no myosin II yet divides by forming cleavage furrows. We previously showed that although F-actin is enriched in the cleavage furrow, it is dispensable for its ingression. Unexpectedly, division of the large chloroplast was delayed in these cells, suggesting a novel role for F-actin in coordinating the timing and location of cell division and chloroplast division. The furrows formed in the absence of F-actin are still associated with microtubules, and pharmacological dissolution of microtubules completely blocked furrowing, indicating that these cytoskeletal elements, unlike F-actin, are essential for furrow formation. Furthermore, the localization and movements of the plus-end-binding protein EB1 and the minus-end-directed kinesin KCBP suggest the possibility that stable rootlet microtubules located in the furrow region act as non-centrosomal organization centers for nucleation of other, more dynamic microtubules that may be involved in membrane addition to the furrow region, similar to the furrow-associated array in animals and the phragmoplast in plants. The implications of these observations for the evolution of eukaryotic division mechanisms will be discussed.

MS72

**Phase Separation by Membrane Protein Lem2 Controls Escrt-mediated Nuclear Envelope Reformation.**

**A. Von Appen**[1], D. LaJoie[2], I. Johnson[1], M. Trnka[3], A. Burlingame[3], K. Ullman[2], A. Frost[1]; [1]UCSF - Department of Biochemistry and Biophysics, San Francisco, CA, [2]Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, [3]UCSF - Department of Pharmaceutical Chemistry, San Francisco, CA.

The open mitosis strategy employed by many eukaryotes requires dramatic membrane remodeling. For proper chromosome segregation, the nuclear envelope dissociates from chromatin and retracts into the contiguous endoplasmic reticulum (ER). At mitotic exit, ER-derived membranes return to chromatin and spread to reform the nuclear envelope within approximately 12 minutes. In a series of dynamic events, the cell must coordinate spindle microtubule disassembly with nuclear envelope sealing. Here, we combine in vivo and in vitro approaches, including light and electron microscopy, to study the molecular principles of nuclear envelope reformation. Our data show how the two-pass inner nuclear membrane protein, LEM2, directs microtubule remodeling and nuclear envelope sealing via the Endosomal Sorting Complexes Required for Transport (ESCRT) pathway. LEM2's amino-terminal proline-arginine-rich low-complexity domain undergoes liquid-liquid phase separation to coat spindle microtubules. LEM2s activity is further spatially restricted to the chromatin disks by a direct binding between the LEM motif and the chromatin-associated barrier-to-autointegration factor (BAF). Once condensed, LEM2 tethers the reforming nuclear envelope the to the spindle and recruits the ESCRT-II/ESCRT-III hybrid protein CHMP7 via LEM2's carboxyterminal winged-helix domain. Together LEM2 and CHMP7 copolymerize around microtubule bundles to form a molecular "O-ring" that promotes nuclear compartmentalization and initiates downstream ESCRT factor recruitment. These results demonstrate how multivalent interactions of a transmembrane protein - including those that mediate phase separation and coordinate localized ESCRT polymerization - enable mitotic spindle disassembly and promote membrane fusion. Consequently, defects in this pathway compromise spindle disassembly, nuclear integrity, and genome stability.

## Microsymposium 13: Actin Filaments: Binding and Assembly

MS73

**Intravital Subcellular and Single Molecule Imaging Reveal Multiple Actin Filament Populations Collaborate in the Remodelling of the Secretory Granule Membrane**

**M. Heydecker**[1], A. Masedunskas[1], S. Ebrahim[2], M. Appaduray[1], A. Shitara[2], N. Bryce[1], R. Weigert[2], P. Gunning[1], E. Hardeman[1]; [1]School of Medical Sciences, University of New South Wales, NSW 2052, Sydney, AUSTRALIA, [2]Laboratory of Cellular and Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD.

The actin cytoskeleton contributes to many cell functions involving the remodelling of membranes. This is achieved both via interaction of actin filaments with myosin motors and through the force generated by branched actin networks. One of these processes is exocytosis of large secretory granules in the salivary gland of rodents. We used intravital subcellular microscopy (ISMic) to identify the types of actin filaments that are required for the granule integration into the apical membrane and intravital single molecule microscopy (iSiMM) to study the molecular behaviour and nanostructures at the basolateral membrane. We find at least two types of actin filaments are associated with exocytosis at the apical membrane. Actin filaments containing tropomyosin Tpm3.1 are used to build a scaffold around the

granule upon initial fusion. Myosin II motors are subsequently recruited to the scaffold independently from F-actin. An Arp2/3 nucleated branched actin filament network emanates from the scaffold and drives rapid remodelling of the granule membrane. Inhibition of Arp2/3 nucleation eliminates the generation of the branched network. Subsequent contraction of the actin filament scaffold displays much slower kinetics to achieve identical remodelling of the granule membrane. This emphasizes the collaboration of multiple actin filament populations in the process of membrane remodelling during regulated granule exocytosis. At the basolateral membrane iSiMM was used to reveal single molecule dynamics of Tpm3.1 and myosin II motors. Molecular recruitment and nanostructures were analysed before and during exocytosis. A decrease in the diffusion coefficient of Tpm3.1 after stimulation indicate that remodelling at both the basolateral and apical membranes take place during exocytosis.

MS74

**The Role of APC-mediated Actin Assembly in Microtubule Capture and Focal Adhesion Turnover**

**M. A. Juanes**[1], D. Isnardon[2], A. Badache[2], S. Brasselet[3], M. Mavrakis[3], B. L. Goode[1]; [1]Brandeis University, Waltham, MA, [2]Centre de Recherche en Cancérologie de Marseille, Inserm, Institut Paoli-Calmettes, Aix Marseille Université, Marseille, FRANCE, [3]Aix Marseille Université, CNRS, Centrale Marseille, Institut Fresnel, Marseille, FRANCE.

Focal adhesions (FA) are highly dynamic structures that assemble at the front of the cell and disassemble at the rear, allowing cells to move directionally. Focal adhesion (FA) turnover depends on microtubules and actin. Microtubule ends are captured at FAs, where they induce rapid FA disassembly. However, actin's roles are less clear. Here, we use polarization-resolved microscopy, FRAP, super-resolution microscopy, live-cell imaging, and a mutant of Adenomatous polyposis coli that is incapable of nucleating actin (APC-m4) and impairs directional cell migration (Juanes et al., 2017; *J. Cell Biology*) to investigate the role of actin assembly in FA turnover. We show that APC-mediated actin assembly is critical for maintaining normal F-actin levels, organization, and dynamics at FAs, along with proper organization of FA components, including Src and FAK Kinases, and Paxillin. Live imaging in wild type cells shows that microtubules are captured repeatedly at FAs as they mature, but once a FA reaches peak maturity, the next microtubule capture event leads to delivery of an autophagosome, triggering FA disassembly. In APC-m4 cells, microtubule capture frequency and duration are altered, and there are long delays between autophagosome delivery and FA disassembly. Thus, APC-mediated actin assembly is required for normal feedback between microtubules and FAs, and maintaining FAs in a state 'primed' for microtubule-induced turnover. In addition, we found that APC-m4 expression or silencing of the formin Dia1 each caused a ~30% reduction in total F-actin levels in cells; however, Dia1 silencing led to only modest defects in FA turnover. Thus, APC-m4 effects on FA turnover do not arise from a general loss of actin assembly in cells, but rather from a specific disruption of APC-mediated actin nucleation at FAs. In summary, this work demonstrates that APC is a *bona fide* actin nucleator *in vivo*, and that this activity of APC is crucial for regulating FA turnover underlying directed cell migration. Importantly, our results do not rule out the possibility of other actin assembly-promoting factors contributing to FA turnover, or APC coordinating actin and microtubule dynamics in other cellular locations besides FAs.

MS75

**SPIN90 Links Arp2/3 Complex Nucleation to Formin Elongation to Control Actin Network Organization**

L. Cao[1], G. Charras[2], A. Jegou[1], **G. Romet-Lemonne**[1]; [1]CNRS - Institut Jacques Monod, Paris, FRANCE, [2]University College London, Lodon, UNITED KINGDOM.

A number of actin filament networks in cells, including the cortex, are generated by a combination of branched nucleation by the Arp2/3 complex, and rapid elongation of barbed ends by formins. The Arp2/3 complex and formins are thought to be activated independently by specific nucleation promoting factors, yet the competition and the synergy between these two machineries is not well understood. Here, we identify a number of nucleation promoting factors responsible for the activation of Arp2/3 and formin mDia1 in the cell cortex. Among them, SPIN90 (also known as DIP/NCKIPSD/WISH) was previously reported to interact with both Arp2/3 and formins, and thus appeared as a potential candidate to regulate the balance between these two machineries.In blebbing melanoma and HeLa cells, we find that, surprisingly, the phenotypes associated to SPIN90 depletion often mimic those associated to formin mDia1 depletion. Using an array of techniques, including in vitro single filament assays, we show that SPIN90 harnesses Arp2/3 nucleation for the benefit of mDia1-assisted rapid elongation, though a combination of mechanisms.In particular, we show that filaments nucleated by SPIN90-Arp2/3 are more likely to recruit formin mDia1 and, consequently, to elongate rapidly. Strikingly, we also show that SPIN90, the Arp2/3 complex and formin mDia1 can form a ternary complex, which efficiently nucleates rapidly growing filaments, with SPIN90-Arp2/3 sitting at their pointed end, and mDia1 tracking the barbed end.Our results globally show that SPIN90 uses Arp2/3 to nucleate linear filaments that are rapidly elongated by formin mDia1, at the expense of branching. As a consequence, SPIN90 regulates cell cortex architecture and mechanical properties. We propose that SPIN90 may play a similar role in other actin networks built using Arp2/3 branching and formin-mediated rapid elongation.

MS76

**Vimentin Intermediate Filaments and F-actin Form Interpenetrating Networks in the Cell Cortex**

**H. Wu**[1], Y. Shen[1], S. Sivagurunathan[2], M. Weber[3], O. Medalia[3], R. Goldman[2], D. Weitz[1]; [1]John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, [2]Department of Cell and Developmental Biology, Northwestern University Feinberg School of Medicine, Chicago, IL, [3]Department of Biochemistry, University of Zurich, Zurich, SWITZERLAND.

