abstracts: oral presentations

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The discussion of American science education is often framed by the questions: Why do American precollege students do poorly on international science assessments and what we are doing wrong? Rather we need to ask: why do so many international students come to US universities for science and what are we doing right? We also need to ask: How do we stay ahead in science education? Our studies of 6,000 high school teachers in 1998 and 2018 provided a pre-COVID snapshot of what is right with American biology education, and documented striking success in retooling classrooms for lab-based instruction in biotechnology. During this time, student exposures to hands-on biotech labs increased 26%, and schools with biotech electives doubled to 35%. However, there was a significant decline in teacher involvement in science extracurricular activities and membership in professional societies. For example, while 65% of 1998 teachers had attended one or more professional meetings, 60% of 2018 teachers had attended none. Consistently over this 20-year period, teachers said they valued week-long or longer summer institutes and workshops at professional meetings as the most important contributors to innovation in the classroom. Traditionally, the National Science Foundation (NSF) has been the major provider of high-quality training for precollege biology teachers. However, analyzing 7,500 entries in a database of NSF grant awards, we found that training opportunities had declined 75% since a peak in 1994. The wide availability of NSF teacher institutes in the 1980s and 1990s helped develop a culture of lifelong learners who responded to the challenge of bringing advances in biotechnology into precollege classrooms. Our study found evidence for erosion of this culture of involved, innovative teachers. Furthermore, we found that the majority of biotech electives are located in zip codes with above-average income. As we emerge from the COVID-19 pandemic, we need to consider how we can help biology teachers return to hands-on instruction and keep up with quickening advances in biological sciences. We also need to level the science playing field for disadvantaged and underrepresented minority students. It is time for NSF to renew its commitment to summer institutes and academic-year workshops as critical contributors to teachers’ lifelong learning and classroom innovation.
**PORTER LECTURE**

**A2**

**Fun experiments you can only do with frogs.**

*R. Heald*; University of California, Berkeley, Berkeley, CA.

To mediate chromosome segregation during cell division, the microtubule-based spindle size adapts to changes in cell size and shape, which vary dramatically across species and within a multicellular organism. However, the nature of scaling events and their underlying mechanisms are poorly understood. To elucidate molecular mechanisms, we take advantage of in vitro systems, particularly cytoplasmic extracts prepared from eggs of the frog *Xenopus laevis* that reconstitute mitotic chromosome condensation and spindle assembly and function in vitro. To study mechanisms of spindle and nuclear size control, we have utilized a smaller, related frog, *Xenopus tropicalis*, to investigate interspecies scaling, and extracts prepared from fertilized eggs at different stages of embryogenesis to study size scaling that occurs during early development. We use phylogenetic comparisons to characterize the key players that define spindle architecture, and are developing the use of other amphibian systems to investigate size control mechanisms at the subcellular, cellular and organism levels. Our studies aim to reveal underlying principles of spindle assembly and biological size control, as well as the molecular basis of variation that contributes to genomic instability and evolution.

**EMBO GOLD MEDAL LECTURE**

**A3**

**Mechanisms of intracellular DNA sensing through the cGAS-STING pathway**

*A. Ablasser*; École Polytechnique Fédérale de Lausanne, Lausanne, SWITZERLAND.

The life of any organism depends on the ability of cells to detect and to respond to pathogens. In order to detect the immense variety of pathogenic entities, the innate immune system of mammals has evolved a range of distinct sensing strategies. One major mechanism is based on the recognition of microbial DNA—an invariant and highly immunogenic pathogen-associated molecular pattern. Host cells, however, contain abundant sources of self-DNA. In the context of cellular damage or metabolic derangement, “out-of-the-context” self-DNA can elicit potentially damaging inflammatory responses. Our research focuses on the so-called cGAS-STING system—an evolutionary highly conserved innate DNA sensing system. On DNA binding, cGAS is activated to produce a second messenger cyclic dinucleotide (cyclic GMP-AMP), which stimulates the adaptor protein STING to induce innate immune responses. While this process was originally discovered as a crucial component of immune defense against pathogens, recent work has elucidated a pathogenic role for innate DNA sensing in a variety of sterile inflammatory diseases. In this talk I will discuss recent findings on cellular mechanisms that regulate cGAS activity and present work on the pharmacological manipulation of aberrant cGAS-STING signaling in the context of inflammatory diseases.
THURSDAY, DECEMBER 9, 2021

E.B. Wilson Award Lecture

A4
A Scientific Journey at the Interface of Cell Biology and Neuroscience: From Membrane Traffic to Neurodegenerative Diseases
P. De Camilli; Yale University School of Medicine/HHMI, New Haven, CT.

Throughout my scientific career I have worked at the intersection of fundamental cell biology and neuroscience, with an emphasis on membrane traffic at neural synapses. Studies of presynaptic nerve terminals, highly specialized cellular compartments, have helped reveal new principles in endocytosis and membrane recycling and have shed light on the critical role of lipid metabolism and transport in the control of membrane dynamics. I will discuss how some of these basic studies are currently yielding insights not only into neuronal physiology but also into pathogenetic mechanisms in neurodegenerative diseases. Conversely, recent clues from the genetics of neurodegenerative diseases have helped open new lines of research into basic cellular mechanisms.

E.E. Just Award Lecture: Race to the COVID-19 Vaccine: Then & Now

A5
Rapid Development of COVID-19 mRNA Vaccine: Then and Now
K. S. Corbett; Harvard, Boston, MA.

A vaccine for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is needed to control the coronavirus disease 2019 (COVID-19) global pandemic. Structural studies have led to the development of mutations that stabilize Betacoronavirus spike proteins in the prefusion state, improving their expression and increasing immunogenicity. This principle has been applied to design mRNA-1273, an mRNA vaccine that encodes a SARS-CoV-2 spike protein that is stabilized in the prefusion conformation. mRNA-1273 has been deemed safe and effective, with a 94.1% efficacy rate and is being used in multiple countries. This seminar will give you an inside scoop of rapid development and forward-moving science of the vaccine.
SYMPOSIA

TUESDAY, DECEMBER 7, 2021

Intra- and Intercellular Communication

S1
Systematic Analysis Of Contact Site Proteomes Reveals Novel Players In Cellular Homeostasis

M. Schuldiner; Weizmann Institute of Science, Rehovot, ISRAEL.

To communicate and work cooperatively, organelles must come into close proximity at membrane contact sites to transfer lipids and small metabolites. Despite our increasing understanding of membrane contact sites, many of their molecular components have yet to be identified, making it difficult to investigate their over-arching roles in cellular and organism function. To overcome this limitation, we established a systematic and high throughput microscopy approach to identify contact site resident proteins in the budding yeast Saccharomyces cerevisiae. Using this method, we have identified multiple new contact site proteins. I will share an example of how mechanistic follow-up on such new contact residents is leading to a new understanding of organelle Biology.

S2
The Neuro-vascular Interactions In The Brain

C. Gu; Harvard Medical School, Boston, MA.

The relationship between the brain and its vasculature is different than that of other organs. The brain vasculature has two distinct features that cater to the brain’s unique functions. First, neurons in the brain are extremely sensitive to their extracellular chemical environment and each neuron is at most 15 microns away from a capillary. Brain endothelial cells forming the blood vessel walls constitute a blood-brain barrier (BBB) to provide a safe and homeostatic environment for the brain. Second, despite representing only 2% of the body weight, our brain consumes 20% of body’s energy at rest and has very limited ability to store energy. So, to meet moment-to-moment changes in regional brain energy demand, neural activity rapidly increases local blood flow, a process called neurovascular coupling. I will present our recent progress on the molecular mechanistic understanding of how the brain vasculature executes these functions, and how unique vascular demands of the brain have led to molecular, cellular, and trans-cellular specializations unlike those found in other tissues.

The Nucleus

S3
Spatially Coordinated Heterochromatinization of Distal Short Tandem Repeats in Fragile X Syndrome

J. Phillips-Cremins1,2,3,4, 1Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, 2Epigenetics Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 3Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 4New York Stem Cell Foundation – Robertson Investigator, New York, NY.
Short tandem repeat (STR) instability is causally linked to pathologic transcriptional silencing in a subset of repeat expansion disorders. In fragile X syndrome (FXS), instability of a single CGGSTR tract is thought to repress \textit{FMR1} via local DNA methylation. Here, we report the acquisition of more than ten Megabase-sized H3K9me3 domains in FXS, including a 5-8 Megabase block around \textit{FMR1}. Distal H3K9me3 domains encompass synaptic genes with STR instability, and spatially co-localize in trans concurrently with \textit{FMR1} CGG expansion and the dissolution of TADs. CRISPR engineering of mutation-length \textit{FMR1} CGG tonormal-length preserves heterochromatin, whereas cut-out to pre-mutation-length attenuates a subset of H3K9me3 domains. Overexpression of a pre-mutation-length CGG de-represses both \textit{FMR1} and distal heterochromatinized genes, indicating that long-range H3K9me3-mediated silencing is exquisitely sensitive to STR length. Together, our data uncover a genome-wide surveillance mechanism by which STR tracts spatially communicate over vast distances to heterochromatinize the pathologically unstable genome in FXS.

S4  
Deciphering the Mechanism of RNA Splicing Reveals the Secret Life of Introns  
\textbf{T. Johnson}; University of California, Los Angeles, Los Angeles, CA.

Pre-messenger RNA splicing is carried out by the spliceosome, a large ribonucleoprotein complex that is functionally conserved across eukaryotes. The spliceosome assembles onto pre-mRNA co-transcriptionally, raising important questions about how the process of transcription through a chromatin template influences spliceosome assembly and splicing outcomes. Moreover, regulation of the fate of both spliced and, unexpectedly, the unspliced RNA provides an elegant mechanism for controlling gene expression. Here we show that regulated intron removal and regulation of the fate of unspliced RNAs are crucial for the cell’s response to its environment, with important implications for human health.

WEDNESDAY, DECEMBER 8, 2021

Biomolecular Condensates

S5  
\textbf{Biomolecular Condensates At The Nexus Of Cellular Stress, Disease And Aging}  
\textbf{S. Alberti}; Technische Universität Dresden, Dresden, GERMANY.

Biomolecular condensates formed by phase separation are membraneless compartments in the cytoplasm and nucleoplasm of cells, which have major roles in cellular organization and physiology. RNP granules are a specific type of condensate that assemble from RNA-binding proteins and RNA. In this talk, I will discuss how the concept of biomolecular condensates has expanded our view of RNP granules and their link to disease, aging and the cellular stress response. I will introduce in vitro reconstitution systems based on the concept of phase separation that now allow us to reconstruct RNP granules in the test tube. Using these reconstitution systems as well as cell biological and genetic approaches, we have gained important insights into the molecular rules of RNP granule assembly, such as the driving forces and amino acids that govern condensation, the conformational changes underlying assembly and molecular mechanisms of condensate regulation and control. I will further discuss how the concept of phase separation has allowed us to dissect the functions of RNP granules and I will demonstrate how
phase separation can be used by cells to sense and respond to changes in the environment and regulate fundamental cellular processes such as protein synthesis.

S6  
**Temperature Adaptation In Phase Separating Systems**  
**A. S. Gladfelter;** University of North Carolina at Chapel Hill, Chapel Hill, NC.

Free-living microbes, plants and cold-blooded organisms survive in the face of temperature fluctuations that arise across many time scales. Climate change is increasing the amplitude and frequency of temperature variations in the natural world and biological phase separation may be a key mechanism of adaptation of the biosphere to climate change. The focus of this presentation will be on our recent work to identify how protein and RNA sequence encodes temperature sensitivity and how material properties of condensates are maintained across temperature fluctuations. For these studies we are using two different temperature sensitive condensate systems. In one area, we are examining the nucleocapsid/genomic RNA interaction required for packaging the SARS CoV-2 genome for replication. In a second area, we focus on a model phase separation protein, Whi3, in the syncytial filamentous fungus, Ashbya gossypii. This protein binds to and regulates specific RNAs important for cell cycle control and cell polarity. In both the viral and fungal systems, we have found sequence variation within the core protein/RNA components are sufficient to induce highly tunable temperature sensitivity for condensation. Sequence elements controlling protein-protein, protein-RNA and RNA-RNA interactions all contribute to modulating higher-order assembly and function in different temperature regimes. These studies indicate that small changes in protein and RNA sequences can promote organism adaptation to different climates providing potential mechanisms for adaptation of the biosphere to climate change.

**Signaling and Metabolism**

S7  
**Feeding-induced Cholesterol Biosynthesis**  
**B. Song;** Wuhan University, Wuhan, CHINA.

Cholesterol is an essential lipid and it costs lots of nutrients and energy to make such a molecule. Therefore, mammals increase cholesterol biosynthesis only after feeding and inhibit the process under fasting condition. However, the regulatory mechanisms of cholesterol biosynthesis at fasting-feeding transition are not fully understood. Here we show that the deubiquitylase USP20 stabilizes HMG-CoA reductase (HMGCR), the rate-limiting enzyme in cholesterol biosynthetic pathway, at feeding state. The post-prandially increased insulin and glucose stimulate mTORC1 to phosphorylate USP20 at S132 and S134, which is further recruited to the HMGCR complex and antagonizes its degradation. The feeding-induced stabilization of HMGCR is abolished in the liver-specific Usp20 deficient mice and the Usp20-S132A/S134A knock-in mice. Genetic deletion or pharmacological inhibition of USP20 dramatically decreases diet-induced body weight gain, reduces lipid levels in the serum and liver, improves insulin sensitivity as well as increases energy expenditure. These metabolic improvements by USP20 inhibition are reversed by the constitutively stable HMGCR(K248R). This study reveals an unexpected regulatory axis from mTORC1 to HMGCR through USP20 phosphorylation and demonstrates USP20 inhibitor as a
potential cholesterol-lowering drug to treat metabolic diseases including hyperlipidemia, liver steatosis, obesity and diabetes.

S8

**New Sides for an Old Coin: Copper as an Intracellular Mediator of Kinase Signaling in Cancer**

D. C. Brady\(^{1,2,3}\); \(^{1}\)Department of Cancer Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; \(^{2}\)Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; \(^{3}\)Abramson Cancer Center Tumor Biology Program, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Kinases respond to and, in some cases, sense inputs such as growth factors, nutrients, and metabolites, in order to relay information that drives complex cellular processes. Aberrant kinase activation disrupts the balance between cell growth and cell death and, consequently, can drive cancer initiation and progression. While kinase inhibitors have dramatically changed the landscape of cancer treatment, the near-universal emergence of resistance limits their clinical durability. Our research program is founded in a new paradigm in nutrient sensing and protein regulation, termed metalloallostery, whereby redox-active metals control kinase activity. Our laboratory’s focus lies at the intersection of kinase signaling and copper (Cu) homeostasis with the goal of defining the mechanisms regulating Cu-dependent kinases in order to target them in cancer through drug development or repurposing.

Previously we discovered that the transition metal Cu, which is acquired as a dietary nutrient and is essential for life, activates the canonical MAPK signaling pathway at the level of the MEK1/2 kinases established an evolutionarily conserved, critical mechanistic function for Cu as an intracellular mediator of signaling. The direct interaction between Cu and MEK1/2 is the first example of Cu enhancing the activity of a mammalian kinase and revealed a novel vulnerability that can be exploited therapeutically in cancers with aberrant MAPK signaling. Beyond the Cu-MEK1/2 interaction, we discovered that Cu binds to, and is required for, ULK1/2 activity, thereby serving as a critical input for ULK1/2-dependent autophagy. Dual targeting of MAPK signaling and ULK1/2-driven autophagy by limiting Cu availability and, therefore, kinase Cu binding, decreased oncogenic KRAS-driven tumor growth and survival. These findings chart new ground in nutrient signaling, cellular energy homeostasis, and metabolic vulnerabilities in cancer, further establish Cu as a signaling molecule, define the molecular basis for a new Cu-dependent cellular process, and exploit Cu-dependent kinases to target oncogene-driven dependencies. The emergence of this new and clinically relevant signaling paradigm has highlighted the need to understand how redox-active metals interact with signaling pathways and underscores the promise of discovering new modes of kinase regulation as orthogonal therapeutic vulnerabilities.
Cell and Tissue Mechanics

S9
Nuclear Mechanotransduction: Regulation of Cell Fate and Beyond
S. A. Wickstrom; University of Helsinki, Helsinki, FINLAND.

Cells are constantly subjected to a spectrum of mechanical cues, such as shear stress, compression, differential extracellular matrix rigidity, and strain, to which they respond to by engaging mechanisms of mechanotransduction. These forces function as important morphogenetic cues that are transmitted to the nucleus to alter genetic programs. On the other hand, excessive mechanical stresses have the potential to damage cells and tissues. In my presentation I will discuss our recent research on how mechanical signals are transmitted from the extracellular matrix into the nucleus to coordinate cell fate changes. I will further discuss how dynamic changes in chromatin organization in response to force change the mechanical properties of the nucleus and chromatin to prevent damage, as well how cells can adapt to differential force environments by changing their structure.

S10
Torsional response of actin filaments
J. P. Bibeau, N. G. Pandit, W. Cao, E. M. De La Cruz; Yale University, New Haven, CT.

Actin filaments experience, sustain and respond to mechanical forces, including compression, extension, bending, and twisting. How actin filaments respond to these forces is central to understanding filament and network mechanical properties, force generation, and mechano-sensing. A great deal has been learned about how filaments and networks respond and adapt to compressive and pulling forces, but less is known about how filaments respond to twisting loads. We used magnetic tweezers and microfluidics to twist actin filaments while visualizing their response, specifically changes in shape, with total internal reflection fluorescence microscopy. Twisting actin filaments about their long axis causes them to loop and fold into plectoneme-like structures. A model in which an actin filament is treated as a twistable worm-like chain accounts for the experimental data, reveals how filament twisting and bending mechanical properties are coupled, and yields the filament bending and twisting stiffnesses. This information is used to understand the mechanical and thermodynamics of filament stability and fragmentation.

Imaging Across Scales

S11
Advanced Imaging Of Cell Division Processes Across Scales.
J. Ellenberg; EMBL, Heidelberg, GERMANY.

The recent resolution revolution in microscopy technologies allows unprecedented insights into the molecular machinery inside living systems. 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 molecular machines to imaging a whole living organism. Combined with machine learning driven image analysis and open sharing of image data, this provides unprecedented opportunities for new insights. This presentation will give examples at different scales where we have used and further developed advanced microscopy approaches ranging from correlative light and electron microscopy (CLEM) and super-resolution microscopy to quantitative live-cell imaging and whole embryo light-sheet microscopy to study assembly of individual protein complexes and protein networks inside cells and reveal the aneuploidy causing cell division errors inside developing mammalian embryos.

S12
Towards A Bright Future For Artificial Intelligence-powered Cell Biology
D. Van Valen; California Institute of Technology, PASADENA, CA.

Recent advances in imaging and genomics have changed the nature and scale of biological imaging data, while concurrent advances in machine learning have made it easier to convert imaging data into interpretable and actionable information. In this talk, I survey the progress the field has made in adapting deep learning methods to a variety of challenges in cellular image analysis, including image restoration, augmented microscopy, cell segmentation, and cell tracking. I describe use cases in which these methods were used to accelerate biological discovery and highlight attempts to incorporate them directly into new experimental designs. Last, I discuss the challenges that must be overcome for imaging-based cell biology to reap the full benefit of the artificial intelligence revolution.

FRIDAY, DECEMBER 10, 2021
Disease and Aging

S13
Organelle Degradation By Autophagy-dependent And Independent Pathways
N. Mizushima; The University of Tokyo, Tokyo, JAPAN.

Eukaryotic cells contain many organelles, with organellar membranes estimated to occupy 20-50 times the area of the plasma membrane. Organelles are constantly synthesized and eliminated. In general, intracellular organelles are thought to be degraded mainly by autophagy, whereby cytoplasm material is engulfed by autophagosomes that then fuse with lysosomes. It is well established that autophagy can selectively degrade organelles such as depolarized mitochondria (mitophagy), damaged lysosomes (lysophagy), and fragments of the endoplasmic reticulum (ER-phagy). Recognition is achieved by autophagy adaptors/receptors that link autophagosomal membranes to organelles. Autophagy adaptors/receptors can be already present on organelles or soluble until recruited to mark organelles. Evidence suggests that defects in organellar autophagy can lead to diseases; for example, mutations in mitophagy-related proteins have been identified in familial Parkinson’s diseases. Although autophagy is the primary means of organellar degradation, organelles can also be degraded in an autophagy-independent manner, as seen during terminal differentiation of the eye lens, when all organelles are degraded. This large-scale organelle degradation is mediated not by autophagy but by cytosolic PLAAT (phospholipase A and acyltransferase) family phospholipases. This newly discovered method of organelle degradation is essential for the lens to acquire optimal transparency. Thus, various mechanisms exist for organelle degradation.
S14

Why so Many Ways to Die? The non-canonical Inflammasome Pathway

V. M. Dixit; Genentech, South San Francisco, CA.

Intracellular lipopolysaccharide (LPS) from Gram-negative bacteria including Escherichia coli, Salmonella typhimurium, Shigella flexneri, and Burkholderia thailandensis activates mouse caspase-11 causing pyroptotic cell death, IL-1β processing, and lethal septic shock. How caspase-11 drives these downstream signaling events is largely unknown. Here we show that Gasdermin-D (Gsdmd) is essential for caspase-11-dependent pyroptosis and IL-1β maturation. A forward genetic screen with ethyl-N-nitrosourea-mutagenized mice linked Gsdmd to the intracellular LPS response. Macrophages from Gsdmd−/− mice generated by gene targeting also exhibited defective pyroptosis and IL-1β secretion induced by cytoplasmic LPS or Gram-negative bacteria. In addition, Gsdmd−/− mice were protected from a lethal dose of LPS. Mechanistically, caspase-11 cleaved Gsdmd and the N-terminal Gsdmd fragment promoted both pyroptosis and NLRP3-dependent activation of caspase-1 in a cell-intrinsic manner. Our data identify Gsdmd as a critical target of caspase-11 and a key mediator of the host response against Gram-negative bacterial infection. At the meeting, I’ll give an update on the discovery of NINJ1, a mediator of plasma cell rupture, the final cataclysmic event in lytic cell death. NINJ1 is a cell surface protein with two transmembrane regions. Its discovery overturns long-held dogma that cell death-related plasma membrane rupture is a passive event.

Modeling Organogenesis

S15

Exploring Mechanisms Of Human Brain Expansion In Cerebral Organoids

M. Lancaster; MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM.

The human brain sets us apart as a species, with its size being one of its most striking features. Brain size is largely determined during development as vast numbers of neurons and supportive glia are generated. In an effort to better understand the events that determine the human brain’s cellular makeup, and therefore its size, we use a human model system in a dish, called cerebral organoids. These 3D tissues are generated from pluripotent stem cells through neural differentiation and a supportive 3D microenvironment to generate organoids with the same tissue architecture as the early human fetal brain. Such organoids are allowing us to tackle questions previously impossible with more traditional approaches. Indeed, our recent findings provide insight into regulation of brain size and neuron number across ape species, identifying key stages of early neural stem cell expansion that set up a larger starting cell number to enable the production of increased numbers of neurons. We are also investigating the role of extrinsic regulators in determining numbers and types of neurons produced in the human cerebral cortex. Overall, our findings are pointing to key, human-specific aspects of brain development and function, that have important implications for neurological disease.
Multicellular organisms are composed of cells and tissues with identical genomes but different properties and functions. They all develop from one cell to form multicellular structures of astounding complexity. During development, in a series of spatio-temporal coordinated steps, cells differentiate into different cell types and establish tissue-scale architectures and functions. Throughout life, continuous tissue renewal and regeneration is required for tissue homeostasis, which also requires fine-tuned spatio-temporal coordination of cells. I will discuss how cellular interactions generate the specific contexts and spatio-temporal coordination underlying development and regeneration and how we specifically investigate what are the molecular and physical mechanisms that allow a cell, in a tissue, to sense its complex environment, to take individual coordinated decisions. Moreover, I will discuss the molecular mechanisms of intestinal organoid self-organization and the role of cell-to-cell variability in populations of differentiating cells during symmetry breaking.
MINISYMPOSIA

EDUCATION MINISYMPOSIUM: SILVER LININGS: RESPONSIVE TEACHING THROUGH MAJOR TRANSITIONS AND BEYOND

EM1
Evaluating the Impact of Student Created Videos in an Undergraduate Biology Classroom
K. Hefferon; Cornell University, Ithaca, NY.