Vimentin intermediate filaments (VIFs) and F-actin are both filamentous cytoskeletal proteins in the cytoplasm but they are typically understood to have vastly different properties and functions. However, there is some evidence that VIFs are involved in cellular functions commonly attributed to actin, which necessitates a closer look at the interplay between the F-actin and VIF cytoskeletons. Using structured illumination microscopy, we image the cortical region of mouse embryonic fibroblasts (MEFs), where we observe close associations between VIFs and F-actin bundles (stress fibers). Furthermore, cryo-electron tomography (cryo-ET) of stress fiber ultrastructure reveals that VIFs are pervasive throughout the structure, suggesting that a stress fiber is an interpenetrating network (IPN) of F-actin and VIFs. Although many F-actin structures are facilitated by crosslinking proteins, we show that a mixture of purified actin and vimentin proteins self-assembles in vitro into an IPN without the help of binding proteins. Finally, we study the functional consequences of having composite VIF-actin structures in cells, and find that VIFs can significantly impact actin dynamics. We use traction force microscopy (TFM) to find that VIFs play an important role in cell contractility even though they are not intrinsically a force-

generating unit; this contribution is likely due to the integrated structure. In addition, fluorescence recovery after photobleaching (FRAP) measurements reveal that VIFs hinder cytoplasmic G-actin diffusion. From these observations, we infer parallels with double-network hydrogels, which are tougher than either of their component gels. These results indicate that the interplay between VIFs and F-actin is an important aspect of cell mechanics, especially as it relates to the contractile nature of cells. These results demonstrate that VIFs play a broad and essential role in cellular mechanics by influencing both the structure and dynamics of actin. Supported by a NIGMS grant (P01 GM096971) awarded to R.G. and D.W. and a Swiss National Science Foundation grant awarded to O.M.

MS77

**Identification of a Novel B Cell Actomyosin Network that Promotes Antigen Contraction During Immune Synapse Formation**

**J. C. Wang**, X. Wu, J. A. Hammer; National Institutes of Health, Bethesda, MD.

B cells are a critical branch of the immune system and drive antibody-based protection. The strength of an antibody response is determined by the ability of B cells to extract and internalize membrane-bound antigens from antigen-presenting cells (APCs). Antigen uptake requires the formation of an immune synapse (IS) where B cell receptor-bound antigens are moved centripetally into a central cluster before being extracted from the APC and internalized. The force provided by the actin motor myosin 2A (M2A) powers antigen extraction. B cells that lack M2A activate aberrantly and mount weak antibody responses. However, the organization of the actomyosin network, its role in IS formation, and the mechanism by which M2A powers antigen extraction in B cells are unknown. Here we test the hypothesis that the actomyosin network drives the events of IS formation that promote B cell activation and antigen uptake. We first define the dynamic organization of actin and M2A at the IS using the super-resolution imaging modalities TIRF/SIM, 3D-SIM and Airyscan. On functionalized glass and planar lipid bilayers, the A20 B cell line forms concentric arcs in the medial portion of the IS. These arcs are rich in M2A based on immunostaining, and on imaging cells in which endogenous M2A was tagged with GFP using CRISPR. 3D-SIM imaging of APCs with primary splenic B cells isolated from M2A-GFP knock-in mice shows that M2A polarizes towards the IS. B cell IS studies are often performed using antigen stimulation only. *in vivo*, B cell integrins provide adhesion to APCs and, via an unknown mechanism, allow for IS formation and antigen uptake with weakly-stimulating antigens. Surprisingly, we found that actomyosin arc formation in primary B cells requires both antigen and integrin costimulation, conditions that reflect physiological B cell activation. These contractile actomyosin arcs are especially prominent in primary B cells such that the actomyosin arcs are the major actin structure at the IS. The contractile nature of the actomyosin arcs that dominate at the primary B cell IS may explain why integrin costimulation boosts B cell responses to weakly-stimulating antigens. Notably, integrin costimulation on bilayers produces actin arcs that sweep peripheral antigen clusters centripetally and is required for contracting low amounts of antigen to form the IS. Moreover, M2A inhibition abrogates the organization of actin arcs and prevents antigen centralization. Therefore, we have identified in primary B cells a novel actomyosin network, which comprises the major actin structure at the IS and promotes robust antigen centralization during IS formation. Current efforts are directed at defining the mechanism by which the actomyosin network drives antigen extraction from APCs using live-cell volumetric imaging.

MS78

**αT-catenin Intramolecular Interactions Regulate Vinculin Binding**

**J. A. Heier**[1], S. Pokutta[2], W. I. Weis[2], A. V. Kwiatkowski[1]; [1]University of Pittsburgh School of Medicine, Pittsburgh, PA, [2]Stanford University School of Medicine, Stanford, CA.

Cardiomyocyte contractions place unique physical and regulatory demands on the protein complexes that join these cells. Cardiomyocytes are connected by intercalated discs (ICDs), specialized junctions that mechanically and electrically couple cells. Mechanical linkage at the ICD is accomplished through the adherens junction (AJ) and desmosome that connect to the actin and intermediate filament cytoskeletons, respectively. The core component of the AJ is the cadherin-catenin complex, and α-catenin serves as the primary link between the AJ and actin. Two α-catenins are expressed in the mammalian heart, αE(Epithelial)-catenin and αT(Testes)-catenin. Tension regulates αE-catenin conformation: actomyosin-generated force stretches the middle(M)-region to relieve autoinhibition and reveal a binding site for the actin-binding protein vinculin. In contrast, little is known about the molecular properties of αT-catenin. Here we describe the biochemical properties of αT-catenin and how intramolecular interactions regulate vinculin binding. Isothermal titration calorimetry (ITC) showed that αT-catenin binds the β-catenin/N-cadherin complex with a similar affinity (low nanomolar) to that of αE-catenin indicating a similar association with the AJ. Limited proteolysis revealed that the αT-catenin M-region adopts a more open conformation than αE-catenin. Consistent with these results, ITC revealed that the αT-catenin M-region binds the vinculin N-terminus with low nanomolar affinity, indicating that the αT-catenin M-region is not autoinhibited and distinct from αE-catenin. However, the αT-catenin head domain binds vinculin 1000-fold more weakly (low micromolar affinity), indicating that the N-terminus regulates M-region binding to vinculin. Notably, β-catenin binding to the αT-catenin head domain does not affect vinculin binding. Together, our results indicate that the αT-catenin N-terminus is required to maintain M-region autoinhibition and modulate vinculin binding. We postulate that the unique molecular properties of αT-catenin regulate ligand binding to meet the demands of intercellular adhesion in cardiomyocytes.

## Microsymposium 14: Cellular Imaging of Cytoskeletal Dynamics

MS79

**Single-molecule Imaging Reveals Distinct Subcomplexes of the *Bacillus Subtilis* Division Machinery**

**M. J. Holmes**, E. C. Garner; Harvard University, Cambridge, MA.

Despite decades of research, the fundamental molecular mechanisms underlying bacterial cytokinesis remain poorly understood. Recently, it has become clear that protein dynamics in the bacterial division complex, and in particular those of the tubulin homolog FtsZ, play a key role in this process. FtsZ polymers localize in a ring at future division sites and recruit cell-wall-synthesis enzymes that build a septum between daughter cells. FtsZ filaments move around this ring by treadmilling: subunits are added to one end of the filament and removed from the other. This results in the filament as a whole moving, but individual subunits remaining stationary. Filament treadmilling is required for the coincident motion of the associated cell wall synthesis enzyme Pbp2B around the division site, as well as efficient cell division. Strikingly, single molecules of Pbp2B move along with the treadmilling filament and are highly processive. Single-molecule processive motion along filaments is typically associated with molecular motors in eukaryotic systems, but neither Pbp2B nor any other protein associated with the

division complex has homology to any known motors. How is this motion achieved? In addition to FtsZ, the division complex (or divisome) contains many other proteins whose dynamics have not been characterized. We imaged single molecule motions of each divisome protein using HaloTag fusions labelled with Janelia Fluor dyes. Two classes of motions were observed. Cytoplasmic proteins that bind directly to FtsZ remain immobile with FtsZ subunits. In contrast, the divisome proteins with periplasmic domains all move around the division site. This latter group contains the other division cell wall synthesis enzyme FtsW, and three non-enzymatic proteins which interact with one another but have unknown function. All four proteins move at the division site with velocities comparable to Pbp2B, and these directional motions require cell wall synthesis. We propose that these periplasmic proteins move around the cell in complex in a manner dependent on treadmilling FtsZ filaments. We are currently working to identify the molecular interfaces between the stationary and moving components. Additionally, we are uncovering how the localization and motion of these mobile proteins depends on specific FtsZ architectures.

MS80

**The Architecture and Dynamics of Podosomes in Macrophage Frustrated Phagocytosis**

**S. Hu**[1], T. Watanabe[1], A. T. Nogueira[1], J. Aaron[2], A. Taylor[2], T. Chew[2], K. Hahn[1]; [1]University of North Carolina At Chapel Hill, Chapel Hill, NC, [2]HHMI Janelia Research Campus, Ashburn, VA.

Cytoskeleton and membrane remodeling are essential for phagocytosis. However, the structure and dynamics of the actin cytoskeleton and its precise regulation are incompletely understood. We set up a temporally and geometrically well-defined “frustrated phagocytosis” model to study the evolution of actin structures and associated proteins during phagocytosis, using several types of super-resolution imaging methods. We first examined engulfment of IgG coated polystyrene beads using 3D structured illumination microscopy (3D-SIM). This showed that podosome-like structures formed perpendicular to the bead surface during phagocytic cup formation, and disappeared when the beads are completely engulfed. We then studied attachment to micro-patterned IgG spots using 3D-SIM, TIRF-SIM and interferometric fluorescent super-resolution microscopy (iPALM), and found similar podosomes at the edge of the spots, often arranged as a ring. Characterizing the podosomes using iPALM with ~ 10 nm vertical resolution showed an almost homogenous layer of actin network covering the IgG spot at ~ 50 to 150 nm height. Sparse radially distributed actin filaments surrounding dense actin cores were found above about 150 nm in height. Strikingly, the podosome had a protruding tip ~ 50 nm lower than the actin network. 3D-SIM revealed that the adhesion protein paxillin surrounds the podosome core at the bottom layer, forming a ring, but alpha-actinin surrounds the podosome core along its entire vertical axis. Myosin II filaments were absent at the podosome bottom but accumulated around podosomes at a higher layer. The dynamics of myosin II filaments were studied using TIRF-SIM; myosin filaments exhibited an inward flow to the center of the IgG spot. Myosin II filaments aggregated over the whole IgG spot at later times when the podosomes disappeared. At this stage, paxillin transformed into to elongated adhesions. Overall, the dynamic 3D architecture of podosome-like structures was revealed during phagocytosis. Contractility driven by myosin II filaments may play a critical role in the transition from podosomes to focal adhesions.