This study examines the effectiveness of a video project aimed at incorporating metacognitive [KH1] [KH2] [KH3] and inclusive teaching practices to support learning in undergraduate Biology courses. Based on the assumption that the process of producing a video explanation of a complicated concept would deepen student learning, instructors in Plant Science and Microbiology courses assigned similar video projects targeting central course topics. The authors incorporated pedagogical theories about promoting inclusivity and metacognitive [AVL4] [AVLS] awareness into an assignment in which students explained a complex course topic in a short video production. We found that students showed pre-post learning and confidence gains in subjects relating to the assignment beyond those due to typical course activities. Further, evidence suggests that the video assignment also enhanced student engagement and self-expression. The video project under study has proven to be useful in both face-to-face and remote settings, as either a required or extra-credit course assignment. We propose that its versatility and effectiveness can enhance many learning environments.

EM2
Student outcomes from a large-enrollment undergraduate introductory course-based undergraduate research experience in remote learning with laboratory kits sent to students
S. M. Lo; University of California San Diego, La Jolla, CA.

Research experiences are important in undergraduate biology education and lead to improved outcomes. Course-based undergraduate research experiences (CUREs) can be designed as part of the standard laboratory curriculum to provide research experiences to more students. Previously, we reported the design and implementation of an introductory large-enrollment introductory CURE on soil microbiomes. The COVID-19 pandemic necessitated the move to remote learning and instruction, where hands-on experiences on campus became impossible. The course was offered in person in Fall 2019 and Winter 2020, remote without hands-on experiences in Spring 2020, and remote with laboratory kits sent to students at home in Fall 2020, Winter 2021, and Spring 2021. The laboratory kits provided equipment to complete about half of the experiments that were in the in-person CURE. Students also sent soil samples to the university for 16S metagenomic sequencing using Illumina technologies. The whole-course microbiome dataset was used for further analyses and hypothesis testing in the course. Here, we describe student outcomes from this CURE using pre- and post-course survey with items from validated instruments on self-efficacy on research skills, project ownership, and sense of community. Enrollment ranged from 400-600 students each quarter, with survey response rates >70%. Survey data are used in a quasi-experimental approach to compare the three versions of the CURE. In the in-person CURE,
Students reported a pre-course rating of 3.76 ± 0.85 (mean and standard deviation) and a post-course rating of 4.33 ± 0.87 for the 25 items on research self-efficacy with an effect size of 0.67. In the remote CURE with laboratory kits, students reported pre- and post-course ratings of 3.85 ± 0.73 and 4.34 ± 0.68, also with an effect size of 0.67. The remote CURE with laboratory kits had comparable self-efficacy gains to the in-person version, suggesting that some elements of the in-person experience could be recapitulated. In contrast, the remote CURE with no hands-on experience resulted in lower self-efficacy gains with an effect size of 0.52. Therefore, our results indicate that remote CUREs with laboratory kits can provide a mechanism to further democratize research experiences to students who are in distance learning programs or may be unable to participate in person as the pandemic continues.

EM3

The Cell Biology Education Consortium. Building an Undergraduate Research Infrastructure
N. S. Reyna, M; Biology, Ouachita Baptist University, Arkadelphia, AR.

To successfully develop a research-based curriculum, faculty must have a viable research program themselves. To address this need, the Cell Biology Education Consortium (CBEC/www.cellbioed.com) created "Cell Blocks" as a means for faculty to share (cancer) cell culture- experiments and projects. Cell Blocks consist of a video protocol, written protocol, and classroom implementation strategies. Most current CURE initiatives focus on students conducting predefined projects. However, this often limits student creativity and the ability to scaffold research throughout a program's curriculum. Because Cell Blocks are similarly formatted, they serve as mix-and-match modules, allowing faculty and students to develop projects that are of interest to them, relevant to their course content, and feasible at their institutions. Examples of student-developed video protocols can be seen on our YouTube channel. By blurring the lines between the classroom and the research lab, the CBEC seeks to improve undergraduate research and impact education. We have taken a collaborative approach to Cell Block development. As a result, we have grown nationally and now incorporates diverse faculty and students throughout the U.S. and Puerto Rico. I will present how Cell Blocks were used to change the focus of my research and better impact my students' learning.

EM4

Challenges and opportunities for students with disabilities in undergraduate research
L. E. Gin, D. A. Pais, K. M. Cooper, S. E. Brownell; School of Life Sciences, Arizona State University, Tempe, AZ.

Individuals with disabilities are underrepresented in postsecondary science education and in science careers, yet few studies have explored this. A primary predictor of persistence in science is participating in undergraduate research experiences (UREs). However, it is unclear to what extent students with disabilities are participating in UREs and what the experiences are like for students who do participate. To address this gap in the literature, in Study I, we conducted a national survey of over 1200 undergraduate researchers to determine the percent of students with disabilities participating in undergraduate research in the life sciences. We found that 12% of undergraduate researchers surveyed self-identified as having a disability, which when compared to the 18% of undergraduate life sciences majors with disabilities indicates that students with disabilities are likely underrepresented in UREs. In Study II, we conducted interviews with 20 undergraduate researchers with disabilities in the life sciences to understand the challenges that students experience in UREs, how students with disabilities navigate
challenges in UREs, the benefits that students with disabilities experience in UREs, and the ways in which students with disabilities uniquely contribute to the scientific community. We used the medical and social models of disability along with the community cultural wealth framework to characterize challenges and opportunities. Two researchers used inductive coding to develop a codebook of themes that emerged from the interviews. Both researchers coded a subset of 25% of the interviews and had an acceptable interrater reliability score (κ = 0.89). One researcher coded the remaining interviews. We identified (1) that students with disabilities experienced unique challenges in UREs including difficulties carrying out specific tasks. Students often described their challenges using the medical model, suggesting that challenges were due to their disability instead of using the social model, which asserts that challenges are due to structural aspects of UREs that exclude individuals with disabilities. (2) Students navigated challenges by advocating for informal accommodations from research mentors, but did not typically receive accommodations from a university office. (3) Students reported that participating in UREs could counteract the narrative that students with disabilities cannot do science. (4) Finally, students reported bringing unique perspectives to the scientific community, such as having a greater sense of compassion for others and drawing upon their lived experiences to inform their approach to research. This work provides an initial foundation for creating UREs that are more accessible and inclusive for students with disabilities.

EMS
Examining how graduate research and teaching affect depression in life sciences Ph.D. students
K. Cooper, L. Gin, N. Wiesenthal; Arizona State University, Tempe, AZ.

In 2018, researchers declared a “graduate student mental health crisis” when graduate students were found to be far more likely to experience depression compared to the general population. National calls to improve graduate student mental health followed. However, few studies have examined how graduate school specifically affects student depression. In this exploratory qualitative interview study, we set out to answer two research questions: (1) How do aspects of graduate research and teaching affect depression in Ph.D. students? and (2) How does depression affect Ph.D. students’ experiences teaching and researching? We interviewed 50 graduate students with depression enrolled in life sciences Ph.D. programs from 28 U.S. institutions. All questions were open-ended, allowing graduate students to describe how depression affected their research and teaching and how research and teaching affected their depression. Three researchers used inductive coding to analyze the data and create a codebook. Two authors used the codebook to code 10% of all interviews and their Cohen’s k interrater score was at an acceptable level; one researcher coded the remaining interviews. The most commonly mentioned aspects of research that negatively affected student depression included failures, obstacles, or setbacks during research (48% of students) and unstructured research experiences (38% of students). The most commonly mentioned aspects of research that positively affected students’ depression were completing small or concrete research tasks (26% of students) and working with others (22% of students). With regard to teaching, aspects that negatively affected students’ depression included time taken from research (47% of students) and negative reinforcement from undergraduates (28% of students), whereas teaching positively affected students’ depression when they received positive reinforcement from undergraduates (58% of students) and because it was a structured task (33% of students). Students reported that depression had an exclusively negative effect on their research, hindering their motivation (64% of students) and self-confidence (58% of students), but helped them be more compassionate teachers (20% of students). We use cognitive, behavioral, and
psychodynamic models of depression to further examine why each of these aspects contributes positively or negatively to depression. This is the first study to identify aspects of both research and teaching that affect depression in graduate students. This work pinpoints specific aspects of graduate school that Ph.D. programs can target to improve mental health among life sciences graduate students.

EM6
**Reflections on Inclusive Excellence 1&2 and building capacity for institutional change in science education.**
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The HHMI Inclusive Excellence initiative 1&2 (IE1&2) comprises a cohort of 57 U.S. colleges and universities that are engaged in the continuing process of building institutional capacity for inclusion and excellence for all students in science. Each school in the IE1&2 cohort has committed to learning through reflection, sharing what is being learned, listening to feedback, and supporting other members of the cohort. HHMI’s IE1&2 grantees include a diverse group of public and private institutions spanning baccalaureate colleges to research universities. Through a collaboration with the Inclusive Excellence Commission (IEC) appointed by the American Association of Colleges and Universities (AAC&U), HHMI is learning how colleges and universities are changing their culture, policies, and practices through faculty, curricular, and institutional development. The IEC developed “Progress towards Inclusive Excellence through Reflection” (PIER) to guide institutional teams in their annual reflection on the impact of their efforts, identifying places where new approaches may be needed, and keeping them focused on achieving sustainable change. Key highlights from the 2020 PIERs include the ways in which the dual pandemics of COVID-19 and incidents of racial injustice prompted many institutions to engage in deeper systemic thinking about inclusion. Each institution’s own sense of urgency and range of responses to address injustices and inequities of the dual pandemics was different. Some PIERs considered expanding the diversity and involvement of different campus voices in their effort to become anti-racist institutions. Some schools were uncertain how and whether the impacts of the COVID-19 and racial injustice, which can be considered as continuing crises, would result in lasting and meaningful changes on their campuses. Through the practice of reflection, faculty and staff in the IE1&2 institutional teams may be able to cultivate a more inclusive academic ecosystem in the sciences to support student learning. HHMI continues to examine how our initiative enables IE1&2 institutions to question their assumptions and reflect on the progress and challenges toward achieving inclusive excellence in the science education.

**MONDAY, DECEMBER 6, 2021**

Cellular Organization of Metabolism

M1
**Combinatorial G x G x E CRISPR screen of mitochondrial SLC25 carriers reveals their metabolic state dependency**
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The human SLC25 carrier family consists of 53 transporters that shuttle nutrients and co-factors across mitochondrial membranes. The family is highly redundant and their transport activities couple to metabolic state. Here, we introduce a pooled, dual CRISPR screening strategy that knocks out pairs of transporters in four metabolic states — glucose, galactose, OXPHOS inhibition, and absence of pyruvate — designed to unmask the inter-dependence of these genes. In total, we screened 63 genes in four metabolic states, corresponding to 2016 single and pair-wise genetic perturbations. We recovered 19 gene-by-environment (GxE) interactions and 9 gene-by-gene (GxG) interactions. A striking GxE interaction is illustrated by loss of the mitochondrial folate carrier (SLC25A32), in which the fitness defect of the KO cells was buffered in galactose due to a lack of substrate in de novo purine biosynthesis. We extended the GxE interaction to ExE interaction and demonstrated that the antifolate methotrexate action can be masked by decreasing glucose metabolism. Our GxGxE screen also recovered a buffering interaction between the iron transporter SLC25A37 and an orphan SLC25A39 carrier, which we then discovered that SLC25A39 regulates OXPHOS and iron homeostasis. Our combinatorial screen underscores the importance of investigating genetic interactions across distinct metabolic environments.

M2
Deciphering the role of mitochondrial redox metabolism in cell proliferation
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While GSH is commonly recognized as a component of antioxidant defense systems, it has wide range of roles in disulfide bond formation, detoxification of xenobiotics, metabolite transport, formation of iron-sulfur clusters and cellular signaling. Eukaryotic cells synthesize glutathione by the consecutive actions of γ-glutamate-cysteine ligase (GCL) and glutathione synthetase (GS), enzymes localized in the cytosol. Despite its exclusive cytosolic production, GSH is also abundantly present in many organelles including peroxisomes, the nucleus, endoplasmic reticulum and mitochondria. In particular mitochondria, the most redox active organelle, contain 10-15% of total cellular GSH. Since mitochondria lack GSH biosynthetic machinery and GSH is negatively charged under physiological conditions, there must be a dedicated transport process for GSH to enter mitochondria. Previous studies using isolated mitochondria support the existence of a mitochondrial glutathione transporter, but the molecular machinery involved in mitochondrial GSH transport has remained elusive. In this seminar, I will talk about our recent work characterizing the mitochondrial GSH machinery and its regulation.

M3
Optogenetic modification of membrane lipids using engineered Phospholipase Ds
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Phospholipase D (PLD) is a lipid-modifying enzyme that hydrolyzes phosphatidylcholine (PC) to produce phosphatidic acid (PA), which is both a central phospholipid biosynthetic intermediate and a multifunctional lipid second messenger. In this latter role, PA is involved in several signal transduction pathways, including mTOR signaling and Hippo signaling. Moreover, the headgroups of phospholipids are important for regulating physical and chemical properties of the membrane, and several functions of PA are thought to be mediated by its small, negatively charged headgroup. Organelle-specific PA pools are believed to play distinct physiological roles, and tools with high spatiotemporal control are
invaluable for unraveling these pleiotropic functions. Here, we present an approach to precisely generate PA on demand on specific organelle membranes. We exploit a microbial phospholipase D (PLD) and the CRY2-CIBN light-mediated heterodimerization system to create an optogenetic PLD (optoPLD). Using optoPLD, we elucidate that plasma membrane, but not intracellular, pools of PA can attenuate the oncogenic Hippo signaling pathway. Further, we develop a high-throughput cell screening system using IMPACT, a chemoenzymatic method for visualizing cellular PLD activity, to obtain optoPLD mutants with increased catalytic efficiency. IMPACT labeling relies on the ability of PLD to catalyze a second reaction, transphosphatidylation, in which the headgroup of PC is replaced with alcohols. By using exogenously supplied synthetic primary alcohols, which can be fluorescently tagged using click chemistry, IMPACT enables a quantitative readout of PLD activity. Using directed evolution of optoPLD based on IMPACT, we produce a panel of super-active optoPLD mutants (superPLDs) with catalytic efficiencies up to 100 times higher than the wild-type optoPLD. Further analysis revealed that superPLDs contain 6-10 mutations in the PLD sequence, which have increased their activity in a multiplicative manner. We demonstrate that superPLDs have a broad substrate specificity and can accept water as well as a variety of primary and, albeit less efficiently, secondary alcohols, making it a promising tool for producing lipids with tailored headgroups on designated organelle membranes and for biocatalytic applications. Collectively, optoPLD and superPLD hold premise as tools for both revealing spatiotemporally defined physiological functions of PA and modifying lipid headgroups to engineer membrane properties and behavior.

M4
Decoupling Mitochondrial Morphology and Activity as a Function of Metabolic State?
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Mitochondrial are highly dynamic organelles that dramatically change their shape and network as a function of metabolic demands. Conventional dogma states that long tubular and interconnected mitochondria indicate high respiration, whereas fragmented mitochondria typically reflect low activity, but this has not been systematically addressed. Using yeast cells growing in distinct carbon sources, which have different metabolic demands, we find that this link between morphology and activity is fluid, and show that mitochondrial morphology and activity are decoupled. The extent of this decoupling depends on the nature of chosen carbon source, and specific metabolic requirements of the cell. In specific carbon sources, cells having hyperfused tubular mitochondria can retain lower respiration capacity, whereas yeast cells that have most of their mitochondria fragmented can show very high activity. Further, our preliminary results suggest that choice of respiratory carbon source can result in a decoupling of the electron transport chain from the TCA cycle, thereby reconfiguring cellular metabolism. Our results bring fresh insights on how carbon metabolism affects mitochondrial form and function, and will inform our understanding metabolic disorders resulting from altered mitochondrial function.

M5
Mitochondrial energy dysfunction induces remodeling of the cardiac mitochondrial protein acylome
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Mitochondria are increasingly recognized as signaling organelles because, under conditions of stress, mitochondria can trigger various signaling pathways to coordinate the cell’s response. The specific pathway(s) engaged by mitochondria in response to defects in mitochondrial energy production in vivo and in high-energy tissues like the heart are not fully understood. Here, we investigated cardiac pathways activated in response to mitochondrial energy dysfunction by studying mice with cardiomyocyte-specific loss of the mitochondrial phosphate carrier (SLC25A3), an established model that develops cardiomyopathy as a result of defective mitochondrial ATP synthesis. Liquid chromatography tandem mass spectrometry targeting the acetylome and malonylome was performed in hearts from Slc25a3loxP targeted (Slc25a3fl/fl) and αMHC-MerCreMer mice expressing a tamoxifen-inducible Cre recombinase under control of the cardiomyocyte specific α-myosin heavy chain promoter (MCM). Proteomics revealed that energy dysfunction-induced remodeling of the acetylome and malonylome preferentially impacts mitochondrial proteins. Upon identification of IDH2 (acetylated and malonylated) and SIRT5 (acetylated) as mitochondrial proteins highly impacted by SLC25A3 deletion, IDH2 and SIRT5 HEK293 KO cell lines were generated by CRISPR-Cas gene editing. IDH2 and SIRT5 activities were measured in isolated mitochondria from IDH2 and SIRT5 KO cells re-expressing acetylation or malonylation constructs. Intriguingly, IDH2 activity was enhanced in SLC25A3-deleted cardiac mitochondria, and IDH2 sites in cells targeted by both acetylation and malonylation revealed that these modifications can have site-specific and distinct functional effects. Finally, we uncovered a novel crosstalk between the two modifications, whereby mitochondrial energy dysfunction-induced acetylation of sirtuin 5 (SIRT5), inhibited its deacylase function. Because SIRT5 is a mitochondrial deacylase with demalonylase activity, this finding suggests that acetylation can modulate the malonylome. Together, our results position acylations as an arm of the cardiac mitochondrial response to energy dysfunction and suggest a mechanism by which focal disruption to the mitochondrial energy production machinery can have an expanded impact on global mitochondrial function.

**M6**

**The p97-UBXD8 complex regulates ER-mitochondria contacts**

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Inter-organelle contacts are appreciated to regulate a number of fundamental biological processes, by serving as platforms for the synthesis of specific biomolecules, transfer of metabolites and organelle division. In particular, the close apposition of the endoplasmic reticulum (ER) and mitochondria is important for calcium transfer and signaling, membrane lipid synthesis and exchange, mitochondrial division, and autophagy. Aberrant formation and dissolution of contacts between these organelles is emerging as an important contributor to the pathobiology of a number of human disorders. However, how contact sites are dynamically re-modeled in a spatio-temporal manner is largely unknown. We sought to explore the role of the ubiquitin proteasome system (UPS) in the regulation of proteins localized to ER-mitochondria contacts and identified a novel role for the p97 AAA-ATPase and its ER membrane-anchored adaptor UBXD8 in regulating the abundance of contacts between the ER and...
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mitochondria. p97 (also known as valosin containing protein, VCP) is an evolutionarily conserved ATP-driven unfoldase, that functions in the UPS to mediate degradation of ubiquitylated substrates. Substrate identification in many instances requires dedicated ‘adaptor’ proteins (such as UBXD8) that recruit p97 to ubiquitylated targets. Our studies indicate that loss of the p97-UBXD8 complex leads to an increase and the abundance of contacts between the ER and mitochondria. Quantitative proteomics of purified ER-mitochondria contacts from wildtype and UBXD8 knockout (KO) cells identified widespread changes in enzymes involved in lipid synthesis. These findings were verified by lipidomic studies that indicated that UBXD8 KO cells had elevated levels of specific phospholipids and an increase in very long chain fatty acids. We show that loss of p97-UBXD8 prevents activation of master lipogenic transcription factor sterol regulatory element binding protein (SREBP1/2) in the ER and contributes to defective lipid metabolism. Importantly, aberrant contacts observed in p97 or UBXD8 depleted cells can be rescued by supplementing cells with specific fatty acids. In summary, our findings suggest that perturbed lipid metabolism may impact inter-organelle contacts and have identified a new role for p97 and its adaptor UBXD8 in modulating ER-mitochondria contact sites.

Cytoskeletal Synergy and Crosstalk for Cellular Function

M7
Branched actin, microtubule motors, and heterochromatin function together to move nuclei through constricted spaces in C elegans
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Cell migration through narrow spaces is critical for many developmental and disease processes, including immune cell intravasation and cancer metastasis. Some cells can squeeze through openings about 5% the resting diameter of the nucleus. Nuclear deformability appears to be the limiting step for cell migration through constrictions. However, the mechanisms that prevent rupture and facilitate nuclear movement through narrow spaces are unclear. We established a model in larval C. elegans using P-cell nuclei which migrate through a narrow constriction as a normal part of development. Three different mechanistic pathways work together to move P-cell nuclei. First, the linker of the nucleoskeleton and cytoskeleton (LINC) pathway recruits microtubule motors, primarily dynein, to the nuclear envelope. Knockdown of dynein heavy chain leads to a P-cell nuclear migration defect. Second, we found that the inner nuclear membrane protein CEC-4, which recruits heterochromatin to the nuclear periphery, is required for normal nuclear migration. cec-4 mutants significantly enhance the nuclear migration defect of LINC complex mutants. We hypothesize that in cec-4 mutants, the nuclear envelope is too weak to endure migration-induced mechanical stress. Third, we identified components of a branched actin network using a forward genetics screen, including CGEF-1, a predicted CDC-42 GEF, that enhanced the nuclear migration defect in LINC complex mutants. Knockdown of CDC-42, ARP2/3, and non-muscle myosin-1 using the auxin-inducible degradation system leads to a P-cell nuclear migration defect, indicating that branched actin networks are necessary for nuclear migration. Other genes of interest identified in the screen include the actin nucleator TOCA-1 and the divergent filamin FLN-2. In our branched actin model, CGEF-1 activates CDC-42, which goes on to activate TOCA-1 to nucleate branched actin via ARP2/3. FLN-2 and myosin-1 are required to organize the actin networks. Complete P-cell nuclear migration failure occurs when all three pathways are simultaneously disrupted. In our working model, dynein is recruited to the surface of nuclei and induces curvature to the nucleus.

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Next branched actin helps move the nucleus through the constriction. Finally, heterochromatin is required at the periphery of nuclei to prevent nuclear rupture during migration.

**M8**

**Differential regulation and fine tuning of kinesin and dynein motility by microtubule-associated septin complexes**

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Intracellular transport is driven by the microtubule (MT) motors kinesin and dynein which mediate plus-end and minus-end directed transport, respectively. MT motor activity and directionality is regulated by cargo and adaptor proteins, microtubule-associated proteins (MAPs) and MT post-translational modifications. Septins are a large family of GTP-binding proteins that oligomerize into higher order complexes, which associate with MTs. We recently discovered that the MT-associated septin 9 (SEPT9) impedes the motility of kinesin-1/KIF5C and enhances kinesin-3/KIF1A, and through this differential regulation promotes neuronal axon-dendrite membrane polarity (Karasmanis et al, Dev Cell 46:204, 2018). Because septins assemble into hetero-oligomeric complexes, we hypothesized that different septin complexes may exert different effects on kinesin and dynein motors. Here, we explored the possibility of a septin code in the regulation of motor motility by comparing effects of the MT-associated SEPT2/6/7 and SEPT5/7/11 complexes to each other and SEPT9 using in vitro single molecule motility assays of kinesin-1/KIF5C, kinesin-3/KIF1A and the dynein-dynactin-BicD2 (DDB) motor complex. We show that microtubule-associated mCherry-SEPT2/SEPT6/SEPT7 (100 nM) and mCherry-SEPT5/SEPT7/SEPT11 (50 nM) impede the motility of kinesin-1/KIF5C in a similar manner to SEPT9; SEPT5/7/11 has a higher MT affinity than SEPT2/6/7. Strikingly, we found that the motility of kinesin-1/KIF1A is differentially modulated by SEPT9, SEPT2/6/7 and SEPT5/7/11. MT-associated SEPT2/6/7 decreased the binding, velocity and run-lengths of kinesin-3/KIF1A, which are all enhanced by SEPT9. In contrast, SEPT5/7/11 did not affect the binding and run-lengths of kinesin-3/KIF1A, but had a mild impact on velocity and pausing. Consistent with previous effects of MT-associated SEPT9, DDB velocity and run lengths were significantly decreased by SEPT2/6/7 (20 nM) and SEPT5/7/11 (10 nM). Notably, SEPT2/6/7 and SEPT5/7/11 were much more potent inhibitors of dynein motility than kinesin, completely blocking MT-DDB binding at concentrations over 50 nM. Although both SEPT2/6/7 (20-50 nM) and SEPT5/7/11 (10-50 nM) inhibited the MT landing rates of DDB, we found that SEPT5/7/11 (50 nM) enhanced the number of immotile DDB particles, which was indicative of a drastic decrease in DDB detachment. SEPT5/7/11 was similarly unique in impeding the detachment of immotile kinesin-1/KIF5 and kinesin-3/KIF1A motors. Taken together, these data demonstrate that septins impact the motility of kinesin-3/KIF1A differentially, and how MT-associated septins fine tune motor motility by modulating individual parameters such as motor detachment in a septin paralog and complex-specific manner.

**M9**

**Septins guide non-centrosomal microtubules to promote focal adhesion disassembly in migrating cells.**

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During angiogenesis, endothelial cells (ECs) utilize signaling molecules and microtubule (MT) associated proteins (MAPs) to control the dynamic and coordinated remodeling of the actin and MT cytoskeleton to
become polarized. Rac1, a signaling molecule of the Rho GTPase family, promotes cell protrusion and enhances MT plus-end assembly into protrusions, thereby driving EC polarization. As ECs polarize, actin filaments assemble and become linked to focal adhesion (FA) complexes that mature at the positions where the cell interfaces with the extracellular matrix. Recent investigations identified that non-centrosomal MTs were solely required for EC sprouting and directed migration, while centrosomal MTs were dispensable. Despite this discovery, how ECs distinguish non-centrosomal from centrosomal MTs to drive fundamental cell functions remains unclear. Non-centrosomal MTs associate with MAPs that promote MT disassembly, like mitotic centromere associated kinesin (MCAK), and with MAPs that spatially guide MT plus-end dynamics and promote MT assembly, like the GTPase septin and the Clip-associated protein (CLASP). Moreover, septins are MAPs that enhance actin associated maturation of FAs and influence MT plus-tip assembly. Collectively, these data point to a potential mechanism used by ECs to delineate MT functions via association with septins. Here, we tested the hypothesis that Rac1 activates septin-mediated guidance of non-centrosomal MT polymerization into FAs. Live-cell fluorescence imaging revealed that MTs that penetrated FAs were predominantly non-centrosomal, and that septin-associated MTs consistently polymerized into FAs resulting in reduced FA size. Optogenetic activation of Rac1 promoted septin localization proximal to peripheral FAs, while myosin-II contractility was required both for maintaining FA-proximal septins and for the effects of non-centrosomal MTs on FA size. Additionally, normal function of the MT depolymerizer MCAK caused increased MT growth into septin and FAs, and reduced FA size, while inactivation of MCAK had the opposite effect. Consequently, removal of the MT stabilizing MAP, CLASP1, lead to an increase in FA size and was increased with excessive MCAK expression. Physiological wound edge experiments revealed non-centrosomal wounds closed faster than centrosomal and control wounds. Inactivation of MCAK or inhibition of CLASP significantly slowed wound closure in non-centrosomal but not centrosomal wounds. Taken together, these data suggest that Rac1 activity drives septin localization proximal to FAs, where septins inhibit MCAK-induced disassembly of non-centrosomal microtubules and recruit CLASP to promote iterative bouts of microtubule growth and shrinkage that induce focal adhesion disassembly.

M10
Vimentin Regulates Collagen Remodeling by Controlling the Aggregation of Myosin 10 at the Termini of Cell Extensions
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Colorectal carcinoma is a leading cause of cancer-related deaths worldwide. Epithelial-mesenchymal transition (EMT) plays an important role in cancer cell invasion and metastasis. Vimentin (Vim) is considered as a hallmark of the epithelial-mesenchymal transition in epithelial cells and increased Vim expression is associated with tumor progression and metastasis in colon cancers. Unconventional myosin 10 (Myo10) is an actin-based motor protein whose expression correlates with cancer metastasis and poor prognosis. However, the regulation of these two significant cancer markers, Myo10 and Vim, in the migrating cell, is not defined. Here we show for the first-time, a direct interaction between Myo10 and Vim in cell extensions. Utilizing STED microscopy and FRET analysis, we found that Myo10 directly interacted with Vim at the termini of cell extensions. The abundance of soluble vimentin was tightly associated with Myo10 trafficking to the tips of cell extensions. Deletion of VIM promoted accumulation
of Myo10 at the edge of the cell membrane. Depolymerization of Vim filaments promoted the retraction of Myo10 from cell extensions to the cell body. In SW480 cells, a colon cancer line, there was marked overexpression of highly insoluble Vim filaments, which resulted in the accumulation of Myo10 behind the leading edge, similar to vimentin-deficient cells. Fluorescence recovery after photobleaching showed that Vim expression regulates Myo10 dynamics. Depletion of Vim strongly reduced the Myo10 mobile fraction in cell extensions. We found that robust Myo10-Vim interactions are essential for collagen remodeling by MMP-14 and that increased Vim expression was associated with deficiencies in the assembly of collagen fibrils. These results indicate that Vim regulates aggregation of Myo10 at the ends of cell extensions and at cell-extracellular matrix contacts, which collectively determine collagen remodeling and cell migration through soft connective tissue and in cancer invasion of soft connective tissues.

M11
Sub-centrosomal mapping identifies augmin-gammaTuRC as part of a centriole-stabilizing scaffold
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Centriole biogenesis and maintenance are crucial for cells to generate cilia and assemble centrosomes that function as microtubule organizing centers (MTOCs). Centriole biogenesis and MTOC function both require the microtubule nucleator gamma-tubulin ring complex (gamma-TuRC). It is widely accepted that gamma-TuRC nucleates microtubules from the pericentriolar material (PCM) that is associated with the proximal part of centrioles. However, gamma-TuRC also localizes more distally and in the centriole lumen, but the significance of these findings is unclear. Here we use expansion microscopy to identify spatially and functionally distinct sub-populations of centrosomal gamma-TuRC. Gamma-TuRC localization on the outside of centrioles is not restricted to the PCM but includes more distal regions. Similarly, nucleation occurs from sites along the entire centriolar cylinder including its distal end. Recruitment of gamma-TuRC at the outside of centrioles is largely mediated by CEP192. In addition, a significant fraction of gamma-TuRC is found in the centriole lumen. Luminal localization is mediated by augmin. Augmin is linked to the centriole inner scaffold through POC5, which we identified as proximity interactor of the augmin subunit HAUS6. Disruption of luminal localization impairs centriole integrity and interferes with cilium assembly. Defective ciliogenesis is also observed in gamma-TuRC mutant fibroblasts from a patient suffering from microcephaly with chorioretinopathy. These results identify a novel, non-canonical role of augmin-gamma-TuRC in the centriole lumen that is linked to human disease.

M12
Microtubule dynamics are required for mammary epithelial cell migration and branching morphogenesis
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Branching morphogenesis closely coordinates cell proliferation and collective cell migration to expand tubular epithelial structures. In the mammary gland, the simple luminal epithelium stratifies into
migratory endbuds during branching before resolving into simple epithelium. This process requires dramatic changes in cell polarity, the actin cytoskeleton, and tissue dynamics. However, little is known about the involvement of microtubules in branching morphogenesis or epithelial migration in 3D environments. Using mammary organotypic cultures, we first examined the organization of the microtubule cytoskeleton during branching. We found that simple luminal cells utilized non-centrosomal apicobasal microtubule arrays, while in stratified luminal cells microtubules radiated from the centrosome. Migratory luminal cells adopted an ameboid-like cell organization with rear-facing nuclear-centrosomal axis. We also examined microtubule dynamics in the stratified endbuds through live imaging of microtubule +tips. We found that cells in the basal-most luminal cell layer had more stable microtubules than cells deeper within the stratified layer. Finally, we tested the requirement for microtubule dynamics during branching using pharmacologic inhibitors of microtubule dynamics. Both microtubule stabilization and destabilization prevented bud formation and arrested duct elongation independent of effects on mitosis. Cell tracking analysis demonstrated that microtubules coordinated the collective migration of luminal cells in elongating buds. Destabilizing microtubules reduced cell directionality, while stabilizing microtubules maintained directionality but reduced cell motility. Our data reveal correct spatiotemporal regulation of microtubule organization and dynamics is essential for collective migration of luminal cells and mammary branching morphogenesis.

Exploring Chromosome Structure and Function by Quantitative Methods

M13

Heterogeneous non-canonical nucleosomes predominate in yeast cells in situ

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The nucleosome is a 10-nm-diameter, 6-nm-thick cylindrical nucleoprotein complex and is the basic subunit of eukaryotic chromatin. Each nucleosome consists of a histone octamer core, wrapped by 147 base pairs of DNA in 1.65 left-handed gyres. This canonical structure was determined 24 years ago and is conserved in vitro in nucleosomes from multiple organisms and in nucleosomes that bear variant histones. We recently showed that a subset of nucleosomes inside human cells do indeed resemble the canonical form. Little else is known about nucleosome structure in situ. We sought to determine the structure of nucleosomes using electron cryotomography (cryo-ET) and 3D classification of chromatin from nuclear lysates (in vitro) and inside thinned frozen-hydrated cells (in situ). To improve our ability to detect nucleosomes in situ, we created a strain in which the sole source of histone H2A is tagged with a green fluorescent protein to serve as an extra cryo-ET density. Analysis of wild-type nuclear lysates revealed that yeast nucleosome class averages have a canonical structure while analysis of H2A-GFP nuclear lysates reveal canonical nucleosomes with up to two density bumps protruding from the nucleosome’s faces. In contrast, canonical nucleosome class averages were absent in cryomograms of chromatin inside cells. Nucleosome-like class averages that have an extra density expected of H2A-GFP bearing nucleosomes were also absent. These results suggest that the budding yeast nucleosomes are conformationally heterogeneous in situ and can be explained by the partial detachment of DNA. The abundance of non-canonical nucleosomes is consistent with biochemical, genomics, and molecular-dynamics studies of yeast chromatin. Non-canonical nucleosomes expose more structural motifs to
M14
Quantitative image-based analysis of organization and reorganization of nuclear structures in hiPSCs and hiPSC-derived cardiomyocytes
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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. The organization of the nucleome—the nucleus, its subcompartments, and the 3D conformation of the genome—is a key determinant of cell-type specific behavior. Much has been learned about nucleome organization, including how it reorganizes as cells differentiate from a pluripotent state to a derived cell type—e.g. heterochromatin expands, the epigenetic landscape changes, and interactions between genomic loci are repositioned. What is still missing is a picture of global nucleome organization that captures the geometry of the nucleus, the organization of nuclear bodies and substructures, and the global 3D structure of the genome—as well as how each of these three variables interface and change during differentiation. We have generated gene-edited hiPSC lines that each express an endogenous, monoallelic EGFP-tagged protein, targeting 17 different nuclear proteins that range from major landmarks (e.g. speckles and nucleoli) to specific chromatin loci (e.g. telomeres) and used established protocols to differentiate these cells into cardiomyocytes (see the Allen Cell Collection at www.allencell.org). We generated high-replicate, 3D image data for thousands of single cells, capturing fluorescence of a given structure (e.g. telomeres via EGFP-TRF2), the plasma membrane and DNA via fluorescent dyes, and deep learning-based label free prediction of the nucleolus. These images are being used by collaborators as spatial constraints in combination with standard genomics-based constraints (e.g. sc-HiC, TSA-seq) to model 3D genome conformations within these imaged nuclei. Finally, we have performed an initial analysis of three of the nuclear structures (nucleoli, nuclear speckles and telomeres), and found both quantifiable similarities and differences in distinct aspects of 3D shape, size and organization for each structure before and after differentiation. For example, we found that hiPSCs maintain a near constant number of telomere clusters as nucleus size increases, while cardiomyocytes show a linear increase in the number of telomere clusters as nucleus size increases, which suggests that there are two distinct modes of telomere organization. Together, the initial observations from this project seem to indicate that the nuclei of hiPSCs and cardiomyocytes are not just different on average, but are also fundamentally different in how their organization changes as they increase in size.

M15
Applying cryo-EM single-particle analysis to Xenopus egg extract system to understand structural mechanics behind chromosome architecture during the cell cycle
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Chromatin and nucleosomes regulate various biological processes of eukaryotic chromosomes. The recent development of cryo-EM facilitated our understanding of the structural basis behind these processes primarily using nucleosomes reconstituted in vitro with recombinant histones and DNA. However, many complexes in vivo chromosomal events, such as cell cycle dependent changes in
chromosome organization and mitosis-specific kinetochore assembly on centromere-associated nucleosomes, cannot be easily reconstituted with purified materials. Thus, understanding the structural mechanics behind these processes remains challenging. Xenopus egg extract system is a unique cell-free system, in which cell cycle-dependent chromosomal processes can be recapitulated using either native chromosomes or recombinant nucleosomes with specific histone variants and/or modifications. Combining this Xenopus egg extract system with cryo-EM, we aim to understand how nucleosome structures are differentially regulated during the cell cycle to control chromosome organization and functions. By establishing several cryo-EM structural analysis pipelines that can determine high-resolution structures from heterogeneous particle mixtures without losing structural variants, we have successfully reconstructed nucleosome structures formed in interphase and metaphase chromosomes at 3.5 Å resolution and their structural variants (Arimura et al. BioRxiv 2020). In addition, this pipeline was applied to determine the novel native structure of alpha-macroglobulin, the key innate immunity protein, and structural variations of ribosome structures from mitotic and interphase extracts. In this meeting, we will report how our approach can be applied to determine structures of multi-nucleosomal organization and nucleosomes with specific histone variants and modifications.

M16
A Systematic Analysis of Argonaute Proteins in C. elegans
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RNA interference (RNAi) pathways, consisting of Argonaute (AGO) proteins and small RNAs (sRNAs) that provide sequence specificity, play key roles in gene regulation across all domains of life. Studies of AGOs in many species have revealed that they impact gene expression at nearly every stage in the life cycle of a transcript—from transcription to translation. Due to their central role in RNAi and profound impact on development and differentiation in numerous organisms, uncovering new and conserved molecular mechanisms of AGOs advances our fundamental knowledge of cellular function and has the potential to provide more precise means to manipulate gene expression, relevant for biotechnology and therapeutics. The C. elegans genome encodes an expanded family of 27 ago genes, 19 of which produce functional proteins. We have undertaken a systematic study of every C. elegans AGO to develop a comprehensive portrait of the molecular mechanisms of these mostly uncharacterized proteins throughout development. Using CRISPR/Cas9 genome-editing, we epitope-tagged each AGO with GFP-3xFLAG. We used confocal microscopy to characterize the expression patterns of each AGO throughout development, and found that 16 AGOs are expressed in the germline. Of these, eight AGOs localized to phase-separated germ granules. High-throughput sequencing of 1) sRNAs associated with each AGO, and 2) total sRNA pools from ago mutants versus wild type uncovered the stratification of subsets of AGOs into distinct gene regulatory modules. Phenotypic analyses of ago mutants under normal and stressful conditions revealed previously unappreciated phenotypes, including a transgenerational loss of fertility known as the Mortal Germline (Mrt) defect, sensitivity to the Orsay virus, and resistance to the bacterial pathogen Pseudomonas aeruginosa. Overall, our systematic and pioneering studies provide an unprecedented view of the sRNA regulatory landscape throughout the development of a complex animal.
**M17**

**Differential Genome Utilization In Early Development**

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In early development, cells transition from maternal to zygotic control, requiring widespread regulated activation of the genome. However, it is poorly understood whether zygotic genome activation (ZGA) is identical in all blastomeres or whether there are specific differences in the timing and fraction of expressed genome from cell to cell. We recently discovered a novel spatiotemporal pattern of ZGA in the model organism *Xenopus laevis*, in which large-scale transcription first initiates in cells at the animal pole, the presumptive ectoderm, and delayed in cells of the vegetal pole which constitute the presumptive endoderm. To investigate spatial and temporal control of genome utilization, we developed a new technique to image and sequence nascent transcription in early embryos. This nascent transcriptomics approach enabled us to monitor the onset of gene expression in whole embryos as well as in regions of the embryo. We identified over 9,000 genes that are nascently transcribed at early-mid ZGA, thousands of which follow the spatially gradual pattern that we initially discovered. Additionally, to determine whether cells achieve differential genome activation, we mapped nascent sequencing reads to the allotetraploid genome. We found striking differences in timing of nascent transcription from specific chromosomes, between the long (L) and short (S) subgenomes, and within expression clusters versus low density regions. At the chromosome level, we observed up to ~3-fold difference in overall expression rate during ZGA. Moreover, genes present on the L and S subgenomes activate at different times with different rates; L forms are largely more active than S forms. To understand how differential utilization of the genome is regulated during ZGA, we focused on the physical association of those highly activated genes and transcription factor binding sites associated with timing of activation. We identified that density of binding sites for a number of pioneer factors correlates with clustered or early gene activation. Intriguingly, a number of these clusters include germ layer specific components, suggesting that the spatiotemporal onset of ZGA may drive patterning of cell specification. Together, our findings provide new insights on the varying nature of genome regulation during ZGA.

**M18**

**Single Molecule In Situ Imaging of DNA Repair in Single Human Cells**

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Cell engineering using Cas/Crispr or other programmable nucleases relies on the endogenous DNA repair machinery to correct genetic defects, perturb functional elements, or introduce exogenous sequences conferring new functions. Currently, it is widely believed that the vast majority of nuclease-induced DNA breaks (95-99%) are repaired using non-homologous end-joining; however, very little is known about the steps cells traverse to resolve DNA breaks at the level of individual DNA alleles. Here we report an approach for visualizing DNA repair via quantitative in situ single molecule tracking of both DNA species and associated repair proteins at a precise genomic location. Using diverse primary and immortalized cells, we show that nuclease-induced double strand breaks overwhelmingly default to a repair mechanism that involves bi-direction, multi-kilobase single strand resection, which is then
reversed and finally resolved with an error-prone end joining process. This state mimics the classical conception of the homology-directed repair pathway, and can be quantitatively abrogated by supply of a homologous template. We show further that this phenomenon varies quantitatively as a function of genome position. Our results suggest that cells experiencing a nuclease-induced cleavage enter a “recombination-ready” state in the absence of a suitable template and remain in this state for an extended period of time before spontaneously resolving a double strand break via an alternative mechanism. Taken together, our results and approach suggest a novel role for strand resection in repair of individual DNA templates in individual cells, and provide a novel platform for imaging DNA repair with broad applications.

Neuronal Cell Dynamics

M19

Neuritogenesis in pyramidal neurons requires spatial coordination of actin protrusion and contractility by a novel network of septin filaments

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The pyramidal neurons of the hippocampus have triangular pyramid-shaped cell bodies, which orient the axon and dendrites along the apical and basal axes, respectively. Neuronal cell body shape is coupled to neurite formation and differentiation into axons and dendrites. In culture, neurites develop from the protrusive circumferential lamella of cell bodies, but how actin polymerization and contractility are balanced during neuritogenesis is not well understood. Importantly, the underlying mechanisms regulating this balance, which is also required to establish neurons with cell bodies of proper size, shape and neurite number is unknown. Here, we show that a novel cytoskeletal wreath-like network of septin GTPases regulates the balance of actin polymerization and contractility, which is necessary for neurite formation at the base of the lamellar protrusions of the neuronal cell body. This septin network, which is enriched with septin 7 (Sept7), colocalizes primarily with myosin IIB at the base of filopodia and inter-filopodia vales, and demarcates a zone of anterogradely decreasing levels of Arp2/3. In immature hippocampal neurons, Sept7 knockdown results in enlarged cell bodies, which are characterized by extensive lamellipodia-like protrusions. Sept7-depleted neurons have an abnormally increased number of neurites, which are highly branched and extend from lamellipodial protrusions rather than directly from a consolidated cell body. Rescue of cell body phenotypes with the Arp2/3 inhibitor CK666 or a dominant-negative cortactin suggest that the septin wreath network suppresses Arp2/3-driven protrusions. Conversely, expression of a constitutively active myosin II regulatory light chain rescued neurite phenotypes, suggesting that the septin wreath network promotes filopodia stabilization, and eventual maturation into neurites. We posit that morphogenesis of pyramidal cell shapes is coupled to neurite formation via a filamentous septin network, which biases protrusive activity toward filopodial over lamellipodial protrusions by favoring actomyosin contractility over Arp2/3 polymerization in the cell bodies of developing neurons.
M20

**Dendritic architecture determines mitochondrial distribution patterns in vivo**

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Neuronal form and function rely on the maintenance of an organized, dynamic mitochondrial network. The molecular mechanisms of mitochondrial dynamics are well described, however little is known about the coordination of mitochondria across large spatial scales (microns to meters) in morphologically complex neurons. We investigated the in vivo relationship between mitochondrial motility and dendritic architecture using well-characterized neurons in the Drosophila visual system called HS neurons. We measured mitochondrial motility using in vivo confocal microscopy and quantified mitochondrial distribution patterns by analyzing ssTEM images of the fly brain (Zheng et al Cell 2018). Mitochondria in HS neurons are highly motile (~ 2 mitochondria per minute move through the primary dendrite) with balanced bidirectional transport yielding ~10% of the total mitochondrial volume in the arbor exchanging through the primary dendrite per hour. Despite high motility rates, mitochondrial distribution patterns are consistent across HS neurons. To determine how HS neurons maintain stable branch-to-branch mitochondrial densities despite high motility rates, we reasoned that the relative amount of mitochondrial movement through one daughter branch scales with the relative size of the subtree supported by that branch. By measuring dendritic subtree lengths, we found that HS dendritic branch patterns are highly asymmetric: at each branch point, one sister subtree is, on average 1.5 times longer than the other. Dendritic subtree length asymmetry is also tightly correlated with the dendritic cross sectional area asymmetry; larger subtrees have proportionally thicker trunks. At each branch point, we found the total dendritic cross-sectional area is conserved such that the thickness of the parent branch equals the total thickness of the daughter branches (“da Vinci scaling”). On its own, da Vinci scaling would result in a constant mitochondrial density throughout the arbor. While we found mitochondrial transport scales with dendrite thickness, with proportionally more mitochondria moving into the thicker daughter branch, we also found that the frequency of motility arrest is inversely correlated with dendrite thickness, leading to more frequent stopping in more distal segments. Da Vinci scaling combined with differential stoppage rates could explain why mitochondrial density increases with distance from the soma, and that after we decompose the dendritic arbor into successive subtrees, the mitochondrial density remains uniform across sister subtrees. Together, our results show that mitochondrial transport scales with dendritic branch patterns, providing a mechanism for maintaining mitochondrial distributions within highly branched dendritic arbors in vivo.

M21

**Presynaptic autophagy is coupled to the synaptic vesicle cycle via ATG-9**

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Autophagy is a cellular degradation pathway essential for neuronal health and function. Autophagosome biogenesis occurs at synapses, is locally regulated and increases in response to neuronal activity. The mechanisms that couple autophagosome biogenesis to synaptic activity remain unknown. In this study we determine that trafficking of ATG-9, the only transmembrane protein in the core autophagy
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pathway, links the synaptic vesicle cycle with autophagy. ATG-9 positive vesicles in C. elegans are generated from the trans-Golgi network via AP3-dependent budding, and delivered to presynaptic sites. At presynaptic sites, ATG-9 undergoes exo-endocytosis in an activity-dependent manner. Mutations that disrupt endocytosis, including one associated with Parkinson’s disease, result in abnormal ATG-9 accumulation at clathrin-rich synaptic foci and defects in activity-dependent presynaptic autophagy, neurotransmission and locomotion behavior. Our findings uncover regulated key steps of ATG-9 trafficking at presynaptic sites, and provide evidence that ATG-9 exo-endocytosis couples autophagosome biogenesis at presynaptic sites with the activity-dependent synaptic vesicle cycle.

M22
Endoplasmic reticulum-lysosome interactions promote autophagy to regulate synaptic growth
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Sites of apposition between organelles, referred to as membrane contact sites (MCSs), are hotspots for intracellular signaling, lipid metabolism, and organelle biogenesis/dynamics in eukaryotic cells. The endoplasmic reticulum (ER) forms an extensive and dynamic network of MCSs with almost all organelles. MCSs between the ER and endo-lysosomes are particularly abundant, suggesting important physiological roles. These contact sites are also observed in neurons. However, their molecular composition and physiological function in the nervous system remain poorly understood. PDZD8 is an intrinsic ER transmembrane protein with a synaptotagmin-like mitochondrial lipid-binding protein (SMP) domain that has been reported to localize to ER-late endosome/lysosome and ER-mitochondria MCSs. The molecular steps involved in the recruitment of PDZD8 to MCSs have been recently elucidated, yet the in vivo relevance of PDZD8 to neuronal function remains unclear. We identified Drosophila PDZD8 in a candidate screen for uncharacterized conserved regulators of synapse formation and function. We used the CRISPR-Cas9 system to generate null alleles and endogenously tag PDZD8. Interestingly, we find that PDZD8 is expressed at synapses throughout the central nervous system and the larval neuromuscular junction (NMJ), where it localizes to ER-lysosome MCSs. We show that activity-induced synaptic growth, neurotransmission, and locomotion are dysregulated in PDZD8 mutants, indicating important roles in nervous system development and function. We further show that PDZD8 regulates synaptic growth via autophagy. In PDZD8 mutants we see accumulation of autophagic proteins as well as autolysosomes. Our analyses indicate that PDZD8 is required for autolysosome maturation to promote autophagic flux in neurons. We propose that PDZD8-mediated ER-lysosome membrane interactions promote autophagy to regulate synaptic growth.