MS81

**The Cellular Mechanism of Structural Color Change in the Zebrafish**

**D. Gur**[1,2], X. Wu[3], J. Hammer[4], J. Lippincott Schwartz[2]; [1]NIH/NICHD, Bethesda, MD, [2]Janelia Research Campus, Ashburn, VA, [3]NIH/NHLBI, Bethesda, MD, [4]NIH\NHLBI, Bethesda, MD.

Many animals, including chameleons and certain species of fish, tune their crystalline-based structural colors to facilitate camouflage, kin recognition, and mate choice. In such systems, remarkable colors are produced by constructive interference of light reflected from stacks of intracellular crystals. The machinery that enables this robust cellular reorganization is unknown. We have used iridophore cells of the zebrafish to reveal the cellular basis of structural color change. Using a combination of super-resolution fluorescence light microscopy, genetic manipulations, and micro-focused X-ray diffraction, we found that zebrafish iridophores are capable of changing their colors in response to stimuli and that this change occurs due to the tilting of the intracellular crystals. We discovered that crystal tilting is facilitated by microtubule motor proteins, which actively pull upon the intracellular crystals. More specifically, we showed that the microtubule minus-end directed motility of dynein is responsible for the red-shift in the iridophore reflected color, while the plus-end directed motility of kinesin is responsible for the blue-shift of the reflected color. Intriguingly, only a subpopulation of iridophores change their color upon stimulation, which results in the disappearance of the animal's famous stripe pattern.

MS82

**Development of An Optogenetic Tool to Reversibly Control Microtubule Acetylation**

**A. Deb Roy**, E. Gross, G. Pillai, A. Kim, T. Inoue; Johns Hopkins University, Baltimore, MD.

Acetylation of α-tubulin at lysine-40 is a highly conserved post-translational modification, which is reported to stabilize microtubule against bending stresses. Microtubule acetylation is exclusively catalysed by α-acetyltransferase-1 (ATAT1), whose only known substrate is α-tubulin. Despite being implicated in a wide array of physiological phenomena including ciliary function, cell motility and mechanosensation, the precise role of microtubule acetylation in these events is not well understood, due to a limited understanding of ATAT1 regulation and lack of tools to specifically control microtubule acetylation. Here we report development of an optogenetic tool to control microtubule acetylation in live cells in a reversible manner. We observed that ATAT1 shuttles between nucleus and cytoplasm but maintains a predominantly cytoplasmic localization by active nuclear export in a Crm1-dependent manner. Unlike wild-type ATAT1, exogenous expression of nuclear localized ATAT1 catalytic domain did not significantly increase microtubule acetylation, suggesting that nuclear export may play a role in regulating ATAT1 access to microtubules and thus regulate acetylation levels. We identified a conserved Leptomycin-B sensitive nuclear export sequence in the disordered carboxy-terminal region, which is critical for nuclear export but not catalytic activity of ATAT1. Based on these findings, we hypothesized that inducible translocation of ATAT1 catalytic domain from nucleus to cytoplasm should allow us to control microtubule acetylation levels. We modified the previously described Light-inducible nuclear export system (LEXY) to reduce its dark-state activity and tethered the catalytic domain of ATAT1 to its carboxy-terminus. LEXY-ATAT1 was predominantly nuclear in dark and was rapidly shuttled to the cytoplasm upon blue light stimulation in a reversible manner. A lit mutant of LEXY(lit)-ATAT1 induced around two-fold increase in microtubule acetylation levels in HeLa cells, as compared to a non-transfected cells or cells expressing a dark mutant of LEXY(dark)-ATAT1. HeLa cells expressing LEXY-ATAT1 exposed to light for 4 hours showed a similar two-fold increase in microtubule acetylation

compared to those kept in dark, validating the functionality of the tool. Thus, we have developed a powerful tool to control microtubule acetylation that will provide various applications exploring cell motility and ciliary functions.

MS83

**Systematic Characterization of a Large Number of Microtubule-Associated Proteins Using Purification-free Tirf-reconstitution Assays**

**A. S. Jijumon**[1,2,3], S. Bodkuntla[1,3], M. Genovo[1,2], M. Bangera[4], F. Maksut[1,2], M. M. Magiera[1,2], C. Janke[1,2,3]; [1]Institut Curie, Paris, FRANCE, [2]University of Paris Saclay, Orsay, FRANCE, [3]PSL University, Paris, FRANCE, [4]Instem, Bangalore, INDIA.

The microtubule cytoskeleton is complex filamentous network involved in diverse functions such as cell division, cell shape, neuronal differentiation, ciliary beating. Strict regulation of microtubule functions is therefore of high importance for the integrity of the healthy cell, and perturbations are often linked to diseases like cancer, ciliopathies and neurodegeneration.In a cellular context, microtubule properties can be controlled by two major mechanisms: by the expression of alternative tubulin genes and post-translational modifications of tubulin, or/and via the interaction of microtubules with a large variety of microtubule-associated proteins (MAPs). Our knowledge of such interactors has been continuously enriched over the past decades, but up to this date no systematic studies exist that aim to describe and categorize these proteins according to their binding mechanisms and structural effects on microtubules. In the present work, we have developed an assay for rapid and systematic analysis of around 40 MAPs, using cleared lysates of cultured human cells in which we overexpress the MAPs of interest. The dynamic behaviour of growing microtubule in presence of different MAPs were monitored over time using total internal reflection fluorescence (TIRF) microscopy. This allows us to study the behaviour of large number of MAPs in a situation close to their natural environment, but eliminating cellular complexity coming from different organelles and crammed cytoskeleton filaments inside the confined intracellular space. Indeed, most MAPs were nicely soluble in our extract approach, while purification often led to protein precipitation. Our novel approach allowed us to define several novel proteins as bona-fide MAPs. We show that previously uncharacterized MAPs have strikingly different effects on microtubule polymerization and microtubule structure, thus creating a variety of distinct microtubule arrays. Also, our cell free TIRF assays provide the evidence for a direct role of many MAPs in the coordination of microtubule and actin cytoskeleton arrays. Moreover, MAPs such as Tau and MACF1 clearly show a preference for PTM-rich microtubules. Our experiments will allow for a better mechanistic understanding of how MAPs and tubulin heterogeneity together control cytoskeleton functions.

MS84

**Three-color Tracking of Dynein Stepping Along Microtubules**

**S. Niekamp**, N. Stuurman, R. D. Vale; University of California - San Francisco, San Francisco, CA.

Cytoplasmic dynein, a minus-end directed microtubule-based motor, is important for the transport of many cargos in cells and plays a key role in mitosis. Unlike the globular and compact motor domains of kinesin or myosin, the dynein motor domain is composed of a small microtubule-binding domain (MTBD) that is spatially separated by a ~135 A long coiled-coil from its large catalytic AAA ring. Using single-molecule tracking, it has previously been shown that the AAA rings of a dimeric dynein move

through uncoordinated stepping. However, from recent structural studies it is clear that the relative orientation of the AAA ring and MTBD is quite flexible. Thus, to fully understand how dynein is walking, it is necessary to follow the MTBD. Moreover, the relative movement of different parts of the dynein motor domain during the stepping cycle is poorly understood. Addressing these questions requires high-resolution, multicolor imaging.We developed a three-color image registration routine that achieves sub-nanometer accuracy. To enable three-color labeling and nanometer accuracy tracking of dynein over a prolonged period of time, we designed ultra-photostable, DNA-based Fluorocubes. These DNA Fluorocubes have single-point attachment to proteins, a ~50-fold higher photobleaching lifetime, and emit ~40-fold more photons than a single organic dye. We are currently applying our three-color image registration method and are utilizing the ultra-photostable DNA Fluorocubes to analyze stepping patterns of dynein and the domain movement within the dynein motor domain during its movement along microtubules.

## Microsymposium 15: Dynamics of the Genome and Epigenome

MS85

**Generation of Regulatory Stable Intronic Sequence Rnas From Conserved Genetic Loci**

**S. Chan**[1], R. B. Ismail[1], J. Heng[2], S. Lim[3], J. Ho[4], J. Pek[1]; [1]Temasek Lifesciences Laboratory, Singapore, SINGAPORE, [2]Raffles Institution, Singapore, SINGAPORE, [3]Temasek Polytechnic, Singapore, SINGAPORE, [4]Ngee Ann Polytechnic, Singapore, SINGAPORE.