M23
Nudc-dependent regulation of the axon terminal cytoskeleton in neurons
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In neurons, Cytoplasmic dynein is the microtubule-based motor known for its function in retrograde cargo transport; however, this motor can also control microtubule stability. In vitro, dynein has been shown to enhance microtubule stability at locations of steric hinderance. In vivo in C. elegans, dynein was shown to be essential for stabilized microtubules in dendrites. How these two functions of the ORAL-29
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dynein, e.g. cargo transport and microtubule stability, are coordinated to work together or in parallel has not been determined. In a forward genetic screen, we identified a zebrafish strain with phenotypes indicative of increased microtubule stability in axon terminals. This strain carries a loss of function mutation in NudC. Currently, NudC has little known function outside of mitosis during which it is essential for kinetochore alignment. NudC has also been shown to interact with dynein and the dynein regulator Lis1. Using live imaging, we show that loss of NudC in neurons causes enhanced microtubule stability in axon terminals and accumulation of autophagosomes therein. Microtubule stability, but not cargo accumulation, can be suppressed by pharmacological inhibition of dynein motor activity in nudc mutants, implicating differential regulation of the motor by NudC. Using in vivo rescue experiments and protein interaction studies, we further show that regulation of motor function by NudC is dependent on its role in maintaining Lis1 protein stability: Both axon terminal microtubule stability and cargo localization phenotypes in nudc mutants can be rescued by exogenous Lis1. Together, our data point to a model in which NudC regulates Lis1 protein stability in axon terminals, which is essential for initiation of retrograde cargo transport. Disrupting this process leads to accumulation of cargo and dynein in axon terminals and it is the elevation of axon terminal dynein motor that leads to the increased microtubule stability in this region. Together, our work illustrates the critical role of dynein motor flux between neuronal compartments for the regulation of not only cargo transport but the maintenance of a dynamic population of axon terminal microtubules.

M24
Brain-derived autophagosome profiling reveals the engulfment of nucleoid-enriched mitochondrial fragments by basal autophagy in neurons
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Neurons depend on autophagy to maintain cellular homeostasis, while defective autophagy and impaired mitochondrial maintenance are pathological hallmarks of neurodegenerative diseases. In order to better understand the role of basal autophagy in maintaining neuron health, and identify the unique functions of autophagy in neurons, we established a reliable method for enriching autophagic vesicles (AVs), including autophagosomes and autophagolysosomes, from healthy mouse brain tissue and neurons in culture. We then performed mass spectrometry to identify the cargos of basal autophagy. We found that mitochondrial proteins comprise 18% of cargos identified from brain, significant by gene ontology analysis (p=9.5E-55). Engulfment of mitochondria occurs under basal conditions in mouse brain, and does not involve selective mitophagy adaptor proteins such as OPTN or NIX, as neither these adaptors nor PINK1 or Parkin were enriched within the AV fraction. Closer investigation of the composition of mitochondrial proteins found within AVs revealed that mtDNA and nucleoid-associated proteins were significantly enriched compared to other mitochondrial proteins, such as components of the electron transport chain. To test for increased engulfment of mitochondrial nucleoids in neurons, we used live cell imaging to assay the autophagic trafficking of nucleoid-enriched mitochondrial fragments in the axons of neurons; 40% of autophagosomes trafficking along the axon were positive for the mitochondrial nucleoid-associated protein TFAM, compared to 20% that were positive for Cox8a. Further, we determined that engulfment of nucleoid-enriched mitochondrial fragments by autophagy requires the mitochondrial fission machinery DRP1 and MFF. Thus, we propose a model that following mitochondrial fission at mitochondria-ER contact sites, nucleoid-enriched mitochondrial fragments are sloughed from the mitochondrial network and non-selectively captured by autophagosomes that form
constitutively from the ER membrane at the axon terminal. We hypothesize that this pathway is unique to neurons and prevents mtDNA accumulation that over time could sensitize neurons to an inflammatory response via cGAS-STING or the NLRP3 inflammasome, conflagrating neurodegenerative disease pathogenesis.

Nuclear Pores and Mitochondria

M25
The innate immunity sensor STING contributes to mitochondria dysfunction and muscular degeneration in a Drosophila melanogaster model of hereditary Parkinson’s disease
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Parkinson’s Disease (PD) is a degenerative disorder characterized by death of striatal dopaminergic neurons. Although most PD cases are sporadic, about 10% are due to Mendelian-inherited mutations. Among these are recessive homozygous mutations in parkin and pink1, which are linked to juvenile onset PD and feature underlying mitochondrial dysfunction. In response to mitochondrial damage, the kinase Pink1 and the ubiquitin ligase Parkin initiate mitophagy - a pathway through which damaged mitochondria are degraded via the autophagosome. This process has been characterized in vitro, however the importance of mitophagy in animal models of mitochondrial diseases remains controversial. Resolving these controversies through a deeper understanding of the molecular mechanism will lead to better knowledge of the disease. Recessive mutations for parkin (park) in the fly model organism Drosophila melanogaster lead to severe physical and locomotion phenotypes attributed to mitochondria dysfunction, which induces muscle defects and progressive degeneration of dopaminergic neurons. In rodents, it was found that degenerative phenotypes in a parkin mutant model could be prevented by blocking the cytosolic DNA sensing cGAS-STING pathway. Innate immunity pathways including STING and NF-kB pathways are conserved in insects, however, the role of innate immune signaling in pink1/parkin mutant fly pathology has not been established. We aimed to determine whether the conserved immunity regulator STING is important in a fly PD model. To test the hypothesis that STING is contributing to the pathology in park null flies, two genetically independent sting and park double knockout stocks were generated. Deletion of sting with either null allele results in significant reduction in the penetrance of the major fly park phenotypes including flight muscle defects, wing posture, and climbing ability. The underlying mitochondria morphology defects in park flies were also suppressed in these double mutant lines. To confirm the specificity for loss of sting, we generated flies carrying a transgenic STING expression construct. Gal4 driven full-body expression of wild type STING in the sting and park animals suppressed the beneficial effects of deleting sting, supporting a direct role for STING signaling in the mitochondria pathology of parkin mutants. This work demonstrates the conservation of a STING-regulated innate immunity pathway acting in the pathogenesis of PD, and it provides a platform with the park fly phenotypes for further studies into the role of innate immunity in mitochondria quality control.
Impaired activity of the mitophagy regulator TBK1 is partially compensated by ULK1 but disrupts mitochondrial quality control in neurons

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Cells perform ‘self-eating,’ autophagy, to clear old and damaged internal components and maintain neuronal homeostasis. This process comprises a highly regulated series of interactions that lead to lysosomal degradation of cellular constituents. Many components of the pathway leading to the selective autophagy of mitochondria known as mitophagy are implicated in neurodegenerative disease and include the mitophagy receptor optineurin (OPTN) and the kinase, TANK-binding kinase 1 (TBK1). TBK1 associates with and phosphorylates OPTN upon mitophagy induction. Together TBK1 and OPTN facilitate mitochondria engulfment by a light-chain 3 (LC3)-positive autophagic membrane. We found that both recruitment of TBK1 and phosphorylation of OPTN by TBK1 at serine 177 were essential for efficient clearance of damaged mitochondria in a cell culture model. However, Unc-51 Like Autophagy Activating Kinase 1 (ULK1) also promoted OPTN phosphorylation and could partially compensate for TBK1 deficiency. Neuronal expression of disease-related TBK1 mutants resulted in mitochondrial network stress under basal conditions, including the accumulation of rounded, fragmented mitochondria and mitophagy intermediates. Neurons expressing TBK1 mutants were less well-equipped to manage further mito-toxic insult, indicating that TBK1 mutations have deleterious consequences for neurological health. Given that TBK1 is also a prominent regulator of inflammatory pathways, we are currently investigating the potential intersection of defective mitophagy and neuroinflammation as they related to neurodegenerative diseases.

Active Mechanisms Prevent Ectopic Condensation of FG Nucleoporins in the Cytoplasm

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The permeability barrier of nuclear pore complexes (NPCs) is created by FG nucleoporins (Nups). FG Nups contain highly disordered phenylalanine/glycine (FG) domains that form a gel-like matrix in the central channel of NPCs. Using the C. elegans model, we find that the intrinsic insolubility of FG Nups makes them highly prone to ectopic condensation in the cytoplasm, and that cells employ a variety of strategies to maintain a soluble pool of FG Nups in the cytoplasm. FG Nups accumulate in cytoplasmic condensates most prominently in growing oocytes and newly fertilized embryos. These condensates are enriched for FG Nups and their binding partners, but do not contain nuclear-facing Nups or scaffolding Nups required for anchoring to the nuclear membrane. In growing oocytes less than 5% of FG Nup molecules are in condensates, the majority are diffuse in the cytoplasm (~70-80%) and in NPCs (~20-30%). The Nup condensates naturally dissolve during oocyte maturation and undergo cycles of re-assembly and disassembly with each mitosis in early embryos. Reduction of individual FG Nups by RNAi reduces condensate formation, suggesting that the concentration of Nups in the cytoplasm is near the solubility limit. We have identified several conditions that enhance Nup condensation, including heat stress, depletion of the kinase MBK-2/DYRK3, mutation of the GlcNActransferase OGT-1, and depletion of the nuclear transport receptor (NTR) CRM1. Together, these observations suggest that cells utilize
several mechanisms to increase Nup solubility in the cytoplasm, including phosphorylation, GlcNAcylation and chaperoning by CRM1. These findings are consistent with *in vitro* studies showing that GlcNAcylation enhances solubility of FG Nups and that interaction with NTRs prevents condensation of aggregation prone proteins. Nups have been reported to localize to stress granules and pathogenic aggregates in cell culture. We find that TDP-43, which mislocalizes to cytoplasmic aggregates in ALS and FTD patients, colocalizes with Nup condensates following cellular stress. Overall, our findings suggest that, while the natural propensity of FG Nups to condense is critical for establishing the permeability barrier of NPCs, it presents a challenge for cells and must be counteracted by energy-consuming mechanisms to minimize the formation of potentially toxic Nup condensates in the cytoplasm.

M28  
**TorsinA regulates nuclear pore complex assembly and localization during neuronal maturation**  
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Nuclear pore complexes (NPCs) are large protein complexes composed of hundreds of nucleoporins that mediate nucleocytoplasmic transport of protein and RNA. NPC abnormalities are implicated in DYT1 dystonia, a neurodevelopmental movement disorder caused by a loss-of-function mutation in the gene encoding torsinA, a AAA+ protein localized to the endoplasmic reticular (ER)/nuclear envelope (NE) endomembrane space. Despite increasing evidence implicating NPC dysfunction in DYT1 and other neurological diseases, NPC biogenesis in neurons remains poorly understood. Furthermore, the biological function of torsinA and the molecular defects underlying DYT1 dystonia remain largely unknown. In mouse primary neurons, we find a steady upregulation in NPC biogenesis during neuronal maturation. While NPCs are uniformly distributed in wild-type neurons, torsinA-null neurons develop mislocalized clusters of NPCs. To elucidate the dynamics of NPC formation and localization, we developed a novel HaloTag-Nup107 mouse line and conducted pulse-chase studies of existing and newly-formed NPCs. We observe that in torsinA-null neurons, new pores form adjacent to existing pores, thereby resulting in increasingly severe abnormal clusters of NPCs as neurons mature. In contrast to the drastic difference in localization between wild-type and torsinA-null neurons, NPC density is normal in torsinA-null neurons, suggesting that torsinA regulates NPC localization, 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 in primary neurons coincides with the formation of mislocalized NPC clusters, thereby implicating a temporal association between these events. To understand the spatial relationship between abnormal NPC clusters and NE buds, we performed multiple-tilt electron tomography. We find that NE buds form in clusters and that the base of NE buds form NPC-like pore structures at the inner nuclear membrane. These data are consistent with a model in which loss of torsinA causes defective NPC assembly, resulting in formation of aberrant NE buds at sites of new NPC formation. Indeed, abnormal NPC clusters demonstrate features of intermediate NPC assembly states, as they contain early- but not late-recruited NPC components. Taken together, our findings suggest a critical function of torsinA in the localization and assembly of new NPCs during a key period of neuronal development and implicate aberrant NPC biogenesis in the pathogenesis of DYT1 dystonia.
M29
RNA-based regulation of TDP-43 nuclear export
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TDP-43 is an essential DNA/RNA-binding protein that preferentially binds GU-rich intronic RNAs and plays a major role in RNA processing and stability. Nuclear clearance and cytoplasmic accumulation of TDP-43 is a pathologic hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Substantial evidence links TDP-43 disruption to the pathogenesis of ALS/FTD, but the cause of TDP-43 mislocalization in disease remains unknown. TDP-43 is primarily nuclear at steady state, but continuously shuttles between the nucleus and the cytoplasm. Ran-regulated TDP-43 nuclear import occurs via binding of its nuclear localization signal (NLS) to importins alpha and beta. TDP-43 nuclear export was initially thought to be mediated by exportin-1 (XPO1) binding to a putative nuclear export signal (NES). However, several groups recently showed that NES deletion does not disrupt TDP-43 export, nor does XPO1 knockdown or inhibition. Here, we used permeabilized and live cell models to investigate the mechanism and regulation of TDP-43 nuclear export. In permeabilized cells, TDP-43 readily exited the nucleus in low-ATP conditions, consistent with passive diffusion through nuclear pore channels. Rapid depletion of NXF1 alone or in combination with XPO1 inhibition failed to disrupt TDP-43 nuclear export in live cells, further excluding a role for active mRNA or protein nuclear export pathways. RNase-mediated degradation of nuclear RNAs in permeabilized cells induced rapid TDP-43 nuclear efflux, suggesting that RNA promotes TDP-43 nuclear sequestration. In permeabilized and live cells, synthetic GU-rich oligomers induced nuclear TDP-43 efflux, likely by competitive displacement of TDP-43 from endogenous RNAs, suggesting that GU-rich RNA binding specifically anchors TDP-43 within the nucleus. Splicing inhibitors induced TDP-43 nuclear accumulation and resistance to nuclear efflux, further linking TDP-43 nuclear sequestration to the availability of intronic pre-mRNA binding sites. Finally, mutation or deletion of TDP-43 RRM domains strongly reduced TDP-43 nuclear enrichment and abolished its RNA-regulated shuttling. Collectively, these findings support a model in which binding to nuclear GU-rich intronic pre-mRNAs sequesters TDP-43 in the nucleus and dictates its availability for passive nuclear export. By maintaining the nuclear abundance of GU-rich intronic pre-mRNAs, the balance of transcription and pre-mRNA processing may therefore be the primary upstream factor determining TDP-43 nuclear localization under physiologic conditions. Moreover, disruption of nuclear RNA homeostasis warrants further investigation related to TDP-43 mislocalization in ALS/FTD.

M30
Ataxin-2 polyQ expansions disrupt the liquid-like properties and anterograde transport of neuronal TDP-43 ribonucleoprotein condensates in the axon and suppress mRNA translation
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Amyotrophic lateral sclerosis (ALS), a debilitating and fatal neurodegenerative disease, is characterized by aggregates of TDP-43, an RNA-binding protein (RBP) with multiple roles in post-transcriptional RNA processing, RNA stability, and transport. Mutations in TDP-43 and other RBPs cause familial and sporadic ALS, highlighting altered RNA metabolism as a common pathogenic mechanism. Recent studies have identified genetic interactions between TDP-43 and Ataxin-2, a RBP with a polyglutamine (polyQ) tract normally 22-23Q in length. Intermediate-length expansions of the Ataxin-2 polyQ tract (27-33Q) confer increased risk for ALS. However, the underlying molecular mechanisms by which Ataxin-2/TDP-43
interactions increase ALS risk are unclear. We hypothesized that Ataxin-2 polyQ expansions aberrantly scaffold TDP-43, stabilize TDP-43/Ataxin-2 interactions within ribonucleoprotein (RNP) condensates and disrupt their dynamic properties, increasing the propensity for pathologic transformation of TDP-43. We used live-cell confocal imaging, photobleaching and immunofluorescence experiments to study the localization, motility and interactions of TDP-43 and Ataxin-2 in rodent primary cortical neurons. Our data show that endogenous wild type Ataxin-2 colocalizes with a subset of TDP-43 positive neuronal RNP granules, which are dynamic liquid-like biomolecular condensates. While TDP-43 positive granules exhibit oscillatory or long-range motility in the axon, Ataxin-2 positive RNP granules are predominantly stationary and localize within the proximal axon. Kymographs were used to analyze the motility of RNP granules containing TDP-43 only and those containing TDP-43 with Ataxin-2 of varying polyQ lengths. We found that TDP-43 granules positive for Ataxin-2 Q30 or Q39 exhibit reduced anterograde run lengths and sequester TDP-43. In addition, our photobleaching experiments show that Ataxin-2 Q30 and Q39 expansions impair TDP-43 dynamics and FRAP recovery. These data suggest that intramolecular mobility and transport of TDP-43 RNP condensates are perturbed in neurons with Ataxin-2 polyQ expansions. Finally, we used puromycylation and single-molecule translation (SunTag) assays to show that translation is suppressed in primary cortical neurons expressing Ataxin-2 polyQ expansions, compared to those expressing Ataxin-2 Q22 or RFP. Our data suggest that Ataxin-2 polyQ expansions perturb TDP-43 anterograde transport and its spatial localization, and impair translation as well. Overall, these results indicate Ataxin-2 polyQ expansions aberrantly sequester TDP-43 and may have detrimental effects on stability, localization, and translation of transcripts critical for axonal and cytoskeletal integrity, particularly in motor neuron axons.

Organelle Structure, Dynamics, and Sub-organization

M31

Taok2 is an ER-localized kinase that catalyzes the dynamic tethering of ER to microtubules
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The endoplasmic reticulum (ER) depends on extensive association with the microtubule cytoskeleton for its structure, function and mitotic inheritance. The identity of molecular tethers that mediate ER-microtubule coupling, and mechanisms through which dynamic tethering is regulated are poorly understood. Here, we identify, Thousand And One amino acid Kinase 2 (TAOK2) as a pleiotropic protein kinase that mediates tethering of ER to microtubules. We show that TAOK2 is a unique multipass membrane spanning serine/threonine kinase localized in distinct ER domains via four transmembrane and amphipathic helices. Using in vitro and cellular assays, we find that TAOK2 directly binds microtubules with high affinity. We define the minimal TAOK2 determinants that induce ER-microtubule tethering, and delineate the mechanism for its autoregulation. While ER membrane dynamics are increased in TAOK2 knockout cells, the movement of ER along growing microtubule plus-ends is disrupted. We show that ER-microtubule tethering is tightly regulated by catalytic activity of TAOK2 in both interphase and mitotic cells, perturbation of which leads to profound defects in ER morphology and cell division. Our study identifies TAOK2 as an ER-microtubule tether, and reveals a kinase-regulated mechanism for control of ER dynamics critical for cell growth and division.
M32

Myo19-mediated F-actin tethering induces mitochondrial fragmentation

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Mitochondria are dynamic organelles that remodel via fission and fusion. In the final step of mitochondrial fission dynamin-related protein 1 (Drp1) constricts the organelle. Upstream of Drp1-mediated constriction there is a role for F-actin at sites of close apposition between mitochondria and the ER, and we probed the involvement of Myosin 19 (Myo19) in this process. This motor protein is a good candidate because it binds F-actin and associates with mitochondria via mito-ER contact-localized Miro proteins. In cells, knock-down of Myo19 causes mitochondria to elongate, while Myo19 overexpression causes mitochondria to fragment. We hypothesize that Myo19 promotes fission as depletion of the motor alters levels of total Drp1/phospho-S616 Drp1. The enhanced fragmentation induced by Myo19 overexpression depends on the direct interaction of Myo19 with actin, as we found overexpression of a Switch II motif mutant predicted to exhibit reduced actin-binding fails to produce mitochondrial fragmentation. Next, we asked if Myo19 might function as a motor or as a tether during mitochondrial fragmentation. Super-resolution stochastic optical reconstruction microscopy (STORM) imaging of native Myo19 indicates a nearest-neighbor distance of ~130nm, a spacing that is likely too far for collective interactions necessary to achieve processive motility. More direct evidence that Myo19 force production is dispensable for mitochondrial fragmentation came when we overexpressed a mutant with a shortened lever arm, and observed mitochondrial fragmentation similar to that induced by overexpression of wild-type Myo19. Thus, we hypothesize that Myo19 acts to tether mitochondria to F-actin, functioning specifically where mitochondria and the ER are in proximity. Support for this hypothesis comes from the observation that mitochondrial fragmentation induced by Myo19 overexpression depends on Spire1C, which generates F-actin at sites of close apposition between mitochondria and the ER. Depletion of Spire1C is sufficient to block the effects of Myo19 overexpression on mitochondrial fragmentation. We also note that upon Myo19 depletion levels of mitochondrial fission factor (Mff) are altered - MFF is the Drp1 receptor that promotes fission at mito-ER contacts. Altogether, our data support a model in which Myo19-mediated F-actin tethering induces mitochondrial fission.

M33

Limiting ER membrane biogenesis prior to cell entry into mitosis ensures its reorganization to allow chromosome motions necessary for mitotic fidelity

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The endoplasmic reticulum (ER) dramatically restructures in open mitosis to become excluded from the mitotic spindle; however, the significance of ER reorganization to mitotic progression is not known. Here, we show that ER membrane biogenesis in interphase coordinates with its mitotic reorganization to prevent chromosome segregation errors that result in the emergence of micronuclei, single encased chromosomes prone to nuclear envelope ruptures and DNA damage. We demonstrate that restricting
ER lipid synthesis ensures that the biophysical properties of the mitotic cytoplasm is not altered from excess ER membranes persisting in the vicinity of prometaphase chromosomes. Excess ER membranes inherited in mitosis increase the effective viscosity of the cytoplasm to slow chromosome motions, which impairs the correction of errors in microtubule spindle attachment to kinetochores. Mechanistically, we show that the understudied but highly conserved protein phosphatase CTDNEP1 (Nem1 in budding yeast/CNEP-1 in C. elegans) establishes and maintains a dephosphorylated pool of a key enzyme in lipid synthesis, lipin 1, upon entry into interphase to limit the expansion of the ER. The absence of this dephosphorylated pool in CTDNEP1 knockout cells makes lipin 1 less stable corresponding to an increase in de novo fatty acid synthesis and overproliferation of ER membranes. Inhibition of de novo fatty acid synthesis in CTDNEP1 knockout cells suppresses both the expansion of the ER in interphase and the formation of micronuclei resulting from chromosome segregation errors. Together, our findings reveal a novel aspect of ER regulation necessary for mitotic fidelity and expand our understanding of how the spatial organization of the ER in mitotic cells controls mitotic processes. Our work further reveals dysregulated lipid metabolism as a potential source of aneuploidy in cancer cells.

M34
Invadopodia formation is spatiofunctionally coupled to the nuclear envelope by septin GTPases
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Cancer cells invade through the extracellular matrix (ECM) by forming actin-rich protrusions termed invadopodia, which degrade the ECM by secreting matrix metalloproteinases (MMPs). Invadopodia formation requires the membrane recruitment and targeting of the scaffolding protein TKS5, cortactin and other actin regulators, and subsequently followed by secretion of MMPs. However, the molecular mechanisms that underlie and regulate TKS5 and MMP targeting to invadopodia are not well understood. A growing number of studies show that over-expression of Septin 9 (SEPT9), a member of the septin family of GTPases, enhances the migratory and invasive properties of cancer cells. Here, we have unexpectedly discovered a novel role for septins in linking the formation of plasma membrane invadopodia to the nuclear envelope. In the metastatic breast cancer MDA-MB-231 cells, SEPT9 isoform 1 (SEPT9_i1) localizes to domains of the nuclear rim and along the ventral surface of the nuclear envelope. SEPT9_i1 is also enriched in areas of gelatin degradation and localizes to the invadopodia of MDA-MB-231 cells undergoing 3D chemoinvasion. We show that SEPT9 isoform 1-specific depletion alters the morphology of nuclei, which diminish volumetrically and appear ruffled with nuclear membrane undulations and indentations. Consistent with a defective nuclear envelope, nuclear import of mCherry-NLS was also significantly diminished. Strikingly, this nuclear envelope phenotype was accompanied with a reduction of invadopodia precursors that localize at the nuclear membrane and rim, and comprise over 60% of total invadopodia precursors. We show that depletion of SEPT9_i1, but not SEPT9_i2, results in dramatic reduction of TK55, cortactin and pTyr421-cortactin clusters from the ventral cell membrane and impairs the degradative ability of MDA-MB-231 cells on gelatin substrates and transwell invasion assays. In SEPT9_i1-depleted cells, TK55 and cortactin are diffusely mislocalized along the nuclear envelope and/or abnormally localize to perinuclear aggregates. In addition, we found that MMP-14 localization shifts from the perinuclear cytoplasm to the peripheral edges of the cell membrane, indicating a potential enrichment in peripheral focal adhesion sites. Taken together, these data suggest that the machinery of invadopodia formation is coupled to the nuclear envelope by a
septin-mediated mechanism that spatially biases ECM degradation to the perinuclear over peripheral regions of the cell membrane, which enables the nucleus to squeeze through the ECM during cell invasion.

M35

Non-vesicular lipid transport mediated by PDZD-8 and TEX-2 promote robust embryogenesis via regulation of endosomal PI(4,5)P₂ homeostasis in C. elegans

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Different types of cellular membranes have unique lipid compositions that are important for their functional identity. PI(4,5)P₂ is enriched in the plasma membrane (PM) where it contributes to local activation of key cellular events, including actomyosin contraction and cytokinesis. However, how cells prevent PI(4,5)P₂ from accumulating in intracellular compartments, despite constant intermixing and exchange of lipid membranes through vesicular transport, is poorly understood. Growing evidence suggests that lipid transfer proteins (LTPs) transport specific lipids between different cellular compartments, thereby helping to maintain unique lipid compositions of cellular membranes. LTPs function primarily at membrane contact sites, where membrane-bound organelles are in close apposition to one another and the PM without membrane fusion. One evolutionarily conserved family of LTPs are proteins that contain the SMP domain (SMP proteins). SMP proteins localize to various membrane contact sites and transport a wide variety of lipid species through their lipid harboring SMP domain. In metazoans there are four classes of SMP proteins: E-Syts, TMEM24, PDZD8, and TEX2. In this study, we used the C. elegans early embryo as our model system and identified a critical role for SMP proteins for endosomal PI(4,5)P₂ homeostasis. By time-lapse imaging the earliest stages of embryogenesis, which is highly stereotypic in C. elegans, and systematically knocking out all four SMP proteins, we found that homologs of PDZD8 and TEX2 (PDZD-8 and TEX-2 in C. elegans) act redundantly to suppress build-up of endosomal PI(4,5)P₂. In the absence of PDZD-8 and TEX-2, PI(4,5)P₂ accumulates within endosomal membranes. Additional depletion of two PI(4,5)P₂ phosphatases, namely OCRL-1 and UNC-26 (homologs of OCRL1 and synaptojanin in C. elegans), exacerbates PI(4,5)P₂ accumulation, leading to massive appearance of abnormally large PI(4,5)P₂-enriched endosomes that ectopically recruit proteins involved in actomyosin contractility. This leads to lack of myosin II-mediated PM contraction, failure of cytokinesis, defects in the degradative capacity of endosomes, and embryonic lethality. PDZD-8 localizes to ER-late endosome contacts in early embryos. Purified SMP domain of PDZD-8 can transport various phosphoinositides, including PI(4,5)P₂, in vitro. Accordingly, specific disruption of the PDZD-8 SMP domain is sufficient to induce aberrant accumulation of PI(4,5)P₂ on endosomes. Our results demonstrate that PDZD-8 regulates endosomal PI(4,5)P₂ levels via its SMP domain, acting together with PI(4,5)P₂ phosphatases and TEX-2 to counteract the build-up of endosomal PI(4,5)P₂. This maintains cellular membrane identities required for normal embryogenesis and cell division.
M36

Membrane Fusion Machinery Mediates Protein Targeting from the Endoplasmic Reticulum to Lipid Droplets

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Lipid droplets (LDs) are cellular organelles specialized in storing lipids. Numerous important metabolic proteins localize to and act at the LD surface. Many of these proteins target from the endoplasmic reticulum (ER) to LDs (ERTOLD), but how this trafficking pathway operates is unknown. Here, we show that some proteins access LDs during their formation at the LD assembly complex (LDAC) in the ER (early ERTOLD), whereas others are excluded from forming LDs by the LDAC and target mature LDs (late ERTOLD). Previous studies indicated that late ERTOLD targeting necessitates the formation of membrane connections between the ER and LDs. By performing a genome-scale imaging screen with a late ERTOLD protein [glycerol 3-phosphate acyltransferase 4 (GPAT4)], we identified the machinery required for late ERTOLD targeting, including ER exit site proteins (e.g., Sar1, Sec16, and Tango1) and membrane fusion proteins (Rab1, Rint1, Syx5, membrin, Bet1, and Ykt6). Consistent with a direct role of this machinery in late ERTOLD targeting, ERES components transiently associate with LDs when late ERTOLD occurs. By determining changes in LD proteomes upon depletion of these ERES or membrane fusion factors, we identified additional late ERTOLD cargo proteins. Together, our results delineate two types of ERTOLD targeting (early vs. late) and suggest a model for late ERTOLD targeting, in which membrane fusion machinery at ERES establishes ER-LD bridges, which in turn allow for the targeting of important cargoes to mature LDs.

Tumor Progression and Therapy 1

M37

Lateral transfer of macrophage mitochondria drives ERK signaling to promote cancer cell proliferation

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In a process called lateral mitochondrial transfer, a donor cell transfers mitochondria to a recipient cell. This phenomenon enhances recipient cell viability and has been observed in many physiological and pathological conditions, including cancer. Generally, it is proposed that transferred mitochondria benefit recipient cells due to their function as an energy-producing organelle, rescuing or increasing cellular health. However, the fate of individual transferred mitochondria and the subcellular mechanism of how the transfer of foreign mitochondria promotes these benefits are unknown. Additionally, how donor cell dynamics influence mitochondrial transfer remains underreported. Here we show that macrophages transfer mitochondria to tumor cells, resulting in enhanced tumor cell proliferation. Using high resolution time-lapse microscopy, we observed that transferred mitochondria do not fuse with the endogenous mitochondrial network and lack membrane potential, indicating that they are dysfunctional. Furthermore, rather than being degraded, the transferred mitochondria accumulate...
reactive oxygen species and promote cellular proliferation by stimulating the ERK pathway. These data indicate that rather than functioning as an energy-producing organelle in recipient tumor cells, transferred mitochondria can act as a signaling source to enhance tumor cell proliferation. Next, we investigated how macrophage plasticity influences mitochondrial transfer. Environmental cues alter macrophage differentiation and function through a process known as macrophage activation. Activated macrophages are classified as M1-like or M2-like macrophages, which promote pro-inflammatory or pro-tumorigenic processes, respectively. Using flow cytometry, we found that M2-like macrophages exhibit enhanced mitochondrial transfer rates to cancer cells. Three-dimensional quantitative analyses of high-resolution images reveal that M2 macrophages have increased mitochondrial fragmentation compared to other macrophage subtypes. We thus hypothesized that mitochondrial fragmentation promotes mitochondrial transfer. To examine this hypothesis, we genetically manipulated macrophages to promoted hyper-fused or hyper-fragmented mitochondrial networks and co-cultured them with tumor cells to determine changes in mitochondrial transfer rates. These co-culture studies reveal that macrophages with either hyper-fused or hyper-fragmented mitochondrial networks exhibit reduced or increased mitochondrial transfer rates, respectively. Collectively, these data help define the mechanism of how lateral transfer of mitochondria alters cellular behavior and reveal an uncharacterized mechanism of M2-like macrophage pro-tumorigenicity.

M38
Investigating EGFRvIII extrinsic regulation of GBM invasion

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Glioblastoma Multiforme (GBM) is the most lethal and common primary brain tumor in adults, with a median patient survival of 12-15 months following current standard of care. This poor prognosis is largely due to highly invasive tumor cells that evade surgical resection and develop multiple mechanisms of therapeutic resistance, leading to patient relapse with recurrent GBM. Further adding to this aggressive character, GBM is made up of a heterogenous population of cells, with 25% of GBM cases containing cells expressing a constitutively active epidermal growth factor receptor (EGFR) truncation of exons 2-7 known as variant III, i.e. EGFRvIII. EGFRvIII cells have enhanced intrinsic migratory properties and can also impart this phenotype on neighboring glioma cells overexpressing wtEGFR through an extrinsic mechanism. We define this ability of EGFRvIII cells to communicate with and convert genotypically different cells into a phenocopy as an “education” process. This cellular cross-talk leads to cooperative invasion, though the mechanisms through which this happens are not well understood. We hypothesized that EGFRvIII cells “educate” wtEGFR cells through the secretion of cytokines, which possibly gives educated wtEGFR cells the ability to propagate this migratory phenotype via transcriptional alterations. To investigate which extrinsic factors secreted from EGFRvIII cells are essential for the education process, we performed a cytokine array on conditioned media (CM) taken from mouse astrocytes overexpressing either EGFRvIII or wtEGFR and identified a subset of cytokines preferentially secreted by EGFRvIII cells, including TNFa. To understand whether TNFa plays a significant role in educating wtEGFR cells, we neutralized TNFa in EGFRvIII CM and subjected wtEGFR cells incubated in this CM to a spinning disk assay, which measures cell adhesion strength by applying fluid shear to a population of cells. We found that inhibition of TNFa failed to reduce the adhesion strength of
wtEGFR cells, indicating that TNFa is essential for the education process, but not necessarily sufficient. In addition to these findings, we explored whether CM from EGFRvIII cells possessing a PTEN deletion could similarly educate wtEGFR cells. Although these cells have an intrinsic adhesion strength profile comparable to EGFRvIII cells with PTEN intact, the CM from these cells did not change wtEGFR adhesion strength, indicating a potential role for PTEN in influencing the secretion profile of EGFRvIII cells and their educating ability. Overall, these studies provide insight into the mechanisms of cooperative tumor cell invasion and possibly suggest new targets for therapeutic intervention.

M39

Mrtf-srf interaction is essential for metastatic colonization

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A switch from dormancy to proliferative state of extravasated tumor cells leads to the formation of micro-metastases that ultimately progress to clinically detectable macro-metastases causing mortality of cancer patients. The goal of this study was to examine the role of myocardin-related transcription factor (MRTF), an important class of coactivators of SRF (serum-response factor), in metastatic colonization of breast cancer cells. Multiplexed quantitative immunohistochemical analyses of clinical samples of invasive breast cancer indicated a general trend of higher MRTF activity in tumor cells compared to stromal cells in the tumor microenvironment, a feature that is conserved across all molecular subtype of breast cancer. Using three-dimensional ECM cultures, we demonstrated that co-depletion of MRTF isoforms (MRTF-A and MRTF-B) inhibits actin cytoskeletal structures that are important for metastatic outgrowth and impairs single-cell outgrowth ability of both estrogen-receptor (ER)-positive and -negative breast cancer cells as well as retards growth progression of established breast cancer cell colonies. Subsequent studies with breast cancer cells engineered for inducible overexpression of fully functional vs various mutant forms of MRTF-A revealed that elevated MRTF-A activity promotes tumor cell outgrowth in vitro and metastatic colonization in vivo and that disruption of MRTF-SRF interaction induces a dormancy-like phenotype in breast cancer cells dramatically impairing metastatic colonization ability of breast cancer cells. Consistent with these findings, we further show that small molecule inhibitor of MRTF/SRF signaling is highly effective in suppressing single-cell outgrowth, progression of pre-established outgrowth and metastatic colonization of breast cancer cells. Global transcriptome analyses show pharmacological inhibition of MRTF/SRF signaling affects multiple biological pathways that are relevant for metastatic growth of cancer cells including actin cytoskeletal and integrin signaling, cell proliferation/survival, metabolism, angiogenesis, and immune cell-regulatory events, and further led us to identify candidate MRTF-target genes for promoting skeletal metastasis of breast cancer cells. Based on these findings, we propose that MRTF-SRF interaction is essential for metastatic colonization of breast cancer cells and therefore, targeting this interaction may be a promising strategy to diminish metastatic burden in breast cancer.

M40

Investigating the molecular mechanisms underlying efficient metastatic extravasation

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Metastasis, accounting for 90% of cancer-related mortality, involves stepwise progression of tumor cells through distinct stages. The cascade initiates as tumor cells migrate within the primary tumor; continues sequentially as cells intravasate, disseminate to a secondary site, and extravasate; then culminates as tumor cells proliferate, forming a secondary tumor. Although extensively studied, the mechanistic details of this process are poorly understood. Previous investigations involved experimental metastasis (EM) models wherein tumor cells are intravenously injected, eliminating the possibility for the tumor cells to undergo conditioning by factors arising at the primary tumor site. Further, technological limitations had precluded longitudinal, single-cell resolution imaging of disseminated tumor cells (DTCs) upon arriving at the secondary site, allowing only for speculation regarding their behavior. With the advent of the Window for High Resolution Imaging of the Lung (WHRIL), it is now possible to definitively determine DTC fate. We employed this approach in two murine models: EM and spontaneous metastasis (SM), where tumor cells spontaneously disseminate from an orthotopic tumor. Comparison revealed more rapid extravasation among SM cells, suggesting signaling at the primary site induces expression of proteins conveying trans-endothelial migration ability. To test this possibility, we examined DTCs of both models for the expression of MenaINV, a splice variant isoform of the actin regulatory protein, Mena, which has previously been shown to be important for tumor cell intravasation within the primary tumor. Immunohistochemistry revealed that SM cells, compared to EM cells, markedly overexpressed MenaINV. Further, subsequent manipulation of EM cells to induce MenaINV overexpression resulted in more rapid extravasation rates, approaching those observed among SM cells. To identify other proteins regulating extravasation in the secondary site, we reasoned that, since EM cells eventually do extravasate, albeit in a delayed manner, expression of those proteins required for extravasation (e.g. MenaINV) are activated later, only after DTC arrival at the secondary site. Utilizing time-controlled injections of a lipophilic dye to isolate intra- and extravascular populations by fluorescence-activated cell sorting (FACS), we aimed to identify responsible proteins by comparing the differential expression patterns (via bulk RNA-seq) among DTCs immediately before and after extravasation. We report the identification of genes differentially regulated as tumor cells cross the lung endothelium to enter the parenchyma. We additionally profile the impact that manipulation of these genes has on each step of the metastatic cascade in the lung.

M41
Measuring Drug-Induced Changes in ERK Activity on a Single Cell Level Predicts ERK Inhibition Efficacy for Combinational Therapy
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ERK activity is frequently increased in cancer cells and is one of the central regulators of cell survival. Direct ERK inhibition is a promising approach to increase therapy effectiveness. However, aside from mutations in ERK upstream kinases (RAS, RAF, MEK), there are few known predictors of ERK-dependent survival. We analyzed the expression of ERK-related genes in more than 11 000 solid tumor samples of different origins and found substantial differences between tumors of epithelial and neural origin. We aimed to explore how different drugs affect ERK activity distribution on a single cell level and how these data can be used to predict new therapeutic options. To measure ERK activity at a single-cell level, we...
used a kinase translocation reporter and created 9 reporter cell lines of different origins, including cancers of epithelial, neural, and other origins. We measured ERK activity and cell viability changes induced by 26 drugs at 7 different concentrations, including common chemotherapy drugs, kinase inhibitors, apoptosis inducers, and epigenome remodeling drugs. In total, we recorded more than 1600 unique profiles for ERK activity and cell viability changes. We compared ERK activity distributions after drug treatment and hierarchical clustering of ERK activity distribution in cell population revealed two drug types. The first group, included HSP90, HDAC, and DNA methylation inhibitors, showed a drug-induced decrease in ERK activity for most cells corresponding with a decrease in cell viability. The second group, which included kinase inhibitors and pro-apoptotic drugs, maintained or even increased ERK activity in a large cell subpopulation even under toxic concentrations. We show that drugs that maintain high ERK activity synergistically reduce cancer cell viability in combination with ERK inhibitor, while drugs that decrease ERK activity on their own show additive or antagonistic effects. Notably, this effect was observed for different cell types, indicating similar mechanisms of ERK-dependent survival. We also

M42

Invadopodia dynamics in prostate cancer; identification of a p-21 activated kinase 4 (PAK4) molecular pathway.

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Invadopodia, actin-rich structures that release metalloproteases at the interface with extra-cellular matrix, in a punctate manner are thought to be important drivers of tumour invasion. Invadopodia formation has been observed in-vitro and in-vivo in numerous metastatic cell lines derived from multiple tumour types. However, prostate cancer cell lines have not been routinely reported to generate invadopodia. We wanted to understand if invadopodia represented a viable therapeutic target in prostate cancer and explore molecular mechanism. We tested the invasive potential of primary prostate adenocarcinoma cell lines and excitingly found that all of these cell lines were capable of spontaneous invadopodia formation and possess a significant degradative ability in-vitro under basal conditions. The establishment of a prostate specific invadopodia study model enabled us to explore the molecular mechanisms that regulate invadopodia activity in these cells. We found that p-21 activated kinase 4 (PAK4) is essential for invadopodia formation and invadopodia-mediated matrix degradation in prostate cancer cells. Moreover, we present evidence that PAK4 can regulate both metalloproteases expression and RhoA activity in these cells. We identify specific PAK4 mediated phosphorylation sites on PDZ-RhoGEF and demonstrate that invadopodia activity is dependent on PAK4 kinase function. Furthermore,
using a RhoA biosensor we localise active RhoA to invadopodia in prostate cancer cells. Importantly, we can now demonstrate, for the first time, that circulating tumour cells isolated from prostate cancer patients exhibit invadopodia-like structures and degrade matrix with visible puncta. Thus, the combination of our studies provides clear evidence that invadopodia activity can play a physiological role in prostate cancer progression and identifies PAK4 as a promising target for migrastatic intervention.

TUESDAY, DECEMBER 7, 2021

Dynamic Membrane Remodeling

M43

In situ structure of VPS13 family proteins

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VPS13 is a lipid-transfer protein expressed in all eukaryotes (Ugur et al. 2020, PMID: 32563856) and encoded by 4 distinct genes in mammals. Mutations in each of these genes result in neurological diseases. Loss of function mutations in the human VPS13C gene, which encodes a protein localized at contacts between the ER and late endosomes/lysosomes, are responsible for rare cases of early onset familial Parkinson’s disease (PD). A low-resolution negative-stain EM structure of purified yeast Vps13p reveals an elongated rod with a loop structure on one end (PMID: 28122955) and a cryo-EM structure of a portion of the rod shows the presence of a groove traveling along its length (PMID: 32182622). However, how VPS13 proteins are arranged at membrane contact sites in intact cells remains unclear. A precise elucidation of this arrangement is critical toward the understanding of the mechanisms of lipid transport by this family of proteins and to validate the hypothesis that they act by providing a hydrophobic bridge for the transfer of lipids between adjacent bilayers. To determine the precise localization and in-situ structure of full length VPS13C in mammalian cells, we used cryo-electron tomography (cryo-ET) combined with correlative light and electron microscopy (CLEM) and cryo-focused ion beam milling (cryo-FIB). Our preliminary cryo-ET data reveals abundant ~30-nm-long rod-shaped bridging densities at ER-late endosome/lysosome contacts in VPS13C-overexpressing HeLa cells. Most of these rod-shaped VPS13C densities are oriented perpendicular to the membranes. Sub-tomogram averaging and classification reveals a rod connecting the ER (frequently ER cisternae with nearly no lumen, referred to as thin ER) to the endolysosome membranes. Ongoing work is aimed at improving the resolution of average structures to reveal the lipid-transfer groove. This structural study will complement other investigations in our lab aimed at understanding VPS13 function and the impact of its absence on cell and organismal physiology. (This work was supported in part by a postdoctoral fellowship from the Parkinson Foundation to SC)
Reconstitution of human atlastin fusion activity reveals autoinhibition by the C-terminus

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Proper endoplasmic reticulum (ER) structure and function in mammalian cells depends on at least one of three atlastin (ATL) GTPase paralogs ATL1/2/3 and mutations in neuronal ATL1 are linked to disease. Human ATLs are thought to promote ER network formation by catalyzing homotypic membrane fusion because the Drosophila ATL orthologue purified from E. coli is sufficient for fusion in vitro. Surprisingly however, human ATL1 purified in the same manner is well documented to lack in vitro fusion activity leaving open the possibility that another factor mediates mammalian ER fusion. Here, we show that ATL1 purified from mammalian cells is indeed sufficient for fusion. ATL1 exhibited fusion activity comparable to Drosophila ATL and disease variants showed alterations in fusion. In contrast, the more ubiquitously expressed ATL2 paralog had negligible fusion activity. This was unexpected because the G domains of ATL1 and ATL2 are 75% identical; the ATL2 soluble domain dimerizes and hydrolyses GTP at rates comparable to ATL1; and most cell types rely on ATL2/3 rather than ATL1. Sequence analysis revealed a non-conserved C-terminal extension on ATL2, and deletion of this extension restored maximal fusion activity suggesting a novel mode of regulation by the C-terminus. Charge reversal of residues in this inhibitory domain of ATL2 strongly activated its fusion activity and overexpression of this disinhibited version caused ER collapse. Finally, neurons express an alternate ATL2 splice isoform whose sequence differs in this inhibitory domain, and this form showed full fusion activity. These findings reveal autoinhibition and alternate splicing as regulators of atlastin mediated ER fusion.

Reticulons initiate the endoplasmic reticulum stress surveillance checkpoint during the cell division

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The endoplasmic reticulum (ER) is essential for many cellular functions including secretory protein production and quality control, lipid synthesis, and calcium storage. As the ER cannot be synthesized de novo, each time a cell divides, functional ER must be inherited from the mother to the daughter cell. While a large body of research has uncovered various cell cycle checkpoints and regulatory mechanisms that ensure proper segregation of nuclear genomic material, much less is known about whether similar regulatory mechanisms or checkpoints operate for the segregation of cytoplasmic components, including organelles like the ER. Previously, we reported a cell cycle checkpoint for ensuring the inheritance of functional ER in S. cerevisiae: the ER stress surveillance (ERSU) pathway. ER stress blocks ER inheritance, causing the septin ring to mislocalize from the bud neck and, in turn, disrupting cytokinesis and halting the cell cycle. Importantly, we found that the ERSU pathway is independent of the well-studied unfolded protein response (UPR). Indeed, cells lacking IRE1, an ER transmembrane UPR-initiating component, fail to activate the UPR but are fully capable of inducing the ERSU response. Conversely, cells lacking the SLT2 MAP kinase are unable to induce the ERSU cell cycle block in response to ER stress but maintain the ability to induce the UPR events. An important remaining question is what are the molecular mechanisms by which the ERSU checkpoint is activated to temporally halt the cell cycle. Specifically, as SLT2 is not an ER transmembrane protein, it remains unclear what components initiate the ERSU at the ER membrane and how ER stress is sensed by such components. To this end, we discovered that the well-known ER-curvature-generating proteins Rtn1 and Yop1 play roles in initiating the ERSU pathway by responding to increased levels of phytosphingosine (PHS) - an early sphingolipid.
synthesized on the ER surface - induced by ER stress. We generated novel mutants of Rtn1 and Yop1 that fail to respond to PHS and are unable to initiate ER inheritance block, but maintain their abilities to generate the ER curvature. These results reveal a novel function of Rtn1 and Yop1 during ER stress, distinct from their well-known ER-curvature generating functions. Here, we will further discuss our findings regarding the molecular mechanisms by which yeast Rtn1 and Yop1 initiate the ERSU response and key molecular events that result in ER inheritance block in response to ER stress. Importantly, similar domains and activities are also present in mammalian reticulons, suggesting that the roles of these proteins in the ER stress response may be conserved throughout evolution.

M46
Imaging the dynamics of vesicle formation supports the flexible model of clathrin-mediated endocytosis
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Clathrin-mediated endocytosis (CME) is an essential cellular process facilitating the internalization of a variety of cargo. Clathrin polymerization and changes in plasma membrane architecture and composition are necessary steps to mediate the formation of clathrin-coated vesicles (CCVs). However, simultaneous analysis of clathrin dynamics and membrane structure in living cells is challenging due to the limited axial resolution of fluorescence microscopes and the heterogeneity of CME. This has fueled conflicting models of vesicle assembly and obscured the roles of flat clathrin assemblies. Here we use Simultaneous Two-wavelength Axial Ratiometry (STAR) microscopy to bridge this critical knowledge gap by quantifying the nanoscale dynamics of clathrin-coat shape changes during vesicle assembly. We found that de novo clathrin accumulations generate both flat and curved structures in living cells. Approximately 80% of clathrin accumulations contributed to successful endocytosis while 20% remained flat in both kidney fibroblast-like (Cos-7) cells stimulated with epidermal growth factor (EGF) and human umbilical vein endothelial cells (HUVECs) stimulated with vascular endothelial growth factor (VEGF). High-throughput analysis revealed that the initiation of vesicle curvature does not directly correlate with clathrin accumulation. We show that clathrin accumulation drives curvature formation at shorter-lived CCVs (<20s), but clathrin undergoes a flat-to-curved transition at longer-lived CCVs (>20s). The broad spectrum of curvature initiation dynamics supports multiple productive mechanisms of vesicle formation and the flexible model of CME. The unique power of STAR microscopy has great potential to reveal the drivers of membrane curvature in the flexible model of clathrin-mediated endocytosis and to link distinct clathrin-coated structures with their impact on cellular logistics and homeostasis.