Stable intronic sequence RNAs (sisRNAs) belong to a class of noncoding RNAs (ncRNAs) comprised of sense intronic transcripts or matured RNAs containing intronic sequences that are not degraded immediately. Recent discoveries reported that ncRNAs, including sisRNAs, may play vital roles in regulating cellular processes, maintaining homeostasis and are involved in pathogenesis. Increasing number of ncRNAs are being identified from simple to complex multicellular organisms and interestingly, some small ncRNAs, such as let-7 microRNA, are highly evolutionary conserved from nematode to human. The evolutionary conservation of these ncRNAs may be attributed to their essential biological functions in the cell, as shown in let-7 microRNA which has important functions in stem-cell renewal and tumor suppression. So far, there are no regulatory long ncRNAs reported to be highly conserved in both invertebrates and vertebrates. Here we report the discovery of regulatory sisRNAs which are transcribed from highly conserved genetic loci found in both *Drosophila* and human. From the genomic annotation of the sisRNAs, we observed that a particular intron from each genetic loci is highly positionally conserved and alternative splicing retaining the intron leads to the production of sisRNAs in both fly and human. To determine the functional roles of sisRNAs, we performed knockdown experiments by using short hairpin RNAs (shRNAs) for fly and antisense oligonucleotides (ASOs) for human breast cancer cells (MCF-7). In *Drosophila*, sisRNAs act to repress the splicing of the cognate pre-mRNAs and are involved in the regulations of important biological processes. We demonstrated that some pre-mRNAs regulated by sisRNAs encode for ribosomal proteins which are crucial for germline stem cell maintenance *in vivo*. Surprisingly in human, sisRNAs adopt a different mode of action by promoting splicing. No significant change was detected on the cognate pre-mRNAs level, however the mRNA level was significantly downregulated. In this case, these regulated mRNAs encode for proteins that are reported to be involved in gene transcription mediated by estrogen receptors and promote growth of breast cancer cells (MCF-7). Based on our results, we propose that sisRNAs modulate cognate

gene expression by regulating the alternative splicing-nonsense mediated decay pathway. In conclusion, our study provides evidence of positional conservation in sisRNAs and their engagement in gene regulatory networks. Owing to their stability and regulatory functions, sisRNAs also serve as a good candidate for studies in human diseases and pathogenesis.

MS86

**_SETD2_ Loss Drives Genomic Instability by Increasing CENP-A Levels and Generation of Dicentric Chromosomes**

**F. M. Mason**[1], E. S. Kounlavong[1], I. Park[2], C. L. Walker[2], W. K. Rathmell[1]; [1]Vanderbilt University Medical Center, Nashville, TN, [2]Baylor College of Medicine, Houston, TX.

Epigenetic regulation is critical for genome maintenance and identity of the centromere, the site of kinetochore assembly and microtubule attachment during mitosis. Alterations to the “epigenetic code” at the centromere can disrupt the organization and function of the histone H3 variant that specifies the centromere, CENP-A. Changes in CENP-A levels or localization lead to errors in kinetochore assembly and chromosome segregation in mitosis causing aneuploidy, a hallmark of cancer. However, genetic perturbations in epigenetic regulators that function as tumor suppressors or oncogenes have not yet been strongly linked to changes in centromere identity or CENP-A function/localization. Previously, we have identified that loss or mutation of the tumor suppressor and H3 methyltransferase *SETD2* causes errors in chromosome segregation and aneuploidy. Yet, it is unclear how loss of SETD2-dependent histone methylation (H3K36 tri-methylation, H3K36me3) directly leads to chromosome mis-segregation. Here, we demonstrate that loss of *SETD2* increases total CENP-A protein levels and CENP-A levels at the centromere. This increase in CENP-A is accompanied by an increase in expression of the DAXX/ATRX histone chaperone complex, which has been previously demonstrated to regulate ectopic, non-centromeric CENP-A localization. Importantly, loss of *SETD2* leads to the generation of chromosomes with two CENP-A containing centromeres (dicentric chromosomes), which are due in part to the formation of neocentromeres (centromeres forming at non-centromeric locations). Staining of metaphase spreads from *SETD2* mutant cells demonstrates that many dicentric chromosomes contain the kinetochore protein Hec1/Ndc80 at both centromeres, suggesting that both centromeres are active and may contribute to chromosome segregation errors. We propose that *SETD2*-dependent H3K36me3 regulates centromeric identity and acts as a tumor suppressor by inhibiting ectopic CENP-A deposition and dicentric chromosome formation, thereby promoting genome stability.

MS87

**Compaction Without Condensin? Using Oligopaints to Investigate Chromosome Territories in the Moth, _Bombyx Mori_**

**L. F. Rosin**[1], J. Gil, Jr.[2], I. A. Drinnenberg[2], E. P. Lei[1]; [1]NIH/NIDDK, Bethesda, MD, [2]Curie Institute, Paris, FRANCE.

Recent technological advances in DNA sequencing and chromosome painting technologies have revealed that the interphase genome is organized into an intricate three-dimensional (3D) structure. Importantly, many features of interphase chromatin organization, including the spatial separation of euchromatin and heterochromatin and the formation of chromosome territories (CTs), are conserved between species as divergent as yeast and humans. This conservation suggests that interphase genome organization facilitates vital cellular processes such as transcription and DNA repair. However, how

these basic principles of nuclear organization have remained unchanged throughout evolution despite large structural and functional changes at the DNA and protein level is unclear. Surprisingly, recent studies revealed that several insect lineages have lost the essential Cap-H2 subunit of the Condensin II complex, which is required for CT formation in *Drosophila*. The organizational consequences of the loss of functional Condensin II remain unknown. Here, we use Oligopaint technology to visualize whole chromosomes in the moth *Bombyx mori* to investigate whether or not CT formation is possible in the absence of Cap-H2. Our preliminary studies show that *B. mori* chromosomes are indeed partitioned into discrete territories. Furthermore, these CTs are highly compact and display both conserved and distinct organizational patterns in the nucleus. These findings indicate the possible emergence of a novel mechanism to regulate chromosome folding and compartmentalization in Lepidopteran insects.

MS88

**Distinct Roles of LINC Complex and Nucleoskeleton Components in Regulating Meiotic Chromosome Dynamics**

**C. Liu**, Z. Lung, A. Dernburg; University of California, Berkeley and HHMI, Berkeley, CA.

Mechanical forces provide important cues for the organization and reorganization of genome inside the nucleus. Forces can be transduced across the nuclear envelope (NE) through the LINC complex and the nuclear lamina, which frequently work together during nuclear movement and nuclear stiffening in response to external forces, and are important for chromosomal processes including genome replication and the DNA damage response.During meiosis, the two sets of chromosomes inherited from both parents are extensively reorganized to segregate into haploid gametes. In early meiosis, homologous chromosomes must pair, culminating with assembly of the synaptonemal complex (SC) between them. Meanwhile chromosomes also undergo DNA double strand break (DSB) formation and repair that lead to crossover recombination. During this time, despite the proximity between chromosomes and NE, it remains unclear how different components of the cytoskeleton and nucleoskeleton systems near NE work together to regulate the dynamic reorganization of meiotic chromosomes.Using the auxin-inducible degradation (AID) system in *C. elegans*, we found that depleting SUN-1 leads to precocious SC assembly between nonhomologous chromosomes. Conversely, upon acute depletion of ZYG-12, the interaction partner of SUN-1, synapsis is delayed and defective despite partial homolog pairing. These data support a model in which SUN-1 inhibits synapsis until homologs pair with their specific partners. Despite being in the same LINC complex as SUN-1, ZYG-12 plays a distinct role: rather than inhibiting inappropriate synapsis, it is important for promoting synapsis once homologous pairing is achieved. Surprisingly, co-depletion of SUN-1 and ZYG-12 results in a phenotype more similar to that of ZYG-12 depletion than SUN-1 depletion, suggesting the existence of additional pathways that prevent nonhomologous synapsis.In contrast to either SUN-1 or ZYG-12 depletion, we found that acute depletion of LMN-1 does not inhibit homolog pairing or synapsis, but does result in altered chromosome morphology, delays in processing of meiotic DSBs, and extensive apoptosis. Of interest, LMN-1 localization at NE is enriched along the length of meiotic chromosomes, and the mobility of synapsed chromosomes is altered in the absence of LMN-1, consistent with a role for LMN-1 in mechanically stabilizing synapsed chromosomes to facilitate DSB processing.Taken together, we have uncovered unexpected, distinct roles of components among the LINC complex and nucleoskeleton systems in regulating early meiotic chromosome dynamics. Further genetic and cell biological analyses will extend our understanding of the molecular mechanisms that work together to ensure accurate chromosome reorganization underlying error-free meiosis.

MS89

**Identification of Regulators of Nuclear Shape**

**A. Schibler**[1], P. Jevtic[2], G. Pegoraro[1], D. Levy[2], T. Misteli[1]; [1]National Cancer Institute, NIH, Bethesda, MD, [2]University of Wyoming, Laramie, WY.

The shape of the human cell nucleus is highly variable amongst cell types and tissues. In addition, nuclear shape changes are associated with aging and disease, including cancer. Despite the very fundamental nature of nuclear morphology, the cellular factors that determine nuclear shape are not well understood. To identify regulators of nuclear shape, we performed a high-throughput imaging-based siRNA screen in two human cell lines using siRNA libraries targeting 867 nuclear proteins including chromatin-associated proteins, epigenetic regulators, nuclear envelope components, and nuclear matrix proteins. Using multiple morphometric parameters, we identified a set of proteins required for maintenance of proper nuclear morphology. Both cell-type specific and general cell shape regulators were identified. Interestingly, most identified factors altered nuclear shape without affecting levels of lamin proteins, know regulators of nuclear morphology. A prominent group of nuclear shape regulators were modifiers of repressive heterochromatin and biochemical and molecular studies uncovered direct physical interaction of specific histone modifications with lamin A. Disease causing lamin A mutations which results in disruption of nuclear shape inhibits these interactions. Our results represent the first systematic exploration of cellular factors involved in nuclear shape determination and identify chromatin related mechanisms as key determinants of nuclear morphology in human cells. The identification of regulators and mechanisms of nuclear morphology has significant implications for our understanding of aging and disease including cancer.

MS90

**Multinucleation Associated Damage Promotes Quiescence Unlike Other Nuclear Atypia**

M. Hart, **V. M. Draviam**; Queen Mary University of London, London, UNITED KINGDOM.

Nuclear atypia is a strong indicator of malignancy. How distinct forms of nuclear atypia differently impact cell fate is however not well understood. Here, we perform single-cell tracking studies to determine the immediate and long-term impact of multinucleation or misshapen nuclei, and compare it to micronucleation, a well-studied catastrophic nuclear atypia which leads to numerous genomic rearrangements. Tracking the fate of newly born cells exhibiting various nuclear atypia shows that unlike other forms of nuclear atypia, multinucleation, but not misshapen nuclei or micronuclei, induces an immediate non-p53 dependent cell cycle arrest. Assessing large scale DNA damage occurring within multinucleate cells revealed the delayed formation of DNA damage repair platforms, 53BP1 nuclear bodies, and the exclusion of transcriptional machinery at DNA damage sites. Ultimately, the onset of replication is blocked, driving multinucleated cells in G1 phase into a long-term quiescence, even in the absence of p53-p21 signalling, a tumour suppressor pathway frequently mutated in cancers. Thus, our study reveals the protective aspect of multinucleation, and calls for the therapeutic need to segregate nuclear atypia on the basis of cell cycle status.