M47
Asymmetric Arp2/3-mediated actin assembly facilitates clathrin-mediated endocytosis at stalled sites in genome-edited human stem cells
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Actin filament assembly facilitates vesicle formation in several trafficking pathways including clathrin-mediated endocytosis (CME). However, how actin assembly forces are harnessed has not been fully revealed for any vesicle forming process. In this study, three-dimensional (3D) super-resolution microscopy, live-cell imaging of triple-genome-edited, induced pluripotent stem cells (iPSCs), and newly
developed machine-learning-based computational analysis tools, were used to comprehensively analyze assembly dynamics and geometry of proteins representing three different CME functional modules. When hundreds of CME events with and without associated Arp2/3-dependent actin network assembly were compared, sites with actin assembly showed a distinct delay between completion of endocytic coat expansion and vesicle scission, consistent with the notion that these were stalled sites requiring actin assembly forces to complete vesicle formation. Importantly, our analysis showed that N-WASP is preferentially recruited to one side of CME sites, where it stimulates actin assembly asymmetrically. These results indicate that in mammalian cells actin assembly is induced at stalled CME sites, where asymmetric forces pull the plasma membrane into the cell much like a bottle opener pulls off a bottle cap.

M48
**The Effects of Class II PI3K Variants During Autophagy**
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Autophagy is an adaptive response to many forms of cell stress. Macroautophagy (autophagy) is a trafficking pathway that relies on delivery of membrane-bound cytoplasmic contents to late endosomes for degradation by the lysosome, called the autolysosome. Thus, the lysosome must accommodate the convergence of cargos from both autophagy and endosomal pathways, as well as coordinate autolysosome maturation with changing levels of basal versus stress-induced autophagy. In Drosophila, we identified two phosphoinositide lipid regulators, class II PI3-kinase (PI3KC2) and Mtm PI3-phosphatase, that act in a shared autolysosome inhibitory pathway to maintain basal levels of autophagy in fat body of fed larvae. Strikingly, we discovered that wildtype flies also co-express a truncated, noncatalytic PI3KC2 splice variant (PI3KC2-short) that, converse to PI3KC2 and Mtm, is required for autolysosome maturation through repression of their catalytic activities. Current studies are addressing different hypotheses on where PI3KC2-short, PI3KC2 and Mtm act to influence autolysosome maturation. In all cases, the deletion mutants exhibit an increase in the number of either Rab5+ early endosomes (∆short) or Rab7+ late endosomes (∆PI3KC2 and ∆mtm), but constitutive Rab7 or Rab5 activity, respectively, is unable to revert normal autolysosome levels. We are currently investigating whether the pathway regulates delivery of specific endosomal-lysosomal cargo(s) involved in autolysosome maturation, and/or balances this cargo delivery with alternative transit through endocytic recycling. In addition, we have shown that defects in autolysosome maturation correlate with aspects of autophagic lysosome reformation (ALR). Our work reveals the importance of coordinated activity between specific phosphoinositide kinase and phosphatase activities, as well as a novel role for their joint regulation by a novel noncatalytic splice variant to respond to changing homeostatic demands in membrane flux.

Immune Evasion and Host Defense

M49
**Human Viruses Rewire Membrane Contact Site Tethering Interactions, Structures, and Functions for Pro-Viral Organelle Remodeling**
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Membrane contact sites (MCSs) link organelles in dynamic networks that coordinate cellular functions across space and time. Although all viruses rely on organelles for their replication cycles, MCSs remain largely unexplored during human infections. Here, we design a targeted mass spectrometry platform for detecting and quantifying MCS proteins at all organelles simultaneously, and define functional virus-driven MCS alterations by both ancient and rapidly evolving human viruses: human cytomegalovirus (HCMV), herpes simplex virus type 1 (HSV-1), influenza A (Infl. A), and the beta-coronavirus HCoV-OC43. Integration with live super-resolution microscopy and molecular virology perturbations reveals that the virus-driven regulation of organelle contacts is time-sensitive and organelle-specific, providing a molecular basis for switching anti- to pro-viral organelle structures and functions. We uncover a stabilized mitochondria-ER encapsulation structure induced by infection, which we term MENC. We also determine that premature ER-mitochondria tethering activates STING and elevates interferon secretion, priming cells against infection. During HCMV infection, ER-mitochondria contact is decreased early, indicating virus immune evasion. Late in infection, MENCs form and recruit asymmetrically localized PTP1P51, which increases in tethering to VAP-B and is required for virus production. At the peroxisome, ACBD5-mediated ER contacts are increased and enriched at infection-derived enlarged peroxisomes, which are plastered along ER membranes and require ACBD5 to form. We find that ACBD5 abundance controls the balance between pro-viral peroxisome biogenesis and membrane expansion, exhibiting a broadly anti-viral role in each infection examined. By characterizing the global virus-directed modulation of MCSs, we uncover the molecular fingerprints of organelle remodeling linked to infection progression and pathogenesis.

M50
The Role of Keratin 16 as a Regulator of the Interferon Pathway
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Robust induction of stress-responsive keratins is an evolutionarily conserved response to epithelial stress and is critical for tissue repair. Keratins have traditionally been studied as a structural component in epithelial cells, yet accumulating evidence indicates that they also act as critical regulators of cellular signaling pathways. Keratin 16 (K16) is induced following cellular stress and its expression is associated with tissue hyperproliferation, cellular differentiation, and innate immune activation. Mutations in KRT16 are responsible for Pachyonychia Congenita (PC), a genetic disorder that features aberrancies in both differentiation and homeostasis within ectoderm-derived epithelial appendages and glabrous skin. Despite myriad observations of K16 in tissue repair and in disorders such as psoriasis and PC, little is known about its interactome and role in cellular signaling, and how it relates to innate immune responses. We compared single-cell RNA-seq from human psoriasis patients with K16 proteomic interactions in keratinocyte cell lines (K16 IP/MS) in an effort to build a K16 interactome. Our analysis identified a potential association between K16 and members of the interferon-alpha/beta signaling pathway. Utilizing a cell line derived from a human keratoacanthoma (HKA-1), we identified a direct interaction between K16 and 14-3-3, a regulator of cellular signaling such as hippo pathway, mTOR, and type I interferon response. We identified K16 to colocalize with the GTP-binding protein Mx1, and ISG15, both effectors of Interferon signaling. In vivo, Krt16-null mice exhibit severe inflammatory response following cellular stress, and this is accompanied by the upregulation of several type I interferon genes.
including Interferon alpha-B, CCL2, CCL5, and OASL2 activator of ribonuclease L (RNase L). These results suggest a role for K16 in the regulation of interferon pathway following cellular stress in human cell lines and mouse models. Efforts are underway to dissect K16’s involvement in type I interferon responses, as well as the functional implications for the cellular phenotypes observed in Krt16-null mice, PC and psoriasis. These studies are poised to significantly expand our understanding of molecular pathways that regulate inflammation, tissue repair and homeostasis in skin and related epithelia. Supported by NIH R01 grant AR044232 (to PAC) and the Center for Plasticity and Organ Design (to EC).

M51
Tunneling nanotubes: "intercellular highways" for Ebola virus dissemination in the host
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Tunneling nanotubes (TNTs), the actin-based long range intercellular communication system that allows direct exchange of cytosolic constituents between cells, can be exploited by pathogens to navigate within the host, largely undetected by the immune system. Ebola virus (EBOV) and other members of the filovirus family continue to pose a significant public health concern due to their ability to trigger increasingly more frequent outbreaks of a systemic, hemorrhagic disease in human populations. EBOV disease is marked by rapid, uncontrolled spread of virus replication to distant tissues and organs by a poorly defined mechanism. In current models, EBOV enters its primary cell target, macrophages, by macropinocytosis, followed by viral gene expression and genome replication in the cytoplasm. Nascent virions bud from the cell surface to infect nearby cells, thus spreading infection locally. Here, we present novel findings that EBOV uses an alternative route to rapidly disseminate replication: TNTs. Using scanning electron and high-resolution quantitative 3D microscopy, we show that EBOV infection dramatically induces formation of TNTs containing virus in primary human homo- and heterologous macrophage cultures. Measuring 0.2-1.7 micron in diameter and up to 110 micron in length, these connections represent different types of TNT populations triggered by the infection. Interestingly, only TNTs containing tubulin and able to transport cellular organelles, including mitochondria, lysosomes, and autophagosomes, contained viral replication units, highlighting the intriguing possibility that EBOV exploits a subset(s) of TNTs to remotely modulate cell health and/or immune responses during infection, further contributing to disease development. We also show that TNTs promote cell-to-cell transfer of the EBOV replication units in the absence of live virus and, in an M-Sec-dependent manner, support efficient replication in cells devoid of factors critical for virus entry into the cell after initial retardation. We will lastly discuss findings we gathered by examining tissues of EBOV-challenged rhesus macaques, an animal model of EBOV disease, for the presence of macrophage TNTs containing virus. Our studies establish an alternate model of EBOV dissemination within its host, laying the groundwork for further investigations into pathogenesis of filoviruses, specifically the molecular mechanism(s) of replication spread and immune evasion. Importantly, these discoveries will stimulate new areas to design experimental countermeasures targeting EBOV and related viruses.

M52
Trex-2 Complex: A New Player in Influenza A Virus mRNA Nuclear Export
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Export of influenza A virus (IAV) mRNAs from the nucleus to the cytoplasm occurs through the nuclear pore complex (NPC) and is a key step in the IAV life cycle. Here, we have investigated the role of cellular mRNA export machinery constituents in nuclear export of IAV mRNA. We used the Auxin-Induced Degron (AID) system to rapidly degrade constituents of the cellular mRNA nuclear export machinery and avoid secondary effects associated with a prolonged mRNA knockdown. We show that rapid depletion of the nucleoporin Tpr, which is localized at the nucleoplasmic side of the NPC, prevents nuclear export of influenza A virus mRNAs while degradation of the other nucleoplasmic nucleoporins (Nup153 and Nup50) does not have a significant effect on viral mRNA export. Tpr is known to have a role in the recruitment of the Transcription and Export complex 2 (TREX-2) to the nuclear basket of the NPC. We found that the components of the TREX-2 complex, GANP and PCID2, are also required for viral mRNA export. RNA-seq of cellular mRNAs from nuclear and cytoplasmic fractions of GANP-depleted cells indicates that nuclear export of mRNAs with fewer exons, low GC content and long exons are dependent on GANP. This is consistent with the observed GANP dependency of the IAV mRNAs that either lack introns or have a small number of introns. In addition, we found that the influenza virulence factor NS1 protein interacts with GANP. Since NS1 protein promotes influenza virus mRNA export of a subset of viral mRNAs, these results suggest that GANP-NS1 interaction may facilitate export of viral mRNAs. In summary, we report a novel role for the TREX-2 complex in nuclear export of IAV mRNAs and of a subset of cellular mRNAs. Additionally, we identified key mRNA features associated with the GANP dependency for nuclear export of cellular and viral mRNAs.

M53
Nutrition is Linked to Immunity Against Bacterial Pathogens via the NRAMP1 Metal Transporter
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The Natural Resistance Associated Macrophage Protein 1 (NRAMP1) is a prominent metal transporter in host defenses against pathogens. Prevailing scholarship holds that macrophages facilitate control of intracellular pathogens via NRAMP1 mediated metal starvation in the phagosome. We found that NRAMP1 in neutrophils also contributes to host control of bacterial pathogens. While investigating how vitamin A deficiency impairs immunity against systemic salmonellosis, we discovered that NRAMP1-deficient animals were equally susceptible to disseminated *Salmonella* as vitamin A-deficient, NRAMP1-proficient, mice. This data suggested that NRAMP1 mediated immunity to disseminated salmonellosis was dependent on vitamin A. Indeed, Vitamin A deficiency impaired infection-induced granulopoiesis, resulting in reduced expression of specific- and gelatinase granule components in neutrophils, including NRAMP1. Adoptive transfer of neutrophils from NRAMP1-proficient donors, but not NRAMP1-deficient donors, reduced the systemic *Salmonella* burden in vitamin A-deficient, NRAMP1-proficient, mice and in NRAMP1-deficient animals. Additionally, NRAMP1-deficient neutrophils displayed diminished killing of *Salmonella* ex vivo compared to NRAMP1-proficient neutrophils. During infection with another vacuolar intracellular pathogen, *Brucella abortus*, we found that NRAMP1 contributed to a reduction in *Brucella* burden of the placenta, in which neutrophils also play a prominent role. Collectively, these data suggest
that NRAMP1-deficiency impairs control of intracellular pathogens by blunting neutrophil-mediated host defenses.

M54  
**Fancc in control of LPS induced septic shock and bone marrow failure**  
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**Objective:** Fanconi anemia (FA) patients are at higher risk of developing bone marrow failure (BMF) or cancer upon exposure to viral infections, bacterial infections, carcinogens and mutagens. Deleterious mutations in FA genes cause impaired ability to repair DNA interstrand crosslink lesions in the nucleus and impaired removal of damaged mitochondria via mitophagy in the cytoplasm. **Fancc**-deficient mice exhibit enhanced inflammatory response and are hypersensitive to lipopolysaccharide-induced septic shock as a result of hematopoietic suppression. Considering the role of inflammation in pathogenesis of Fanconi anemia and bone marrow failure diseases in general, we asked whether inflammasome inhibition could be a promising therapeutic for the treatment of FA. **Methods:** MCC950 is a small molecule selective inhibitor of the NLRP3 inflammasome. To determine if suppression of inflammasome signaling protects against bone marrow failure in an animal model of FA, we have investigated the therapeutic potential of MCC950 in protecting against the hematopoietic suppression and lethality induced by lipopolysaccharide (LPS) in FA complementation group C gene (**Fancc**)−/− mice. **Results:** Our studies demonstrate that MCC950, a small molecule inhibitor of the NLRP3 inflammasome, can protect **Fancc** null mice from LPS-induced hematopoietic suppression and septic shock as evidenced by increased survival rate of **Fancc**−/− mice pretreated with MCC950. NLRP3 inhibition rescued the hypersensitivity of **Fancc**−/− mice to LPS-induced septic shock. MCC950 partially rescues the thrombocytopenia in peripheral blood. Analysis of the composition of hematopoietic stem cells (HSCs) and HSC-derived progenitors shows that MCC950 partially restores the loss of megakaryocyte-erythroid progenitor (MEP) and granulocyte-macrophage progenitor (GMP) pools upon LPS challenge. Further, Treatment with MCC950 significantly improved the bone marrow colony-forming capacity of **Fancc**−/− bone marrow cells in vitro. **Conclusion/Clinical applicability:** Despite currently available treatments for Fanconi anemia including bone marrow transplantation and androgen therapy, poor outcomes highlight the need to explore novel therapeutic options. Our studies demonstrate that MCC950, a small molecule inhibitor of the NLRP3 inflammasome, can protect **Fancc** null mice from LPS-induced hematopoietic suppression and septic shock. MCC950 prevents hematopoietic cell loss, and animal mortality in **Fancc**-deficient cells and animals treated with LPS, and partially normalized important hematopoietic parameters in our **Fancc**−/− mouse model upon LPS challenge. These data indicate that NLRP3 inhibition shows promise and warrants further evaluation as a potential therapeutic strategy in FA.

**Mechanics of Cell Adhesion and Cell-Cell Interactions**

M55  
**Mechanical stability of Afadin-based adhesion complexes**  
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The formation of mechanically robust, polarized epithelia typified by intricately organized intercellular adherens junction and tight junction adhesion complexes is critical to the construction of multicellular
life. However, the molecular mechanisms that underlie the construction and stratification of these junctions remain unclear. Afadin is an actin-binding protein that is associated with both adherens and tight junctions, and is essential during embryogenesis and epithelial barrier function in adult tissues. Afadin binds via its PDZ domain to the intracellular C-termini of Nectins and JAMs, two abundant classes of cell-adhesion proteins which may template the assembly of adherens and tight junctions, respectively. Although indirect evidence suggests that Afadin may transmit forces between cellular adhesions and the actin cytoskeleton, nothing is known about the ability of the linkages of Afadin with either Nectins or JAMs to transmit or respond to mechanical load. Using a single-molecule magnetic tweezers assay, we probed the mechanical stability of the bonds between the Afadin PDZ domain and the intracellular domains of Nectin-1 and JAM-A. We found that both ligands formed remarkably stable bonds with Afadin-PDZ at forces up to 10 pN. In contrast, preliminary data derived from solution-phase, biochemical measurements, showed that bond lifetimes for Nectin-1/Afadin were much longer than for JAM-A/Afadin at close to zero load. Our data suggest that the complexes of afadin with JAM-A and Nectin-1 may function in parallel with the much more intensively studied cadherin-based adhesions to transmit forces across cell-cell junctions. We speculate that differences in force responsiveness of Nectin- and JAM-afadin complexes may underlie the presently poorly understood roles of Afadin in controlling the architecture of epithelial tissues.

M56
Programming cellular interactions and organization with synthetic cell adhesion molecules
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Cellular adhesion molecules have evolved to control an array of intracellular signaling functionalities that are critical to the regulation of multicellular organisms. In order to design artificial multicellular systems capable of complex organization and behavior, we have engineered a toolkit of synthetic cellular adhesion molecules with programmable recognition domains that retain endogenous signaling. For example, some of these synthetic receptors mediate tight cell-cell interactions via cytoskeletal reorganization, while others facilitate receptor enrichment through the recruitment of scaffold proteins. We find that the identity of the intracellular domain, and how it engages the cytoskeleton and other intracellular machinery, dominates in determining the strength and morphology of the resulting cell-cell interfaces. Furthermore, through incorporating diverse extracellular interactions, both heterotypic and homotypic, this receptor toolkit enables the formation and reorganization of structures with custom cellular arrangements. These synthetic adhesion interactions can be used to predictably program the assembly of novel multi-cellular architectures, the modification of native tissues, or immune cell adhesion to target tissues. We are presently expanding the applicability of synthetic adhesion to control complex multicellular assembly and the localization of immune cells to tissue both in vitro and in vivo.
M57

Lights, Camera, Action: Investigating the activation of the Rho flare tight junction repair pathway using optogenetics

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Driven by actomyosin contractility, epithelial cells undergo various cell shape changes, including cell division and shape changes associated with developmental morphogenesis, which challenge cell adhesion and barrier integrity. The small GTPase RhoA is activated at cell-cell junctions in short-lived, local accumulations, termed “Rho flares”, which repair local leaks in barrier function through an actomyosin-dependent junction contraction that reinforces tight junctions (TJs). However, the upstream mechanisms leading to Rho flare activation remain unknown. Because Rho flares are preceded by junction elongation and a TJ breach, we predict that junction elongation may act as a mechanical stimulus for Rho flares. By using the TULIP optogenetic system in Xenopus laevis embryos, we find that regional light stimulation induces a region of contractility, leading to an increased frequency of Rho flares at nearby junctions. Junction elongation occurs upstream of these optogenetically-induced Rho flares, serving as a mechanical stimulus for Rho flare activation. Cells can mediate mechanical stimuli through heterotrimeric Gα12/13 proteins, which activate specific GEFs to promote downstream RhoA signaling. We find that p115RhoGEF localizes to the apical perimeter of cell-cell junctions and strongly accumulates at Rho flares, increasing in signal intensity after the TJ breach and before reinforcement of the TJ protein ZO-1. p115 knockdown (KD) reduces baseline junctional active Rho intensity as well as ZO-1 signal in comparison to controls. Current work will determine if p115 KD affects Rho flare activation, reinforcement of TJ proteins, and TJ barrier function. Together, these results reveal key players that participate in Rho flare activation to maintain TJ barrier integrity as cells change shape within a developing vertebrate epithelium.

M58

Mechanical stresses accelerate Red Blood Cell aging

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Red blood cells (RBCs) experience alterations in cell size, cell shape, and plasma membrane components during their lifespan. RBCs are filtered through the spleen, where senescent cells become trapped in the narrow inter-endothelial slits and are cleared by macrophages. To assess how the cyclic physical forces caused by splenic filtration affect RBC aging, we circulated cells through microfluidic devices with narrow slits (<1 micron wide). We found that cumulative passing through narrow slits elicited major biophysical and molecular alterations on the RBCs. Of note, vesiculation was significantly accelerated, promoting a more spherical RBC morphology and a loss of cell deformability. To study how vesiculation modified the protein contents in the RBC membrane, we performed a comprehensive proteomics analysis of RBCs that had previously circulated through our microfluidic device and controls. This analysis revealed significant changes in three protein clusters associated with the antioxidation system, structural integrity, and metabolic processes. Consistent with the proteomics data, we found that cyclic loading significantly accelerated the appearance of RBC aging hallmarks like hemoglobin oxidation and intracellular ATP depletion. We found that not only the onset but also the shutdown of vesiculation was accelerated by cyclic physical forces, which is relevant given that vesiculation has been proposed to
serve as a self-protecting mechanism. We submit that vesiculation limits itself by the decreasing distance between membrane-cytoskeleton linkages as membrane material is lost, which the bilayer’s spontaneous fluctuations and deformations under external forces. We confirmed this hypothesis using STORM microscopy to show that the distance between molecular linkages Ankyrin-1 and Band 4.1 is significantly lower in cells circulated through our microfluidic device than in controls. Furthermore, we found that membrane fluctuations indeed decreased significantly in circulated cells. Finally, we studied the interaction of macrophages with RBCs circulated through our microfluidic device, observing that phagocytosis increased compared to control RBCs, mostly due to an increase in RBCs stiffness coupled with autoantibody opsonization. Overall, this work provides new insight into how physical forces accelerate the aging of RBCs and their clearance by macrophages.

M59

**Shaping of the C. elegans gonad by growth-induced pressure, matrix remodeling, & differential adhesion of a leader cell.**

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Organ morphology is critical for its function. Biochemical signals along with mechanical cues influence multiple cell and tissue behaviors to shape an organ during development. Although significant advances have been made in understanding the genetic regulation of 3D organ development, the role of mechanical forces has remained largely unexplored. Here we use C. elegans, a free-living nematode, as a model system to understand the mechanical basis of gonad development. The C. elegans gonad has two symmetrical U-shaped arms, each with a single somatic cell known as Distal tip cell (DTC) at its tip. The DTC functions as a leader cell guiding multiple follower germ cells to form the U-shaped architecture. The gonad initially elongates on the ventral surface away from the midbody (Phase I), then initiates a U-turn towards the dorsal surface (Phase II), finally moving back towards the midbody (Phase III). Akin to a migrating cell, we found that the DTC is polarized with ARX-2, an actin-related protein, enriched at the front and NMY-2, non-muscle myosin-II, present at the rear. However, it lacks any actin-rich protrusions. Moreover, DTC-specific depletion of NMY-2 or ARX-2, the contractile force-generating machinery, did not show any defect in gonad morphogenesis, suggesting that autonomous movement of the DTC is not required for gonad elongation. Instead, we found that the gonad elongates due to a pushing force generated by proliferating germ cells confined behind the DTC and a concomitant release of pressure in the front due to DTC-induced matrix degradation. Strikingly, we found that spatiotemporally controlled differential adhesion between the DTC and its environment can explain gonad folding. Thus, stronger cell-matrix adhesion on the dorsal side of the DTC creates a torque which leads to the U-turn of the gonad. Taken together, our study provides novel mechanistic insight into C. elegans gonad development.

M60

**The mechanobiology of bacterial biofilms growing on soft substrates**

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During chronic infections and in microbiota, bacteria predominantly colonize their hosts as multicellular structures called biofilms. A common assumption is that biofilms exclusively interact with their hosts biochemically. However, how substrate mechanics influence biofilm formation and, more specifically,
how they form on soft, tissue-like materials, remains unknown. During early colonization, \textit{P. aeruginosa} uses a surface-specific motility mode known as twitching. This early-stage exploration coordinates group behavior to seed multicellular structure of nascent biofilms. Cells power twitching by successive extension and retraction of type IV pili, long and thin protein filaments. We investigated the effect of substrate mechanics on the surface motility of \textit{P. aeruginosa}. By tracking single cells on substrates of different stiffness, we observed that the twitching speed was not correlated with the modulus of the hydrogels, but rather with their mesh size. We hypothesize that type IV pili attaches more efficiently to hydrogels with smaller pore size, thereby improving overall displacement. The mechanically-dependent regulation of twitching motility in turn regulates the architecture and spatial organization of nascent biofilms: \textit{P. aeruginosa} forms isolated mushroom like structures on hydrogels where motility is limited, while they spread out more intensely on substrates where they can twitch, limiting the height of the multicellular structures and enhancing the mixing with neighbouring colonies. Furthermore, we observed that biofilms appeared to deform the soft substrates they were growing on. We could observe this phenomenon for \textit{P. aeruginosa} but also in biofilms of \textit{V. cholerae}, which lack surface motility. By combining mechanical measurements and mutations in matrix components, we established a model for which biofilms deform by buckling, and that adhesion transmits these forces to their substrates. We demonstrated that \textit{V. cholerae} biofilms can generate sufficient mechanical stress to deform and even disrupt soft epithelial cell monolayers, suggesting a mechanical mode of infection. Altogether, our results bring a new perspective on the study of biofilms in a context that more closely resemble the infection environment. We show that the mechanical microenvironment experienced by bacteria during colonization has a strong impact on the development and physiology of bacterial biofilms.

**Microtubule Motors: Emerging Phenomena and Methods**

**M61**

\textbf{Single-molecule imaging of cytoplasmic dynein in vivo reveals the mechanism of motor activation and cargo capture}

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Cytoplasmic dynein 1 (dynein) is the primary minus end-directed motor protein in most eukaryotic cells. Dynein remains in an inactive conformation until the formation of a tripartite complex comprising dynein, its regulator dynactin and a cargo adaptor. How this process of motor activation occurs is unclear, since it entails the formation of a three-protein complex inside the crowded environs of a cell. Here, we employed live-cell, single-molecule imaging to visualise and track fluorescently tagged dynein. First, we observed that dynein that bound to the microtubule engaged in minus end-directed movement only \textasciitilde 30\% of the time and resided on the microtubule for a short duration. Next, using high-resolution imaging and correlative light and electron microscopy, we discovered that dynactin remained persistently attached to microtubules, and that endosomal cargo remained in proximity to the microtubules and dynactin. Finally, we employed two-colour imaging to visualise cargo movement effected by single motor binding. Taken together, we discovered a search strategy that is facilitated by dynein’s frequent microtubule binding-unbinding kinetics: (1) in a futile event when dynein does not encounter cargo anchored in proximity to the microtubule, dynein unbinds and diffuses into the
cytoplasm, (2) when dynein encounters cargo and dynactin upon microtubule-binding, it moves cargo in a short run. Several of these short runs accumulate to give rise to long-range cargo transport. In conclusion, we demonstrate that dynein activation and cargo capture are coupled in a step that relies on reduction of dimensionality to enable minus end-directed transport in vivo.

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Cephalopod RNA Recoding Sites Modulate Dynein and Kinesin Function

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RNA editing is a pervasive epigenetic mechanism that can diversify transcriptomes. In humans, most RNA editing occurs in non-coding regions, and editing rarely results in mRNA recoding. However, mRNA recoding is unusually prevalent in cephalopods (squid, cuttlefish, and octopus), with ~60% of all proteins recoded through RNA editing. Most cephalopod recoding sites have not been characterized, but RNA recoding may be an important epigenetic strategy in these animals to functionally regulate proteins. Sites of RNA recoding may therefore represent a “nature-guided” roadmap to reveal functionally important residues or sites of modulation that are difficult to predict by homology and structural analysis alone. Proteins involved in microtubule-based transport are heavily edited and provide a uniquely tractable model system to characterize the effects of RNA recoding on protein function. Using total internal reflection fluorescence (TIRF) microscopy, we have characterized sites of RNA editing in the motor proteins dynein and kinesin and have found that individual recoding sites can significantly alter motility in vitro. Additionally, we are exploring the role of RNA recoding on the function of squid kinesin. We have identified tissue-specific combinations of RNA edits along the squid kinesin motor domain from the optic lobe and stellate ganglion of the market squid Doryteuthis pealeii. These tissue-specific variants display significantly different motile properties across different temperatures, which supports the idea that RNA recoding may be employed to modulate motor function in response to environmental stimuli such as temperature. Overall, this study furthers our mechanistic understanding of dynein and kinesin and highlights the utility of analyzing cephalopod RNA editing sites as a general strategy to probe protein function.

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A single-stranded DNA based high-throughput force sensor for molecular motors

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Cellular cargo like peroxisomes, mitochondria, etc. are transported by the action of multiple molecular motors attached to the cargo. Multiple motors on the cargo exert forces to steer it to the destination. Measuring Pico-Newton forces of individual motors while they are collectively transporting the cargo is important for understanding intracellular transport. Existing force sensing techniques, such as optical traps, effectively quantify responses of single motors to the applied force but cannot reach a single motor resolution during collective transport and suffer from low data throughput. Quantifying forces exerted by individual molecular motors during multi-motor transport with the existing force measurement techniques is challenging. In this study, we have designed a high-throughput assay involving nanometer-resolved multicolor-fluorescence and a denatured ssDNA (d-ssDNA)-based force-
sensor. This assay can measure picoNewtons (pN) of forces exerted by the individual kinesin motors, driving a common microtubule. Our assay allowed high throughput data acquisition as forces by hundreds of kinesins could be measured in a single movie. We immobilized quantum dot labeled kinesin motors on the surface using d-ssDNA linker. Kinesins were immobilized sufficiently away from each other such that individual kinesin motors could be observed in the microscope. We used multicolor fluorescence microscopy to simultaneously observe kinesins, microtubules, and roadblocks in different channels. Due to the stretchability of d-ssDNA, kinesins could be displaced from the attachment point of the d-ssDNA when multiple kinesins drove a common microtubule. By quantifying the extension of the d-ssDNA, we could estimate the forces of individual kinesins accurately. We also benchmarked the force-extension properties of d-ssDNA by performing optical trap measurements. Optical trap measurements showed that our force sensor could measure forces accurately in the 0-20 pN range. Kinesins primarily exerted less than 1 pN force for transporting a common microtubule. By introducing roadblocks, i.e., immobilized nanospheres of varying size (20-100 nanometers), we directly observe that individual kinesins can transiently modulate their forces upon encountering physical hindrances while keeping their average forces below 1 pN. Our assay is high throughput and can readily be applied to other types of molecular motors.

Kinesin-binding protein remodels the kinesin motor to prevent microtubule-binding

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Kinesins are tightly regulated in space and time to control their activation in the absence of cargo-binding. Kinesin-binding protein (KIFBP) was recently discovered to bind the catalytic motor heads of 8 of the 45 known kinesin superfamily members and inhibit their interaction with microtubules. In humans, mutation of KIFBP gives rise to Goldberg-Shprintzen syndrome (GOSH), but the kinesin(s) that is misregulated to produce clinical features of the disease is not known. Understanding the structural mechanism by which KIFBP selects its kinesin binding partners will be key to unlocking this knowledge. Using a combination of cryo-electron microscopy and crosslinking mass spectrometry, we determined structures of KIFBP alone and in complex with two mitotic kinesins, KIF15 and KIF18A, revealing regions of KIFBP that participate in complex formation. KIFBP adopts an alpha-helical solenoid structure composed of TPR repeats. We find that KIFBP uses a 2-pronged mechanism to remodel kinesin motors and block microtubule-binding. First, KIFBP engages the microtubule-binding interface and sterically blocks interaction with microtubules. Second, KIFBP induces allosteric conformational changes to the kinesin motor head that displace a key structural element in the motor head (α-helix 4) required for microtubule binding. We identified two regions of KIFBP necessary for in vitro kinesin-binding as well as cellular regulation during mitosis. Taken together, this work establishes the mechanism of kinesin inhibition by KIFBP and provides the first example of motor domain remodeling as a means to abrogate kinesin activity.
The conserved MAP kinase scaffold JIP3 prevents excess lysosome accumulation in axons of vertebrates and invertebrates. Whether and how JIP3’s interaction with microtubule-based motors contributes to this critical organelle clearance function is unclear. Using purified recombinant proteins, we show that dynein light intermediate chain (DLIC) binds to the N-terminal RH1 domains of JIP3, its paralog JIP4, and the lysosomal adaptor RILP. A point mutation in a hydrophobic pocket of the RH1 domain, previously shown to abrogate RILPL2 binding to myosin Va, abrogates the binding of JIP3/4 and RILP to DLIC without perturbing the interaction between the JIP3 RH1 domain and kinesin heavy chain. We use this separation-of-function mutation to show that the interaction between JIP3 and DLIC is required for JIP3-mediated organelle clearance in the anterior process of Caenorhabditis elegans touch receptor neurons. Unlike JIP3 null mutants, JIP3 that cannot bind DLIC causes prominent accumulation of endo-lysosomal markers at the neurite tip, which is rescued by a disease-associated point mutation in JIP3’s leucine zipper that abrogates kinesin light chain binding. This study highlights that RH1 domains are interaction hubs for cytoskeletal motors and suggests that JIP3-bound dynein and kinesin-1 participate in bi-directional organelle transport.

In neurons, mitochondria are transported to distal regions for supplying energy and buffer Ca2+. Mitochondrial transport is mediated by Miro and TRAK adaptors that recruit kinesin and dynine-dynactin. To understand how mitochondria are transported by these opposing motors and stalled at regions with elevated Ca2+, we reconstituted the mitochondrial transport machinery in vitro. We show that the coiled-coil domain of TRAK activates dynein-dynactin motility, but kinesin requires an additional factor to efficiently transport Miro/TRAK. Unexpectedly, TRAK adaptors that recruit both motors move towards kinesin’s direction, whereas kinesin is excluded from binding TRAK transported by dynein-dynactin. The assembly and motility of the transport machinery are not affected by Ca2+. Instead, the mitochondrial docking protein syntaphilin is sufficient to oppose the forces generated by kinesin and stall the motility. Our results provide mechanistic insight into how mitochondria are transported by the coordinated action of motors and statically anchored to regions with high neuronal activity.
Nutrient and Growth Signaling

M67

Subcellular localization of mTOR mRNA in neuronal growth control

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mTOR is a serine threonine kinase known to control cell growth and size, and to regulate protein translation. In neuronal cells mTOR activation was shown to increase axonal growth following injury. However, the function of mTOR in the different subcellular compartments is not known. We previously showed that the mTOR 3’ UTR plays a role in the transport of mTOR mRNA to axons and that mTOR axonal translation is important to determine cell survival after injury (Terenzio et al. 2018). Here we used fluorescence in situ hybridization of a reporter transcript to identify multiple axonal localization motifs within the mTOR 5’ and 3’ UTRs. We found that stretches of guanine nucleotides within these motifs help to recruit nucleolin, an RBP previously shown to play a role in mRNA axonal transport and cell size regulation. We generated a number of gene-edited mice with deletions within the mTOR 5’ and 3’UTRs that disrupt axonal transport of the mTOR transcript. Large deletions in the mTOR 5’UTR lead to the generation of mTOR hypomorph mice, but more subtle mutations reduced mTOR translation levels in the axons but not in the cell body. Notably, specific reduction in axonal mTOR causes an increase in axonal outgrowth, suggesting that subcellular regulation of localized translation pathways is involved in neuronal size sensing and growth control.

M68

Spatiotemporal regulation of AMPK revealed by a sensitive kinase activity reporter

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AMP activated protein kinase (AMPK) is a key regulator of cellular energy homeostasis, but mechanisms behind subcellular AMPK activity remain elusive. To monitor the dynamic activity of AMPK, we developed a single-fluorophore excitation-ratiometric AMPK activity reporter (ExRai AMPKAR) which exhibits an excitation wavelength change upon phosphorylation by AMPK, providing a specific readout of AMPK activity in living cells. Using subcellularly localized ExRai AMPKAR, we found AMPK activities at the lysosome and mitochondria are differentially regulated. While different stress signals, irrespective of their effects on ATP, robustly yet gradually increases mitochondrial AMPK activity, lysosomal AMPK activity accumulates with much faster kinetics. Genetic deletion of the canonical upstream kinase serine/threonine kinase 11 (LKB1) resulted in slower AMPK at lysosomes but did not affect the response amplitude at either location, in sharp contrast to the necessity of LKB1 for cytosolic AMPK activity. Nuclear-localized ExRai AMPKAR unveiled the presence of AMPK activity in the nucleus and led to a model of LKB1-mediated cytosolic activation of AMPK followed by nuclear shuttling to allow accumulation of nuclear AMPK activity. Thus, a new, sensitive reporter for AMPK activity, ExRai
AMPKAR, in complement with mathematical and biophysical methods, captured subcellular AMPK activity dynamics in living cells and unveiled complex regulation of AMPK signaling within subcellular compartments.

M69
The amino acid sensor General Control Non-derepressible 2 (GCN2) suppresses TOP mRNA translation via La related Protein 1
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Background La-related protein 1 (LARP1) has been identified as a key translational inhibitor of 5′ terminal oligopyrimidine tract (TOP) mRNAs. Mammalian mRNAs that encode ribosomal proteins and translation factors contain 5′TOP motif. In addition, recent studies identified TOP like motifs on SARS-CoV2 RNA. LARP1 inhibits TOP mRNAs downstream of the nutrient sensing protein kinase complex mTORC1 by binding to the mRNA cap and the adjacent 5′TOP motif, resulting in the displacement of the cap-binding protein eIF4E from TOP mRNAs. Another nutrient sensing protein kinase GCN2 has been previously linked to regulation of TOP mRNA translation, by an unknown mechanism. In the present study, we characterize the role of GCN2 kinase in regulation of TOP mRNA translation via LARP1.

Methods We have utilized Chromatin immunoprecipitation followed by sequencing (ChIP-seq) to identify genome-wide binding sites of transcriptional regulator ATF4 in WT and GCN2 KO cells. We employed ChIP-seq, ChIP-qPCR and RT-qPCR analysis of WT, GCN2 KO and ATF4 KO cells to establish LARP1 as a bona fide target of GCN2-ATF4 pathway. We also utilized polysome profiling, protein co-immunoprecipitation, western blotting and imaging to investigate the role of GCN2 kinase in regulation of TOP mRNA translation through LARP1. Results In this study, we show that GCN2 inhibits TOP mRNA translation via ATF4-dependent transcriptional induction of LARP1 and GCN1-mediated recruitment of LARP1 to stalled ribosomes. Furthermore, we show GCN2 and LARP1 suppress SARS CoV-2 replication.

Conclusions Our study reveals a novel link between the two nutrient sensing kinases, mTORC1 and GCN2, to co-regulate the activity of LARP1 and consequently TOP mRNA translation. While mTORC1 controls the activity of LARP1 through phosphorylation of LARP1, GCN2 controls transcription of LARP1 through translational upregulation of ATF4. Our study also indicates involvement of GCN2-LARP1 pathway in inhibition of SARS CoV-2 replication. These data suggest that mammalian cells use similar mechanism to suppress translation of host mRNAs encoding translation machinery and SARS CoV-2 RNA encoding viral proteins.

M70
Metabolic crosstalk in the skin: how adipocytes and immune cells communicate in the wound bed
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How metabolism regulates cell fate in mammals is widely unknown. Understanding the underlying mechanisms is especially important during regeneration, where cells are presented with a homeostatic challenge in terms of biomass production and high energy demand. After injury in the skin, a timely inflammatory response involving blood-derived circulating monocytes is essential for tissue repair. Once in the wound bed, these cells differentiate into macrophages during the inflammatory phase, a process
that goes awry during aging in chronic non-healing wounds. Cell differentiation is a highly energy-demanding process, and metabolic substrates present in the wound bed niche are important for the wound healing outcome. We previously demonstrated that dermal adipocytes release fatty acids into wound beds after injury to promote inflammation, yet the function of adipocyte-derived fatty acids in the initiation of the inflammatory response after the injury is not known. To unveil the role of adipocytes as providers of fatty acids used to fuel the metabolic requirements of immune cell differentiation, we set out to evaluate their role on monocyte to macrophage differentiation in the skin wound bed. Here, we utilize in vivo mouse models of skin injury and metabolic assays to reveal that monocytes utilize fatty acids to fuel metabolic programs that induce macrophage differentiation. We show that extracellular vesicles (EVs) loaded with lipids are actively taken up by monocytes in vitro and in mice in vivo. Using real-time respirometry, we show that these fatty acids activate the β-oxidation metabolic pathway in monocytes and its inhibition leads to the abrogation of macrophage differentiation. Furthermore, we show that with age, not only adipocyte-derived EV production was impaired, but more importantly, these particles were less effective in rewiring monocyte metabolism towards oxidative phosphorylation. In summary, our findings reveal an essential adipocyte-monocyte metabolic axis that controls inflammation and healing in the wound bed niche.

M71
A metabolic CRISPR screen identifies essential genes for nutrient generation through macropinocytosis and lysosomal catabolism
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The cells of mammalian organisms must acquire essential amino acids from exogenous sources to support biomass formation. While mammalian cells were thought to depend on uptake of monomeric amino acids, most amino acids in the extracellular space are contained within proteins. Cells can access the copious nutrient stores of extracellular proteins through macropinocytic uptake and lysosomal catabolism. Several oncogenic signaling pathways upregulate these processes, which enables cancer cells to feed on proteins and thereby grow in nutrient-poor tumor microenvironments. To characterize the underlying mechanisms, we have conducted a genome-wide CRISPR screen in a pancreatic cancer cell line under metabolic conditions where cells can only proliferate by generating amino acids through macropinocytosis and lysosomal catabolism of extracellular proteins. This screen comprehensively identified processes that become essential when cells rely on extracellular proteins as an amino acid source, including endocytic trafficking regulators, nutrient sensors and growth factor signaling pathways. In addition, multiple genes without known functions in cell metabolism were identified, including the uncharacterized transmembrane protein TMEM251. Genetic deletion of TMEM251 completely blocked lysosomal proteolysis, both of macropinocytic and autophagic cargoes. Mechanistically, this phenotype resulted from a failure to deliver acid hydrolases to the lysosome, which were instead secreted as immature pro-enzymes into the extracellular space. Our findings suggest that TMEM251 functions as a novel component of the mannose 6-phosphate pathway, which mediates lysosomal trafficking of newly synthesized hydrolytic enzymes from the Golgi. These results identify TMEM251 as a critical component for the generation of functional lysosomes, and suggest the lysosomal enzyme trafficking machinery as a novel target in cancer metabolism.
Environmental nutrients alter SREBP-mediated lipid metabolism in pancreatic cancer cells

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Environmental nutrients alter SREBP-mediated lipid metabolism in pancreatic cancer cells. Cellular metabolism is not only dictated by the cell-intrinsic metabolic network, but is also heavily influenced by cell-extrinsic environmental conditions. Environmental factors such as nutrient availability, oxygen availability and environmental pH are all known regulators of metabolism. However, compared with our knowledge of how cell-intrinsic factors regulate metabolism, much less is known about how cell-extrinsic factors regulate cellular metabolism. To understand how one cell-extrinsic constraint, namely abnormal nutrient availability in the tumor environment, influences cancer cellular metabolism, we previously measured nutrient availability in the microenvironment of murine pancreatic tumors. We used these measurements to generate a novel tissue culture media that faithfully recapitulates nutrient concentrations present in the tumor microenvironment, termed Tumor Interstitial Fluid Medium (TIFM). We have subsequently cultured pancreatic cancer cells both in TIFM and standard laboratory conditions (RPMI-1640 medium) and used mass spectrometry-based metabolomics and flux analyses to identify metabolic properties of pancreatic cancer cells specifically driven by cell-extrinsic pathophysiological nutrient challenge. We found that TIFM culture dramatically reduced lipid synthesis in pancreatic cancer cells such that the cells rely upon lipid uptake to meet their lipid needs. In contrast, pancreatic cancer cells grown in RPMI-1640 do not require exogenous lipids for growth and readily synthesize lipids. Mechanistically, transcriptomic analysis of mouse pancreatic cancer cell cultured in TIFM revealed a significant decrease in SREBP target genes and genes associated with lipogenesis compared to the same cells in RPMI-1640. Furthermore, we found that we found that pancreatic cancer cells under standard nutrient conditions upregulate SREBP and lipogenesis when starved of lipids, whereas TIFM-cultured cells do not. Thus, nutritional constraints of the tumor environment transcriptionally rewire the lipid metabolic network of pancreatic cancer cells to require lipid uptake over synthesis for proliferative needs. Thus, we posit that tumor nutritional cues are sensed by SREBP and we are working to identify the TIFM nutrient(s) regulating SREBP and how these nutrients influence SREBP activity. This work highlights the critical role of cell-extrinsic factors, especially nutrient availability, in regulating cellular lipid metabolism, and suggests identifying the metabolic requirements (and thus effective targets for metabolic therapies) of cells will require careful consideration of the environment of the cells.

Spindle Assembly and Chromosome Segregation

Distinct classes of lagging chromosome underpin age-related oocyte aneuploidy in mouse

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Chromosome segregation errors that cause oocyte aneuploidy increase in frequency with maternal age and are considered a major contributing factor of age-related fertility decline in females. Lagging anaphase chromosomes are one such age-associated segregation defect, but whether they actually missegregate and cause aneuploidy in mouse oocytes is unclear. Here we used high resolution live-imaging confocal microscopy to monitor chromosome behaviour during meiosis-I (MI) and show that lagging chromosomes in mouse oocytes comprise two mechanistically distinct classes of chromosome
motion that we refer to as ‘Class-I’ and ‘Class-II’. Whilst Class-I lagging chromosomes originated from fully congressed bivalents and exhibited reduced velocity of poleward movement during anaphase, Class-II laggards originated from mildly misaligned bivalents and travelled with comparable velocity towards the spindle pole following anaphase onset. Live-imaging revealed that although both classes increase in frequency with age (from 5.0% to 36.8% for Class-I, \(p<0.00005\) and 13.3% to 29.8% for Class-II, \(p<0.03\) in young \(n=60\) and aged \(n=57\) oocytes), only Class-I laggards directly lead to aneuploidy and Class-II laggards are largely benign. Surprisingly, a comparison of kinetochore-microtubule attachments between young and aged oocytes revealed that merotelic attachments, responsible for lagging chromosome formation in mitotic cells, were no more abundant in aged oocytes and present at negligible level late in MI (0.6-2.3%). Instead, a large proportion of kinetochores (20-30%) in aged oocytes lacked stable microtubule attachments shortly prior to anaphase-I onset, suggesting that chromosomes in aged oocytes do not establish their attachments in a timely manner. We show here that experimentally controlled prolongation of MI provides enough extra time for chromosomes to stably attach to microtubules which specifically limits Class-I lagging and reduces aneuploidy in aged oocytes (from 36.7% to 14.3% in control \(n=30\) and APCin-treated \(n=35\) group, \(p<0.04\)). Our study also revealed an age-associated deterioration in kinetochore MCAK protein level, the restoration of which improved chromosome alignment and limited Class-II lagging chromosome formation but had no impact on aneuploidy in aged oocytes (30.4% vs 28.0% in GFP control \(n=23\) and MCAK-GFP \(n=25\) group, \(p>0.85\)). Taken together, our data reveal lagging chromosomes to be a cause of age-related aneuploidy in mouse oocytes and suggest that manipulating the cell cycle length could increase the yield of useful oocytes in some clinically relevant contexts.