## Microsymposium 16: Intracellular Trafficking & Membrane Recycling

MS91

**Membrane Homeostasis During Exocrine Secretion Is Maintained by Membrane Crumpling and Sequestration**

K. Kumari, N. Scher, T. Biton, E. D. Schejter, B. Shilo, **O. Avinoam**; Weizmann Institute of Science, Rehovot, ISRAEL.

Dynamic membrane trafficking comprises the essence of cellular homeostasis in every living eukaryotic cell. However, membrane dynamics becomes especially challenging in secretory epithelial tissues, where the continuous fusion of vesicles with the apical membrane takes place. This is most dramatically demonstrated in exocrine tissues that secrete viscous cargoes from giant vesicles, ranging up to 8 microns in diameter. Secretion of such giant vesicles adds large amounts of membrane to the apical surface of cells. Yet, it remains unclear how homeostasis of the cell surface in terms of size, shape, and composition is maintained under these extreme circumstances. To address this question, we used as a model system the *Drosophila* larval salivary gland, which secrets a viscous mucin-like protein called 'glue' via giant vesicles. After fusion with the apical membrane, a contractile actomyosin network is recruited to the surface of the vesicles to expel their content, and could thus be used as a marker to follow the fate of the vesicular membrane over time. Using live super-resolution microscopy, we observed that the vesicular membrane does not simply collapse into the apical surface during content release, but instead presents a crumpled appearance. To determine the precise ultrastructure of the vesicular membrane at different phases of secretion, we used correlative fluorescence and 3D-electron microscopy (CLEM). We observed that the vesicle membrane becomes increasingly crumpled and folded as secretion progresses, indicating that the membrane is not incorporated into the apical surface. To quantify this phenomenon, we used a correlative block-face and FIB-SEM approach and found that the compacted membrane indeed accounts for most of the original vesicular membrane before secretion. Furthermore, we found that the sequestered membrane recruits the clathrin-mediated endocytosis machinery, which recovers the membrane over a prolonged period of time post secretion. These results indicate that membrane homeostasis is maintained by actomyosin mediated crumpling and sequestration of the vesicular membrane. This novel mechanism for maintaining membrane homeostasis may be ubiquitous and essential for exocrine tissue physiology.

MS92

**Studying Dynamics and Mechanics of Clathrin-mediated Endocytosis in the Native Tissue Context**

**M. Mund**, A. Picco, M. Kaksonen; University of Geneva, Geneva, SWITZERLAND.

Clathrin-mediated endocytosis (CME), an essential cellular function in eukaryotes, has been extensively studied using adherent cultured cells. These cells, however, are artificially flat and lack the physiological tissue environment, which influences the mechanical properties of the plasma membrane. Thus, the question how CME is adapted to the functional and architectural diversity of cells and tissues, remains poorly understood.Here, we aim to understand how CME functions in the native tissue context. Generally, we ask whether CME is performed by an adapted machinery and occurs with specific spatiotemporal dynamics in different cells and tissues. We used *Drosophila melanogaster,* which combines the advantages of a versatile genetic toolkit, a comparably small genome and accessibility to a variety of imaging modalities. Using CRISPR we created fly lines where endocytic proteins are

endogenously tagged with fluorescent markers. We then used fluorescence microscopy and particle tracking to follow individual endocytic events and measure their dynamics with high spatiotemporal resolution.In our experiments, we imaged intact *Drosophila* pupae, which are non-motile. We focused on the single-layered epithelium at the notum, which is at the very surface and thus well-suited for microscopy. Within this epithelium, individual precursor cells undergo stereotypic divisions to form mechanosensory organs, each consisting of a bristle, socket, neuron and sheath cell. We use this organ as model system to image CME within tissue, throughout cell division and differentiation.This approach allowed us to record individual endocytic events in living fly pupae with a similarly high resolution as in cell culture, allowing us to precisely determine their lifetimes and dynamic trajectories. We find that endocytic events are temporally highly regular, and observed intriguing changes in the dynamics of endocytic events in bristle cells. We hypothesize that in these cells, CME is reprogrammed during development, potentially in response to changing cellular function and architecture. To investigate this question in more detail, we will study how actin and actin-binding proteins contribute to force generation during CME, how changes in membrane tension affect endocytic dynamics, and how CME adapts during differentiation also in the neuron and the socket cell of the mechanosensory organ.

MS93

**Expanding the Realm of Small GTPase Function: Evidence for Rab40b/Cul5 Mediated Rap2 Regulation During Cell Migration**

**E. D. Duncan**, E. Linklater, R. Prekeris; University of Colorado Anschutz Medical Campus, Aurora, CO.

One of the most fundamental questions in cell biology is how cells migrate through three-dimensional (3D) spaces. A defining feature of cell migration is the formation of actin-rich protrusions at the leading edge that are necessary for remodeling of the extracellular matrix (ECM). Extension of these actin-rich protrusions, known as invadopodia, combined with the targeted secretion of matrix metalloproteinases (MMPs) facilitate degradation of the ECM, allowing cells to migrate and invade through their surrounding environment. However, what remains poorly understood is how cells coordinate MMP secretion and actin polymerization during the formation and extension of invadopodia. Our lab has identified Rab40b as a regulator of invadopodia formation, targeted MMP secretion, and cell migration. Rab40b is unlike any other small GTPase because in addition to the canonical Rab domain, it also contains a Suppressor of Cytokine Signaling (SOCS) domain, a binding motif known to act as a bridge between E3 Ubiquitin (Ub) Ligases and protein substrates. In this study we demonstrate that Rab40b binds to Cullin5 (Cul5), a known E3 Ub Ligase module critical for protein ubiquitylation and degradation. We have also identified Rap2 as a putative substrate of the mammalian Rab40b/Cul5 complex. Importantly, the Rap family of small GTPases, including Rap2, have been linked to the regulation of cell-cell adhesions and actin cytoskeleton dynamics. Here we show that Rab40b/Cul5 binds and regulates Rap2 by mediating its mono-ubiquitylation. Interestingly, we demonstrate that inhibition of Rap2 mono-ubiquitylation leads to endocytic removal from the plasma membrane and rapid lysosomal degradation, thus, terminating Rap2 signaling. Based on our preliminary data, we propose that Rab40b is a dual-functioning Rab GTPase, given its co-regulation of vesicular MMP trafficking as well as Rap2 activity, and such co-regulation plays a key role in driving 3D cell migration and invasion. Results gained from this study will broaden our scientific understanding not only of Rab GTPase function but will also help uncover novel machinery governing cell migration.

MS94

**Spontaneous Clathrin Lattice Curvature Without Triskelia Replacement.**

B. Heine, **K. A. Sochacki**, J. W. Taraska; National Institutes of Health, Bethesda, MD.

Clathrin mediated endocytosis is a major route for the capture and internalization of membrane bound material in eukaryotic cells. This pathway plays a key role in signal transduction, cellular homeostasis, antigen presentation, and synaptic transmission. Clathrin triskelia bind adaptor proteins to form a polygonal lattice on a patch of membrane which buds into the cell to form a transport vesicle. Clathrin lattices assemble on flat membrane patches as well as curved pits or vesicles. It has only recently been confirmed that flat clathrin structures with a primarily hexagonal lattice can transition into curved pentagon-containing lattices in living cells. This lattice rearrangement is thought to require dynamic cytoplasmic clathrin replacement and ATP-dependent chaperones like auxilin. Here, we show that flat clathrin structures curve spontaneously without the presence of cytoplasmic clathrin or chaperones. Specifically, mammalian cells were unroofed to isolate adherent membranes and remove cytoplasm and all sources of energy. Sequential time points at and after unroofing were observed with platinum replica electron microscopy and fluorescence microscopy. Quantification of these data reveal minimal loss of membrane bound clathrin and near complete curvature of all flat clathrin lattices within 2 minutes. We further use drug treatments and pH changes to control the flat to curved transition. We propose that (1) clathrin lattice curvature does not require cytoplasmic clathrin interchange, (2) clathrin lattices spontaneously curve, and (3) flat lattices must be maintained by a flattening force.

MS95

**Escrt-III Heteropolymers Utilize Non-specific Lateral Interactions to Stabilize Curvature of Spiraling Polymers**

**S. Banjade**, S. Tang, Y. Shah, S. D. Emr; Cornell University, Ithaca, NY.

The ESCRT (endosomal sorting complexes required for transport) system controls vital cellular processes including multivesicular bodies biogenesis, viral budding, cytokinesis, nuclear envelope reformation, plasma membrane repair and many others. While “early” ESCRTs - 0, I and II are not essential in all of these functions, self-assembly of the ESCRT-III proteins into spiraling polymers is a critical step in all known ESCRT-dependent events. ESCRT-III heteropolymers adopt variable architectures to remodel membranes in these events, but the mechanisms of inter-subunit recognition in the heteropolymers to create flexible architectures remain unclear.In our work, we demonstrate *in vivo* and *in vitro* that the *Saccharomyces cerevisiae* ESCRT-III subunit Snf7 uses a conserved acidic helix to recruit its partner Vps24. *In vivo*, charge-inversion mutations in this helix inhibit Snf7’s ability to recruit Vps24, and induce functional defects in cargo sorting through multivesicular bodies. Through directed-evolution based selection approaches, we discovered mutations in Snf7 that rescue the defects of the acidic helix mutants. Mutations that rescue these defects balance the acidic nature of that helix. Using the same approach, we also identified charge-inversion mutations on Vps24 in a basic region that rescue the functional defects *in vivo*. Crosslinking experiments provided evidence that the residues we identified on Vps24 reside in close proximity to the Snf7 helix in the polymer.Using electron microscopy assays of polymer assembly on lipid monolayers, we find that the same motif of Snf7 is involved in Snf7’s lateral interaction with Vps24 and another ESCRT-III subunit Vps2. Addition of Vps24/Vps2 leads to architectural changes in the ESCRT-III polymers, changing 2D flat spirals into 3D spirals. Lateral association of the polymers is most likely a major driver of such a transition, which would allow

membrane-deformation to occur.These data suggest that heteropolymers of ESCRT-III proteins associate through lateral electrostatic interactions between filaments, an assembly mechanism in which the interacting proteins lack residue-to-residue specificity. We propose a model in which cooperative electrostatic interactions in the polymer propagate to allow for specific inter-subunit recognition, while sliding of laterally interacting polymers enable changes in architecture at distinct stages of vesicle biogenesis. Our data suggest a mechanism by which interaction specificity and polymer flexibility can be coupled in membrane-remodeling heteropolymers. This mechanism of recognition and assembly also suggests a molecular explanation to the question of how ESCRT-III subunits are able to adapt to spirals of different curvatures.

MS96

**Neuronal Traffic Jams: Mechanistic Insights Into Mutant Mammalian Prion Aggregate-mediated Intracellular Transport Impairments**

**T. Chaiamarit**, A. Verhelle, R. Chassefeyre, S. E. Encalada; The Scripps Research Institute, La Jolla, CA.

A ubiquitous hallmark of neurodegeneration is the accumulation of misfolded protein aggregates inside axons. Intracellular aggregates can impair or block the proper transport of cellular components from the soma to the synapse, but the mechanisms of transport impairment remain unclear. Using an *in cellulo* neuronal system expressing a mutant prion protein ($PrP^{PG14}$) that forms aggregates inside axons and causes prion diseases in humans, we tested the hypothesis that $PrP^{PG14}$ aggregates poison neurons by creating traffic jams to intracellular transport. Using high-resolution quantitative imaging approaches, we showed that $PrP^{PG14}$ aggregates selectively interfere with the anterograde axonal transport of pre-synaptic vesicles and mitochondria by sequestering kinesin-1 away from cargoes, and thus disrupting the association between kinesin-1 and cargo adaptors as these complexes move through the aggregates. Our findings reveal a novel mechanism by which intracellular protein aggregates selectively disrupt axonal function and transport through sequestration of molecular motors. This study suggests potential targets for the development of strategies to combat intracellular prion aggregation, as well as the aggregation of misfolding-prone proteins involved in other neurodegenerative diseases.

## Microsymposium 17: New Perspectives in Cell Biology: Frontiers of Microscopy

MS97

**Tissue Architectural Cues Drive Organ Targeting of Tumor Cells in Zebrafish**

**C. D. Paul**[1], K. Bishop[2], A. Devine[1], E. L. Paine[1], J. R. Staunton[1], S. M. Thomas[1], J. R. Thomas[1], A. D. Doyle[3], L. M. Miller Jenkins[1], N. Y. Morgan[4], R. Sood[2], K. Tanner[1]; [1]Laboratory of Cell Biology, National Cancer Institute, Bethesda, MD, [2]Translational and Functional Genomics Branch, National Human Genome Research Institute, Bethesda, MD, [3]National Institute of Dental and Craniofacial Research, Bethesda, MD, [4]National Institute of Biomedical Imaging and Bioengineering, Bethesda, MD.

Clinical patterns of cancer metastasis are non-random, with certain types of cancers preferentially metastasizing to certain secondary organs in a process termed organotropism. Genetic profiling of cancer cell lines that metastasize to the brain or bone marrow in mice have elucidated important genetic drivers of organ colonization. However, the impact of the microenvironment on organ selectivity during metastasis is less understood, particularly because of a lack of models to concurrently visualize the early stages of metastasis in multiple tissues within the same animal. Here, we used a zebrafish

xenograft metastasis model to determine drivers of organ selectivity during early metastasis. The zebrafish is amenable to imaging at single-cell resolution in multiple organs in addition to possessing blood vessels on the scale of human capillaries, a brain with structural and cellular similarity to the mammalian brain, and hematopoietic tissue in the caudal vein plexus (CVP) analogous to bone marrow. Breast cancer cells that target specific murine organs (brain and bone marrow) ultimately colonized analogous tissues (brain and hematopoietic tissue in the CVP) in larval zebrafish after injection to the circulation. This pattern was conserved across human MDA-MB-231 brain- and bone marrow-targeting subclones (231BR and 231BO, respectively), brain-seeking human Bt474m1 cells, and murine 4T1 bone marrow- and brain-seeking cells. Initial cell dissemination was non-random, with more cells arresting in the complex vasculature of the CVP than the brain, but was independent of cell type, with brain- and bone marrow-targeting cells arresting at identical rates in a given organ. Similar results were obtained with inert beads and upon knockdown of β1 integrin in 231 cells. Using a combination of live-cell imaging, mechanical mapping of tissue properties, confined cell migration assays, and quantification of vascular architecture, we determined that vessel topography was the key determinant of initial cell arrest. Following arrest, a significantly higher fraction of 231BO compared to 231BR cells extravasated in the CVP, leading to the observed organ targeting. Mass spectrometry of 231BO and 231BR cells indicated that pathways involving β1 integrin were upregulated in bone marrow-targeting cells, while myosin 1B was overexpressed in brain-targeting cells. Knockdown of integrin β1 reduced extravasation in the CVP and redirected cell targeting toward the brain. Conversely, knockdown of myosin 1B decreased extravasation in the brain and lead to greater targeting of the CVP. In conclusion, while non-random targeting during initial dissemination was driven by differences in organ vasculature, organ selectivity was dependent on extravasation ability following cell arrest.

MS98

**Live Cell Histology for Classification of Melanoma Cell Population Based on Single Cell Actions**

**A. Zaritsky**[1,2], A. R. Jamieson[2], A. Nevarez[2,3], E. S. Welf[2], G. Danuser[2]; [1]Ben-Gurion University of the Negev, Beer-Sheva, ISRAEL, [2]UT Southwestern Medical Center, Dallas, TX, [3]University of California San Diego, San Diego, TX.

It has long been speculated that metastatic cells adopt distinct morphodynamic states that are predictive of their potential to disseminate and survive in remote tissues. However, this hypothesis has not been systematically tested, due to the challenges in associating clinically-documented outcomes of metastatic development in patients to behavior of individual cells. By combining a unique cell system, high-content imaging pipeline and a novel analytical approach we demonstrate that morphodynamic behaviors, or *cell actions*, are indeed characteristic traits of the metastatic efficiency of individual cells. We coin this new assay *quantitative live cell histology*. We built on a transplantation xenograft model of melanoma that maintains, with high reproducibility, the clinical signature of tumors extracted from patients. Some tumors disseminated to distant locations (stage IV - high metastatic potential), while others did not (stage III - low metastatic potential). We established an experimental and imaging pipeline to harvest primary human melanoma from xenograft transplants originating from 8 patients, created primary culture, and live imaged with label-free phase contrast microscopy under conditions that mimic the tumor microenvironment. We implemented a computational pipeline to transform label-free live cell images to a cell-action high-dimensional space. We found that traditional description of cell action by cell shape- and shape-dynamics is restrictive, limiting the potential of the method. Instead, we implemented an unsupervised deep neural network (autoencoder) followed by supervised machine

learning to capture the subtle details of cell actions that discriminate between cells from high- versus low-metastatic potential tumors. The same methodology could distinguish between established melanoma cell lines versus primary-derived cell systems, between transformed and untransformed cell lines, and even between two different cell lines or primary tumors. Altogether, we demonstrate that the dynamics of unstructured textural data extracted from live melanoma imaging encapsulates information predictive of the cell origin, including the metastatic efficiency. Beyond the potential of becoming an invaluable assay for the discovery of metastasis-promoting pathways, quantitative live cell histology may become the backbone assay to investigate various aspects in cellular heterogeneity, plasticity, and identification of molecular players governing particular cell functions.

MS99

**Multiplexed Fluorescence Lifetime Imaging Microscopy Reveals Dynamic Stem Cell Niche Metabolism in Lgr5-gfp Intestinal Organoids**

**R. Dmitriev**, D. Papkovsky, I. Okkelman; University College Cork, Cork, IRELAND.

Intestinal organoids enable culturing the live intestinal epithelium with functional crypts and villi, study its interactions with nutrients, drugs, metabolites and model the gut-microbiota interactions. Organoids produced from Lgr5-GFP mice allow visualizing stem cell niche and, in combination with different culturing conditions, studying its dynamics in embryonic and adult states. However, metabolic needs and the roles of different metabolites within intestinal epithelium are still poorly understood, which prompted us to study $O_2$ dynamics and mitochondrial function in the culture of Lgr5-GFP organoids. First, we optimized methodology to measure real-time $O_2$ dynamics by the phosphorescence and fluorescence lifetime imaging microscopies and respective probes ($O_2$-PLIM and FLIM) in the intestinal organoids. This approach helped us to visualize the dynamics and heterogeneity of $O_2$ in live organoid culture and correlate it with distribution of stem cells by Lgr5-GFP expression. By using extracellular flux (XF) analysis, we confirmed such strong heterogeneity of organoids grown under differentiation conditions. We further challenged organoids with low (0.5 mM) (LG) and high (10 mM) (HG) glucose in the medium, measured their oxygenation and analyzed the difference between Lgr5-GFP+ and 'no GFP' cells. Paired *t*-test revealed statistically significant differences in oxygenation between stem cell niche and differentiated cells, exposed to different concentrations of glucose: 91±21 μM for stem cell niche and 86±22 μM for differentiated cells in high glucose medium ($p<0.1$) and 95±18 μM for stem cell niche and 88±18 μm for differentiated cells in low glucose medium ($p<0.05$). Interestingly, these differences were influenced by glucose content without effect on the overall organoid oxygenation (89±21 μM in HG and 92±18 μM in LG). In low glucose we observed a tendency in increase of oxygenation differences between stem cell niche and mature cells (6.5±4.7 μM in LG versus 4.5±8.9 μM $O_2$ difference in HG), which was consistently replicated in two independent experiments. We also performed FLIM of mitochondrial membrane polarization and cell proliferation within Lgr5-GFP-enriched regions and found strong heterogeneity of mitochondrial structures within the stem cell niche. Thus, our data support the idea that *stem cell niche* cells display more dynamic and adaptable repertoire of energy production pathways, highly dependent on their environment and available nutrients. Altogether, our study emphasizes both the power of real-time multiplexed FLIM detection of cell metabolism in organoids and identifies the existing limitations in data processing / analysis. This work was supported by the Science Foundation Ireland (SFI) grants 13/SIRG/2144 and 18/IF/6238.

MS100

**Identifying Patterns of Protein Organisation in 3d Super-resolution Datasets**

A. Curd[1], R. Hughes[1], A. Cleasby[1], M. Baird[2], Y. Takagi[2], J. Ries[3], H. Shroff[2], **M. Peckham**[1]; [1]University of Leeds, Leeds, UNITED KINGDOM, [2]National Institutes of Health, Bethesda, MD, [3]EMBL, Heidelberg, GERMANY.

Super-resolution microscopy techniques can now precisely locate specific molecules within cells and cellular structures, to within ~10 nm. However, proteins within macromolecular complexes are organized at length scales that are still very difficult to access. Single-particle averaging techniques adopted from electron microscopy show some promise, but are of limited use in many fluorescently-labelled samples. As well as limits on precision for the location of each detected molecule, low labelling density of a sample, and heterogeneity between and internal to macromolecular complexes, pose significant problems. We have developed a novel approach to determine protein organization with these complexes that overcomes these problems, by aggregating the relative positions between localisations to reveal common organizational features. We applied this new analysis technique to super-resolution data acquired for several different molecular organizations. We first used 3D STORM data for a nuclear pore (Nup107) protein, which has known rotational symmetry, and provides a good test for our approach. We successfully extracted accurate organizational information (symmetry and distances) from this data, demonstrating the strength of our technique. Next, we tested 3D STORM data for cardiomyocyte Z-disks. A major component protein of the Z-disc, α-actinin-2 (ACTN2) was labelled using a novel small non-antibody binding protein (Affimer, Tiede et al., 2017;6:e24903), conjugated to Alexa 647. Z-disks are crucial for muscle structure and contractility, and contain a repeating complex, which has a dense, inhomogeneous, ~20-nm tetragonal lattice structure. We successfully uncovered this known repeating pattern of ACTN2 across the Z-disc (~20nm repeat) along the long axis of the muscle fibre. A similar analysis of 3D PALM data using mEos-tagged ACTN2 in Z-discs was also successful despite low labelling and detection efficiency of the labels, and an average localization precision comparable with the lattice length scales. 3D PALM data for Eos-tagged myopalladin and LIM-nebulette, or LASP2) showed novel organizational features, suggesting a new model for the localization of LASP2. Using these, and other examples, we have demonstrated that this tool can be used to find pattern information from samples with unknown structures, and expect this analysis to be applied to study regularity and symmetry in many other complexes.

MS101

**Single-molecule Imaging and Analysis of the Dynamic Organization of Vascular Endothelial Growth Factor 2 (VEGFR-2) on the Surface of Live Endothelial Cells.**

**B. Da Rocha-Azevedo**[1], S. Lee[2], A. Dasgupta[1], T. Kim[1], M. Kittisopikul[3,1], A. Vega[1,4], L. De Oliveira[1], K. Jaqaman[1,4]; [1]Biophysics, University of Texas Southwestern Medical Center, Dallas, TX, [2]Korea University, Seoul, KOREA, REPUBLIC OF, [3]Cell and Molecular Biology, Northwestern University Feinberg School of Medicine, Chicago, IL, [4]Bioinformatics, University of Texas Southwestern Medical Center, Dallas, TX.

VEGFR2 is a receptor tyrosine kinase thatplays an essential role in promoting angiogenesis in response to its ligand VEGF. The canonical model has been that VEGF binds to VEGFR2 monomers, leading to VEGFR2 dimerization, autophosphorylation and downstream signaling. Recent studies suggest that VEGFR2 can dimerize in the absence of VEGF, and that VEGF affinity is higher to pre-dimerized VEGFR2. However, little is known about VEGFR2 spatiotemporal organization, including its oligomeric state, in its

native plasma membrane environment in live endothelial cells. Therefore, we undertook a quantitative single-molecule study of VEGFR2 on the surface of live endothelial cells, labeling endogenous receptors and imaging the cell surface using TIRF microscopy, followed by automated single-molecule tracking and statistical data analysis. We found that VEGFR2 exhibits two movement types: (i) freely diffusing (~35%), with a mean diffusion coefficient of 0.18 $um^2$/sec; and (ii) confined/immobile (~65%). We observed a heterogeneity in VEGFR2's oligomeric state, with monomers, dimers and higher order oligomers/clusters. The freely diffusing molecules underwent more frequent interactions, but the confined/immobile molecules were on average of a higher oligomeric state. Addition of VEGF caused an almost immediate reduction in the diffusion coefficient of freely diffusing VEGFR2 molecules, followed by a more gradual shift (within 10 min) in the population from free to confined/immobile. Co-imaging of VEGFR2 and VEGF revealed that VEGF binds both monomeric and pre-oligomerized/clustered VEGFR2, with a preference for the latter, including those in the confined/immobile subpopulation. It also reduced the dissociation rate (to about one-half) of the dynamic interaction events. To understand better the link between VEGFR2 activation, its mobility and oligomeric state, we treated endothelial cells with the VEGFR2 phosphorylation inhibitor AAL993. We found that AAL993 treatment increased the probability of free diffusion and the frequency of dynamic interactions both in the absence and presence of VEGF, suggesting that phosphorylation plays a critical role in the spatiotemporal organization of VEGFR2 at rest and upon ligand binding. Together, these observations depict a dynamic organization of VEGFR2 on the surface of endothelial cells that both affects and depends on VEGF binding and downstream activation.

MS102

**Waiting to Die: Nucleation-limited Signalosome Assembly Renders Human Cell Fate Decisions Inevitable**

**R. Halfmann**[1,2], A. Rodriguez Gama[1], T. Kandola[1], S. Venkatesan[1], J. Wu[1,2], M. Hu[1]; [1]Stowers Institute for Medical Research, Kansas City, MO, [2]University of Kansas Medical Center, Kansas City, KS.

Multiple signaling proteins of the innate immune system exert their cellular activities by assembling into large macromolecular complexes known as signalosomes. We previously discovered that two such proteins - the pyroptosome scaffold ASC, and the antiviral signaling protein MAVS - each assemble through self-templating polymerization that is reminiscent of infectious protein particles known as prions (Cai et al. 2014; PMID: 24630723). More recently we revealed that prion-like activity broadly arises from structurally encoded kinetic barriers to nucleation - the probabilistic formation of a self-templating multimer de novo (Khan et al. 2018; PMID: 29979963). For proteins like MAVS and ASC, the nucleation barrier is so high that their soluble inactive states persist despite physiological concentrations that are highly supersaturated with respect to the assembled active state. To identify other innate immune signaling proteins that may function in this manner, we used DAmFRET, a flow cytometric cell-based assay of nucleation barriers, to screen 138 candidate prion-like modules from 129 human proteins that broadly function in programmed cell death and innate immune signaling. We discovered 36 of these proteins that are inherently capable of supersaturation and switch-like self-templating activation in living cells. We have further discovered a network of nucleating interactions between them, wherein polymerization of one protein nucleates the polymerization of specific additional proteins, resulting in a cascade of irreversibly activating protein switches. This widespread kinetic control over cell fate indicates that cells are literally waiting to die -- pyroptosis, necroptosis, and alternative cell fates downstream of these proteins are thermodynamically favored, and therefore inevitable with time. Surveying known disease-associated mutations in one such signalosome, we show that the nucleation

barrier for BCL10 is balanced between hypo-activation and immunodeficiency on the one hand, and hyper-activation and lymphoma on the other. Finally, I will discuss our investigations into the implications of this phenomenon for aging-associated inflammation and innate immune memory in human monocytes.

## Microsymposium 18: Stem Cell Differentiation and Techniques

MS103

**Visualizing the Proliferation/differentiation Decision Using a CDK Activity Sensor During Metazoan Development.**

**A. Q. Kohrman**[1], R. C. Adikes[1], J. J. Smith[1], A. S. Aydin[1], S. J. Collins[1], O. Ahmed[1], N. J. Palmisano[1], C. Tian[2], M. A. Martinez[1], T. Medwig-Kinney[1], S. Tank[1], S. Liu[3], W. Zhang[1], R. A. Morabito[1], N. Kim[1], E. Feiner[4], N. Weeks[1], G. H. Thomsen[1], T. Miller[1], L. A. Davison[5], S. L. Spencer[2], B. L. Martin[1], D. Q. Matus[1]; [1]Stony Brook University, Stony Brook Univesity, NY, [2]University of Colorado, Boulder, Boulder, CO, [3]University of California, Santa Cruz, Santa Cruz, CA, [4]Princeton Univesity, Princeton, NJ, [5]University of Pittsburgh, Pittsburgh, PA.

During organismal development, differential regulation of the cell cycle is critical to many cell biological processes, including cell fate specification and differentiation. While the mechanisms of the cell cycle are well studied, how the control of cell cycle is linked to differentiated cell behavior remains poorly understood. To truly understand this interplay, we must be able to directly and precisely measure cell cycle state. In order to characterize cell cycle state in living animals, we adapted a CDK2 biosensor for *in vivo* use. We generated transgenic strains in the nematode, *C. elegans*, and zebrafish, *D. rerio*, and performed transient RNA injections in the frog, *X. laevis*. The biosensor reports Cyclin-Dependent Kinase (**CDK**) activity as the cytoplasmic/nuclear localization ratio of a GFP-tagged human CDK2 substrate, DNA Helicase B (**DHB**). Phosphorylation of the sensor by CDKs causes its translocation from the nucleus to the cytoplasm. We have modified this sensor to allow for automated assessment of cell cycle state using a U-Net based automated image segmentation approach. Unlike other live cell cycle imaging tools (e.g., FUCCI), DHB can be used to distinguish actively cycling cells in the G1 phase of the cell cycle versus quiescent or terminally differentiated cells in G0. We have used this biosensor to quantify lineage specific differences between cycling cells, and to examine the link between quiescence and differentiation. We examine the interplay between proliferation, differentiation, and morphogenesis across multiple metazoan species and provide new biological insights into the control and timing of the metazoan cell cycle during specification and differentiation.

MS104

**Definitive Cell Fate Identification Reveals Dynamic Growth in Oncogenic Clones**

**M. V. Sandoval**[1,2], S. Beronja[2]; [1]University of Washington, Seattle, WA, [2]Fred Hutchinson Cancer Research Center, Seattle, WA.

Tissue growth is a highly coordinated process, subject to regulation by both cell autonomous mechanisms and signals from the extracellular environment. Together, these molecular inputs ensure a balance between growth promoting and growth suppressing mechanisms. In the skin epithelium, balanced growth is thought to be achieved by regulating stem cell fate choice, which has a direct effect on the size of the proliferative progenitor/stem cell pool. Cell fates are determined as the progenitors

undergo symmetric renewal, symmetric differentiation, or asymmetric division. If daughter cells undergo differentiation, thus losing mitotic potential, the progenitor pool decreases and tissue growth declines. When this process is disturbed, a single mutant cell can aberrantly divide and grow into a cancer. Understanding the mechanisms that govern normal growth and how these processes are dysregulated in disease is of vital importance.To study how murine tissues respond to activation of a common oncogene, we developed a novel cell fate identification (CFI) assay. CFI uses sequential EdU and BrdU incorporation to label dividing progenitors and then analyzes the fates of the two daughter cells. To model an oncogene-driven type of tissue growth, we induced $Hras^{G12V}$ expression in single cells in the adult epidermis. The immediate effect of $Hras^{G12V}$ expression was to increase the rate of progenitor cell renewal, consistent with an expected and positive effect on tissue growth. Interestingly, by following expanding $Hras^{G12V}$ clones over several weeks, we observed that the rate of progenitor renewal eventually decreases to WT levels. We show that this is driven by a switch in the mode of cell fate choices, where increased renewal was replaced by increased differentiation. This switch could be caused by the tissue responding to oncogenic expansion, suggesting that differentiation could be a method to stall oncogenic growth.In order to identify the mechanism of $Hras^{G12V}$-driven differentiation, we used an shRNA-mediated screen of Ras-effector genes to functionally probe the molecular pathways that govern differentiation of the epidermis. This screen identified Rassf5 as a gene of interest. Our validation studies demonstrated that shRNA knockdown of Rassf5 expression caused $Hras^{G12V}$ epidermis to undergo more renewing divisions. Conversely, $Hras^{G12V}$ epidermis that overexpresses Rassf5 undergoes more differentiating divisions. This study establishes an accessible assay with the ability to specifically determine cell fate and quantify the rate of tissue growth. Using this technology we demonstrated that oncogenic clones undergo dynamic growth, giving support to the idea that the epidermis is transiently tolerant of oncogene driven overgrowth.

MS105

**Understanding Principles of Stem Cell Differentiation in the Regenerating Epidermis**

**K. Cockburn**, V. Greco; Yale University, New Haven, CT.

Maintenance of highly regenerative epidermal tissues relies on the ability to produce differentiated cells to replace those that are lost. How resident stem cell populations generate the correct numbers of differentiated cells and how these cells are properly integrated into an adult tissue are not well understood. Here we use 2-photon intravital imaging of the mouse interfollicular epidermis to elucidate the early steps of stem cell differentiation. Using a combination of cell tracking and 3-dimensional morphological analysis, we find that cells exit the stem cell compartment through a delamination process that is not mediated by actomyosin extrusion and instead involves slow vertical elongation followed by lateral spreading over their former basal neighbors over the course of 1-2 days. By combining our analysis with a live reporter of differentiation status, Keratin10, we find that stem cells begin to differentiate up to 36h before they initiate the delamination process. Surprisingly, during this window of time a large proportion of these early differentiating cells divide, producing two daughter cells that complete the differentiation trajectory and exit the stem cell compartment. Finally, to ask whether these divisions occur as part of an obligate transit amplifying program we block them and find that the tissue can recover from their loss by non-autonomously increasing the number of stem cells that initiate the differentiation process. Together, these results define epidermal differentiation as an inherently flexible process, with differentiating stem cells able to divide if needed and the overall tissue able to modulate differentiation rates to meet demand.

MS106

**Isolation and Characterization of Putative Skeletal Stem Cells From the Effluent of Autologous Bone Graft Preparations**

**B. Le**, A. Wessel, J. Florez, III, W. Yao, M. Lee, F. A. Fierro; University of California Davis, Sacramento, CA.

The recently identified human skeletal stem cells (hSSCs) are essential for maintaining bone homeostasis and for promoting bone repair. Therefore, they present a tremendous potential for the treatment of many orthopaedic applications. However, a major bottleneck is the limited sources for these rare cells. A common method for the harvest of autologous bone graft is the Reamer Irrigator Aspirator (RIA) system, where the intramedullary canal of a long bone (often femur) is reamed, irrigated and aspirated with a filter trap to collect semi-solid stroma/bone material used for grafting procedures. During this procedure, a large volume of liquid and small particles is discarded as waste. Here we show that this waste fluid (RIA effluent), is rich in CD45-CD51+CD200+ cells, which are putative hSSCs. Using single cell RNA sequencing, we demonstrate that this cell population is quite homogeneous, with differentially expressed genes associating with mitochondrial genes and cell signaling. In immune deficient mice undergoing bone fracture, intramuscular injection of hSSCs leads to a significant improvement in bone repair as shown by microCT and calcein staining. Of note, we show that these cells home preferentially to bone and bone marrow after intravenous injection, suggesting that the cells may be applicable to treat systemic skeletal defects, such as osteoporosis. Altogether, our data shows that RIA effluents are a rich source of potential hSSC, which could lead to the development of new regenerative medicines for orthopedic applications.

MS107

**Recapitulating Biological Signaling Scenarios with Spatiotemporal Control Using a Multiplexed, DNA-Patterning Approach**

**O. J. Scheideler**, D. V. Schaffer, L. L. Sohn; University of California, Berkeley, Berkeley, CA.

Dissecting the complex action of the biological signals that orchestrate single-cell fate decisions as well as tissue function/dysfunction is challenging due to the spatiotemporal parameters modulating their actions. This complexity is further compounded by the fact that these signals—soluble ligands, extracellular matrix-sequestered cues, and cell surface-presented cues—comprise a larger network in which multiple signals are coordinating with each other across time and space. In order to model and investigate such dynamic signaling scenarios *in vitro*, we have engineered a DNA-based patterning platform that imparts unprecedented spatial and temporal control over individual and multivariable signaling scenarios. Specifically, we utilize photolithography to generate a patterned photoresist (PR) template that guides the conjugation of DNA oligonucleotides onto a surface. The PR-template can be stripped and a new one patterned without damaging the initial DNA layer. As we demonstrate, we can subsequently register and layer up to 10 orthogonal DNA strands, remarkably without sacrificing lithographic resolution. The resulting, multiplexed DNA patterns are highly functional and can hybridize to complementary DNA sequences that have been tagged to cells and/or solid-phase signaling ligands. The precision of the DNA patterns is thus transferred to the assembled *in vitro* model, imparting micron-scale control in recapitulating single-cell to bulk-tissue signaling environments. To demonstrate our platform's utility in de-coupling the spatial dependencies of biological signals, we investigated how two competing niche signals, fibroblast growth factor-2 (FGF-2) and ephrin-B2, coordinate spatially to

instruct adult neural stem cell fate decisions at the single-cell level. By modulating their spatial presentation, we observe a strong spatial bias toward FGF-2 that, in some unexpected cases, did not translate to the predicted phenotype of high proliferation and maintenance of stem cell identity but rather high neuronal differentiation. To achieve temporal control within our platform, we demonstrate various nuclease-based strategies to modulate the action of DNA-hybridized signals. With these spatiotemporal capabilities in-hand, our DNA-directed approach provides opportunities to investigate the underlying signaling mechanisms of key processes across a wide range of biological disciplines.

MS108

**High-throughput Microfluidic Platform for in Vitro Vascularization of Human Organoids and Tissue Explants - Cell Cultures' Missing Component?**

**D. Kurek**[1], S. Previdi[1], F. Bonanini[1], S. De Ruiter[1], S. J. Trietsch[1], A. Nicolas[1], D. Hendriks[2], D. Dutta[2], H. Hu[2], H. Clevers[2], H. Lanz[1], P. Vulto[1], J. Joore[1]; [1]MIMETAS B.V., Leiden, NETHERLANDS, [2]Hubrecht Institute, Utrecht, NETHERLANDS.

Vasculature is a crucial ingredient of human organs and tissues. In addition to being a vehicle for blood circulation, exchange of angiocrine factors contribute to tissue function and development. The challenge in creating better biomimetic models lies in capturing the 3D morphology, heterogeneity and boundary aspects of tissues. Microtissues such as spheroids or organoids show often limited growth potential and an immature phenotype due to the lack of circulation. One way to improve vascularization of organoids or explants is by transplanting them into a host (*i.e.* mouse). However, the low predictivity of animal models, boost the development of *in vitro* alternative strategies. Microfluidic techniques are increasingly recognized as an important toolbox able to add physiologically relevant cues to traditional cell culture models. Here, we introduce a microfluidic platform that is capable of vascularization of tissues such as organoids, spheroids and tissue fragments. The platform comprises 64 independent microfluidic chips arranged in a microtiter plate format. Each chip comprises an extracellular matrix gel that is patterned by means of a surface tension technique called PhaseGuiding. In each chip two blood vessels are grown on lateral side of the free-standing ECM gel. A gradient of angiogenic factors is applied to induce vascular bed formation. Tissues are grafted onto the vascular bed by placing it on top of the ECM gel, containing the vascular bed. Tissue dependent vessels remodeling and stabilization can be monitored overtime by real time imaging and barrier integrity. When liver spheroids or organoids are used, vessels became stabilized and fully leaktight in the system. Moreover, the expression of $CD31^+$ cells around and in within the spheroids proves that endothelial cells migration and tissue envelopment occurred during co-culture. The high number of units (up to 64 chips in 384 well format) enables functionality studies and compound screening in a robust and automated way. We propose the use of the Organoplate® Graft as a vessels grafting platform for multiple 3D tissues allowing drug discovery and disease modeling in a more physiological environment. The method provides an in vitro alternative to current xenograft techniques and may fill up a crucial gap in current day cell culture.