M74

Opposing Effects of Tubulin Isotypes on Spindle Function During Yeast Mitosis

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Faithful segregation of chromosomes depends on the assembly and proper function of a bipolar spindle. The spindle is built from microtubules (MTs) which are polymerized from tubulin, a heterodimer of \(\alpha\)- and \(\beta\)-subunits. Eukaryotic organisms express multiple isotypes of \(\alpha\)- and \(\beta\)-tubulin, and their discrete contributions towards mitosis is poorly understood. We employed genome editing to engineer cells expressing single tubulin isotypes, Tub1-only or Tub3-only, corresponding to the two \(\alpha\)-tubulin isotypes encoding the yeast microtubule cytoskeleton. Using genome-wide screening, we find that these isotypes show distinct genetic interactions with mitotic spindle motors that display antagonistic activities during spindle movement. Confirmed by genetic crossing and functional assays, Tub3-only spindles prematurely terminate during anaphase, whereas Tub1-only spindles are hyper-elongated. Tub1-only spindles grow relatively faster during the fast phase of spindle elongation as opposed to Tub3-only spindles, which grow both slower and shorter, phenotypes reminiscent of the antagonistic activities of kinesin-5 and kinesin-14, respectively. Intriguingly, the budding yeast homologs of kinesin-14, Kar3, and kinesin-5, Cin8, display biased localization to Tub3- and Tub1-only MTs, respectively, during distinct phases of anaphase by quantitative fluorescent microscopy. In wildtype, the antagonistic activities of these proteins are properly balanced, enabling the spindle to achieve the sufficient length necessary for duplicated chromosomes to be equally segregated between daughter cells. Our results show that spindle-associated proteins such as motors become misregulated in the absence of either isotype, resulting in compromised spindle function. Altogether, our study demonstrates for the first time how tubulin isotypes influence the activities of MAPs to maintain genome integrity.
M75
Unique midbody structure in mouse oocytes is associated with asymmetric cytokinesis
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Cytokinesis is the last step of the cell cycle by which two euploid cells are formed. In addition to ensuring the symmetric division of somatic cells, cytokinesis leads to the formation of a transient, membrane-less organelle called the midbody (MB). For completion of cytokinesis, the MB must be clipped on both or either of its ends. Although abscission often results in MB release, in some cellular contexts MB abscission results in inheritance when the clipping is asymmetric, or uptake when clipping is symmetric and the MB is phagocytosed by a daughter cell or a neighboring cell. Uptake or inheritance of MBs correlates with cell fate determination, and changes in stemness and tumorigenicity. From the two mechanisms of MB acquisition observed, asymmetric abscission is considered uncommon in somatic cells. On the other hand, mammalian oocytes undergo asymmetric divisions during meiosis, suggestive of asymmetric cytokinesis and abscission. In addition, oocytes must sustain embryo development and acquire totipotency after fertilization. Although the MB is associated with changes in cellular function, its presence and role in oocytes has not been explored. In this work, we identify MB structures in mouse oocytes analogous to those of the mitotic ones based on the localization of key structural proteins MKLP2 (arms), PRC1 (core), and MKLP1 (ring) using super resolution microscopy. We also show that these regions of oocyte MBs are prone to disruption by 1,6-hexanediol treatment, suggesting similar physical phase-separated properties to the mitotic MB. Interestingly, we observe that the midzone spindle and ring structure have a unique, asymmetric morphology not observed in mitosis. We hypothesize that the asymmetry is driven by the forces exerted by the microtubules during polar body extrusion. To test this hypothesis, we first induce symmetric division of the oocytes by compression and observe that compressed cells possess a more symmetrical MB. Next, acute depolymerization of the midzone spindle using nocodazole reduces the asymmetry observed in control oocytes. Together, these data elucidate, the presence of meiotic midbodies in mammalian oocytes and highlight qualities distinctive from what is reported about the mitotic MB and that correlate with an asymmetric cytokinesis.

M76
Pathogenic Mutations in the Chromokinesin KIF22 Disrupt Anaphase Chromosome Segregation
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KIF22 (or Kid) is a kinesin motor that contributes to the movement of mitotic chromosomes. Point mutations in the motor domain of KIF22 dominantly cause a skeletal developmental disorder characterized by short limb bones and short stature, and we recently identified a patient carrying a point mutation in the coiled-coil domain of KIF22 with similar phenotypes. To investigate whether pathogenic mutations in KIF22 affect the function of the motor in mitosis, we have built stable cell lines that permit inducible expression of KIF22-GFP with motor and coiled-coil domain mutations. KIF22 uses plus end-directed motility and direct binding to chromosome arms to contribute to chromosome congression and alignment in metaphase. Surprisingly, mutant KIF22 localizes normally and is capable of generating forces to move chromosomes toward microtubule plus ends in prometaphase, indicating that mutant motors are active and that pathogenic mutations are not simply loss of function mutations.
As cells proceed through mitosis, however, we observe a dramatic defect in anaphase chromosome segregation. Specifically, live imaging demonstrates chromosome recongression: chromosomes begin to segregate in anaphase, then reverse direction to move back toward the center of the spindle rather than continuing toward the spindle poles before decondensing. The spindle poles themselves also move back toward the center of the spindle during this recongression. The observed anaphase defects cause reduced proliferation, abnormal daughter cell nuclear morphology and, in a subset of cells, cytokinesis failure. Nuclear morphology defects are not observed when cells expressing mutant KIF22 enter and exit mitosis with depolymerized microtubules, indicating that the effects of these mutations are dependent on force generation within the spindle. This phenotype could be explained by a failure of KIF22 to inactivate in anaphase. Continued generation of forces toward the plus ends of microtubules could cause chromosomes to move back toward the center of the spindle rather than segregating to the poles. Consistent with this model, a phosphomimetic mutation within the tail of KIF22 (T463D), which constitutively activates the motor, phenocopies the effects of pathogenic mutations. We are currently exploring how these defects may affect the division, proliferation, and tissue-level organization of chondrocytes, as these cells set the scale of skeletal development.

M77

Kinetochore-microtubule detachment is independent of depolymerization for powering poleward chromosome movement

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Faithful chromosome segregation is essential for cell viability. To ensure accurate segregation, sister kinetochores must have proper, bioriented attachments to the plus ends of spindle microtubules. In human cells, depolymerization of kinetochore-bound microtubule plus ends acts as a driver of chromosome poleward motion. Depolymerization can also lead to kinetochore-microtubule (k-MT) detachment from chromosomes. This detachment promotes error correction in the orientation of k-MT attachments to prevent chromosome segregation errors. This study aims to investigate the potential relationship between poleward chromosome motion and microtubule detachment from kinetochores, and seeks to answer the question, does k-MT depolymerization associated with chromosome movement influence the efficiency of k-MT detachment. Live imaging of mitotic U2OS and HeLa cells was used to measure multiple parameters including: the velocity of poleward chromosome movement, the detachment rate of k-MTs, the rate of poleward microtubule flux, inter-centromere distance, and pole-to-pole distance, in a variety of conditions. In untreated human U2OS cells, poleward chromosome velocity is equivalent in prometaphase and metaphase (1.94 µm/min and 1.97 µm/min, respectively), although k-MT detachment rate, measured as a read out from k-MT half-life, significantly decreases from prometaphase to metaphase (t1/2 = 2.31 min and 3.79 min, respectively). To explore this question further, we specifically manipulated the activities of MCAK and Kif18A, proteins known to be involved in the regulation of k-MT attachment stability and chromosome velocity. Overexpression of Kif18A reduced poleward chromosome velocity to 0.80 µm/min, as previously shown, but had no effect on k-MT detachment rate (t1/2 = 3.0 min) compared to untreated metaphase cells. Stimulation of MCAK activity with the compound UMK57 did not significantly alter k-MT detachment rate (t1/2 = 3.21 min) and decreased velocity to 1.55 µm/min. In cells overexpressing Kif18A, the addition of UMK57 displayed chromosomes that were virtually immobile despite the presence of robust k-fibers. The half-life of k-MTs under these conditions was 2.14 min and equivalent to prometaphase cells. Immunofluorescence imaging also shows that changes to MCAK and Kif18A protein levels do not affect the localization of one
another. Taken together, these data indicate that independently acting mechanisms regulate k-MT detachment rates and depolymerization for powering chromosome poleward movement. Moreover, there is an unexpected functional relationship between the activities of Kinesin-8 and Kinesin-13 families of proteins on chromosome movement.

M78  
**Augmin regulates kinetochore tension and mitotic fidelity by nucleating bridging fibers**  
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The mitotic spindle in human cells consists of microtubules mainly nucleated at the centrosome and on the lateral surface of existing microtubules by the augmin complex. However, it is unknown how the augmin-mediated nucleation affects functionally distinct microtubule bundles and consequently the forces within the spindle. Here we show, by using stimulated emission depletion (STED) microscopy and siRNA depletion of HAUS6 or HAUS8 subunits of the augmin complex that augmin plays a crucial role in the proper formation of bridging fibers, which laterally link sister kinetochore fibers. After depletion, the structure of bridging fibers was severely impaired with the majority of bridging fibers either missing or improperly attaching to sister kinetochore fibers. Confocal microscopy revealed that the augmin depletion reduced the number of microtubules within bridging fibers by around 80% and in kinetochore fibers by 40%, suggesting that the bridging microtubules are mainly nucleated at the surface of present microtubules. Moreover, the interkinetochore distance decreased preferentially for kinetochores that lack a bridging fiber, independently of the thickness of their k-fibers, implying that augmin affects forces on kinetochores largely via bridging fibers. Without augmin, the decreased number of bridging fibers was accompanied by their spatial rearrangement within the spindle and slower poleward flux of k-fibers. Augmin depletion also resulted in chromosome segregation errors, nucleus fragmentation and the appearance of micronuclei. We propose a model in which augmin promotes tension on kinetochores and mitotic fidelity by generating bridging microtubules from the lateral sides of kinetochore microtubules.

**Subcellular and Tissue-scale Approaches to Morphogenesis**

M79  
**Dissecting the role of the Par6/aPKC polarity complex in epithelial tissue architecture with optogenetics**  
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Epithelial architecture relies on the apical-basal polarity machinery to create functionally distinct cortical domains and to position adhesive cell-cell junctions that connect individual cells. Accordingly, loss of epithelial polarity is known to cause tissue disorganization. To understand how apical-basal polarity maintains epithelial architecture, we used optogenetics and chemical genetics to disrupt the conserved Par6/aPKC polarity complex with high temporal control. This allowed us to monitor how the epithelial
tissue becomes disorganized and determine key events controlled by apical-basal polarity. Optogenetic inactivation of the apical Par6/aPKC complex leads to loss of epithelial integrity and multilayering in the *Drosophila* follicular epithelium. Live imaging analysis revealed that epithelial gaps form in under one hour of aPKC inactivation when dividing cells detach from neighbour cells. We show that this phenotype results from increased apical constriction in non-mitotic cells, which pull on mitotic cells until detachment. Accordingly, disruption of actomyosin-driven contractility restores epithelial integrity, while induction of apical constriction by light-induced recruitment of RhoGEF2 also induces tissue rupture. Our work highlights aPKC as a key negative regulator of apical contractility and shows that unbalanced apical constriction threatens tissue cohesion in proliferative epithelia.

M80

**Sarcalumenin regulates myoblast-myotube fusion via modulating calcium homeostasis between ER and cytosol**

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Myoblast fusion is essential for regeneration of vertebrate skeletal muscles. Upon injury, muscle precursor cells (satellite cells, SCs) activate and proliferate, giving rise to myoblasts that differentiate and fuse to injured pre-existing muscle fibers. Myomaker (MYMK) is a muscle-specific protein that is essential for myoblast-to-myotube fusion in vertebrates. Moreover, fibroblasts expressing MYMK can fuse with myoblasts indicating the MYMK in sufficient to induce fusion between fibroblasts and myoblasts. Based on the hypothesis that genes in the same pathway are likely co-regulated, we asked whether other genes involved in myoblast fusion can be identified using features of the MYMK promoter. We generated promoter models for MYMK using the evolutionarily conserved regions and identified genes with homologous promoters in several mammalian genomes. The list of genes that we obtained included many proteins involved in calcium signaling and endoplasmic reticulum (ER) remodelling. This was consistent with recent findings that cytosolic calcium and calcium-responsive genes like the ryanodine receptors (RyRs) are essential for myoblast-to-myotube fusion. We confirmed the co-expression of genes in the homologous promoters list to MYMK using RNA-seq and observed that multiple calcium-responsive genes express differentially between myoblasts and myotubes. Of these, we assessed the role of sarcalumenin (SRL) as it is a dynamin-like domain containing calcium-responsive muscle-specific ER-localized protein. Based on the previous correlation of SRL with Store Operated Calcium Entry (SOCE) channel proteins, we hypothesized that SRL may regulate fusion by influencing calcium release from the ER to the cytosol. Knockdown of SRL in cultured primary myoblasts resulted in higher cytosolic calcium than WT cells and exhibit hyperfusion without affecting differentiation. To test whether SRL regulates myoblast-to-myotube fusion *in vivo*, we have generated SRL KO mice and are currently analyzing their recovery post injury. Taken together, we have identified SRL as a novel regulator of myoblast fusion. Moreover, our results show that myoblast fusion and muscle contraction, are initiated by the same calcium-handling pathway and share promoter homology with MYMK suggesting that these processes co-evolved in vertebrates to assure contraction and fusion do not occur at the same time.
M81
A sub-apical network of non-muscle myosin 2C constrains cell- and tissue-scale morphology in the intestinal epithelium
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The surface of the intestinal tract exhibits morphological adaptations at multiple levels of scale, which collectively serve to increase surface area available for nutrient absorption. Large folds of tissue called villi are covered with transporting epithelial cells, known as enterocytes. These cells in turn extend an array of microvilli from their apical surface, which further expands the surface of interaction with the luminal contents. Although cytoskeletal mechanisms have been invoked to explain how these adaptations arise during development and differentiation, the molecular details remain unclear. Previously, we identified the actin-based motor non-muscle myosin-2C (NM2C) as a force-generating component of the enterocyte sub-apical terminal web, a highly crosslinked filamentous meshwork at the base of microvilli, where it works to control the length of microvilli via contractility-dependent actin turnover. NM2C is also found in the circumferential actin belt that surrounds the cell at the level of the terminal web, which had been previously demonstrated to be under tension. Based on this localization and previous data, we proposed that NM2C may play a role in generating and/or propagating mechanical forces that are needed to control cell and tissue morphology of the small intestine and potentially, collective epithelial migration up the crypt-villus axis. To investigate the role of NM2C in vivo, we are characterized the phenotype of NM2C knockout (KO) mice. NM2C KO mice display abnormal villus architecture, with enterocytes that exhibit elongated and, in some cases, compressed profiles when viewed laterally. Additionally, NM2C KO tissues exhibit “ruffled” junctions, which are less linear compared to WT junctions and suggest reduced levels of mechanical tension in these structures. Furthermore, using organoids generated from KO mice, we found that epithelial cells lacking NM2C display ruffled ZO-1 signal, which indicates weakened cell-cell junctions. These organoids also demonstrated increased permeability to 4kDa and 70kDa dextran. Consistent with a decrease in barrier function due to weakened junctions, NM2C KO mice and organoids present with tuft cell hypertrophy within the epithelium. Collectively, these findings demonstrate that NM2C forms a contractile network, which spans the enterocyte at level of the terminal web and generates tension needed for maintaining normal cell and tissue-scale morphology of the small intestinal epithelium.

M82
The cofilin regulator LIM-kinase controls actin dynamics during embryonic wound closure
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Embryos display a striking ability to repair wounds rapidly, with no inflammation or scarring, in a process driven by collective cell movements. Upon wounding, the cells adjacent to the wound accumulate filamentous actin and the molecular motor non-muscle myosin II at the interface with the wounded cells, forming a supracellular cable around the wound which contracts and coordinates cell movements to close the lesion. Myosin-II binds actin and generates contractile forces through its motor function. However, when myosin motor activity is disrupted wounds still close—albeit more slowly—suggesting that there are alternative mechanisms of force generation. During cytokinesis, actin filament disassembly by the actin-binding protein cofilin coupled with filament crosslinking can induce contractile forces independent of myosin motor activity. We are investigating the role that actin filament disassembly plays in wound healing in embryos of the fruit fly Drosophila melanogaster, which are
amenable to live imaging as well as genetic, pharmacological, and biophysical manipulations. Using time-lapse microscopy and quantitative image analysis, we found that cofilin inactivation by overexpression of the cofilin upstream regulator LIM-kinase (LIMK) reduced the rate of wound closure by 33% with respect to controls. F-actin levels at the wound edge increased faster when LIMK was overexpressed, with half-times of F-actin accumulation 60% shorter than in controls. We used fluorescence recovery after photobleaching to quantify actin dynamics around the wound, and we found that the mobile fraction of actin decreased by 30% when LIMK was overexpressed, suggesting that actin was stabilized as a consequence of a reduction in cofilin activity. Consistent with this idea, the distribution of F-actin around the wound was significantly more uniform in LIMK-overexpressing embryos, further indicating that F-actin remodelling around the wound was defective. Our results suggest that cofilin activity is necessary for proper actin turnover at the wound edge and rapid embryonic wound repair. We are currently investigating the mechanisms by which cofilin impacts force generation during embryonic wound healing. A better understanding of these mechanisms will enable the development of therapeutic interventions to treat chronic wounds and other pathological conditions associated with defects in collective cell migration, including cancer metastasis and many congenital disorders.

M83
Symmetry breaking of tissue mechanics in wound induced hair follicle regeneration of laboratory and spiny mice

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Tissue regeneration is a process that recapitulates and restores organ structure and function. Although previous studies have demonstrated wound-induced hair neogenesis (WIHN) in laboratory mice (Mus), the regeneration is limited to the center of the wound unlike those observed in African spiny (Acomys) mice. Tissue mechanics have been implicated as an integral part of tissue morphogenesis. Here, we use the WIHN model to investigate the mechanical and molecular responses of laboratory and African spiny mice, and report these models demonstrate opposing trends in spatiotemporal morphogenetic field formation with association to wound stiffness landscapes. Transcriptome analysis and K14-Cre-Twist1 transgenic mice show the Twist1 pathway acts as a mediator for both epidermal-dermal interactions and a competence factor for periodic patterning, differing from those used in development. We propose a Turing model based on tissue stiffness that supports a two-scale tissue mechanics process: (1) establishing a morphogenetic field within the wound bed (mm scale) and (2) symmetry breaking of the epidermis and forming periodically arranged hair primordia within the morphogenetic field (μm scale). Thus, we delineate distinct chemo-mechanical events in building a Turing morphogenesis-competent
field during WIHN of laboratory and African spiny mice and identify its evo-devo advantages with perspectives for regenerative medicine.

**M84**

**Building the muscular wall in the atrial chamber of the heart involves cell elongation and reorganization of tissue polarity**

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Cardiomyocytes form the contractile unit of the heart. During vertebrate cardiac development, cardiomyocytes undergo cellular rearrangements important for the formation of complex myocardial structures, including the pectinate muscles in the atrium and the trabecular network in the ventricle. Previous studies have mostly focused on the formation of the ventricular trabeculae, which increase cardiac pumping efficiency. However, the morphogenesis and rearrangements of the cardiomyocytes that drive atrial myocardial complexity, which is crucial to propagate the action potential necessary for cardiac contraction, has largely been overlooked. Our study uses zebrafish larvae to elucidate cardiomyocyte behaviors during the maturation of the atrium, as they allow for high-resolution live imaging and are easily amenable to genetic modifications. Using high resolution live imaging of zebrafish hearts, combined with mosaic labelling and temporal tracking of individual atrial cardiomyocytes, we found that atrial myocardial morphogenesis is driven by a complex set of cell behaviors distinct from ventricular cardiomyocyte development. Specifically, we observed that atrial cardiomyocytes in zebrafish larvae form membrane protrusions, lamellipodia-like and filopodia-like, and adopt an elongated shape in a non-stochastic orientation that establishes atrial tissue-level polarity. These shape changes lead to partial multilayering between neighboring cardiomyocytes, and to the formation of new cell contacts, resulting in populations of elongated cardiomyocytes that span the entire atrium in an orientation parallel to the direction of blood flow. Notably, these atrial cardiomyocytes behaviors appear to be independent from factors important in ventricular morphogenesis, including mechanical stress brought on by cardiac contraction and blood flow, and Erbb2 and Notch signaling. Altogether, these data suggest that atrial morphogenesis is driven by unique cardiomyocyte behaviors, as well as distinct molecular and environmental/physical factors, all of which are currently under investigation.

**THURSDAY, DECEMBER 9, 2021**

**Emerging Systems**

**M85**

**Molecular and anatomical transformations in cilia and centrosomes underlie ciliary retraction during cerebellar granule cell differentiation**

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Granule cells (GCs) in the cerebellum are the most abundant neuronal cell type in the brain and abnormal GC proliferation causes sonic hedgehog (Shh)-subtype medulloblastoma. GC progenitors
divide in response to cilia transduced Shh signals in the external granular layer prior to differentiating into neurons and migrating to form the inner granular layer. Unlike cilia retraction that has been studied in cultured cell systems upon cell cycle re-entry, reduced ciliation in GCs appears to be associated with differentiation. However, how and why ciliary changes occur in the GC life cycle is not well understood. Here we used fluorescence and large-scale 3D electron microscopy volumes from developing and adult cerebella to understand cilia and centrosomal dynamics during GC maturation. In mature GCs, cilia are largely absent, yet mother centrioles are docked at the cell surface. As GCs differentiate and migrate, most cilia remain internal and gradually reduce in length and frequency. Furthermore, cilia in progenitor cells form through an intracellular biogenesis pathway during which many are concealed from external signaling. Intermediate structures suggest that cilia reduction is accomplished both by limiting cilia formation and by promoting cilia resorption. To identify molecular candidates contributing to the observed anatomical changes, we queried transcriptomic data from developing cerebella for expression changes in centrosome and cilia related genes. Accompanying their decreased strength of hedgehog signaling, maturing GC progenitors have reductions in pericentriolar material, centriolar satellite components, and intraflagellar transport proteins, which were further validated by immunofluorescence imaging. Shh signaling competent cilia-centrosomal complexes also persisted in proliferative GC nests prior to tumorigenesis. Furthermore, we observed centrosomal cap proteins relocalized at docked centrioles in the mature GC neurons which likely prevents reciliation. These results indicate that not only are cilia being retracted as a programmed part of neuronal differentiation, but GC centrosome composition changes as mother centrioles dock at the plasma membrane. The regulated abatement of cilia mediated signaling is likely important for granule cell maturation and is dysregulated during medulloblastoma pathogenesis.

M86
A unicellular walker controlled by a microtubule-based finite state machine
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Cells are complex biochemical systems whose behavior emerges from interactions among myriad molecular components. Computation is often invoked as a general framework for navigating this cellular complexity. However, the manner in which cells might embody computational processes such that theories of computation, including finite state machine models, could be productively applied, remains to be seen. Here we demonstrate finite state machine-like processing embodied in cells using the walking behavior of Euplotes eurystomus, a ciliate that walks across surfaces using fourteen motile appendages (cirri). We found that cellular walking entails highly regulated transitions between a discrete set of gait states. The set of observed transitions decomposes into a small group of high-probability, temporally irreversible transitions forming a cycle and a large group of low-probability time-symmetric transitions, thus revealing stereotypy in sequential patterns of state transitions. Taken together, these findings implicate a finite state machine-like process. Cirri are connected by microtubule bundles, and we found that the dynamics of cirri involved in different state transitions are associated with the structure of the microtubule bundle system. Perturbative experiments revealed that the bundles mediate gait coordination, suggesting a mechanical basis of gait control.
Non-centrosomal microtubule assembly at division site regulates actin-independent cleavage-furrow formation in *Chlamydomonas*

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Myosin-II and the canonical contractile actomyosin ring are conserved only in animals, fungi, and related species. In contrast, microtubules (MTs) and tubulin(-like) proteins are involved in cytokinesis in a wide swath of organisms, from the FtsZ ring in bacteria and archaea, the phragmoplast in plants, to the furrow-associated MT array in animals. However, the evolution and conservation of their roles - particularly in cleavage-furrow formation - remain unclear. The unicellular green alga *Chlamydomonas reinhardtii* is phylogenetically close to plants yet divides like animals by cleavage-furrow formation, and this process does not involve myosin-II or require F-actin. We studied the roles of MTs in *Chlamydomonas* to gain insights into the evolution of cytokinesis in eukaryotes from their common ancestors, in which the actomyosin ring was unlikely to be present. Through time-lapse imaging of the plus-end-binding protein EB1 labelled with mNeonGreen, we found that *Chlamydomonas* cells have two types of dynamic MT structures involved in division: the preprophase arc (PPA) and the furrow microtubule array (FMA). The PPA is formed before mitosis around the future division plane, similar to the preprophase band in land plants. FMA is formed after spindle breakdown at the division site and remain associated with the ingressing furrow, similar to structures reported in some animal embryos. The PPA and FMA partially colocalize with and depend on the gamma-tubulin ring complex protein GCP2 and a homolog of human augmin-like complex subunit. Our results suggest there is a division-site associated non-centrosomal microtubule-organization center (ncMTOC) that is responsible for formation of PPA and FMA, which in turn are required for the timely onset and normal rate of furrow ingresson. Two kinesins, the minus-end directed KCBP (conserved in plants) and the plus-end directed KIF11 (a homolog of metazoan Eg5), localize along the PPA and FMA and are required for normal furrowing, suggesting that force generation and/or cargo trafficking by these motors are important for actin-independent cytokinesis.

A cryptic, bacteria-dependent collective invasion in *Fonticula alba* reveals a new multicellularity in fungi-animal tree

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Multicellularity emerged in Opisthokonta from its amoeboid ancestor and resulted in the distinct fungal and animal organizations. However, the evolutionary history of these multicellularities is challenging to unravel given their striking mechanistic differences. *Fonticula alba* is an understudied, early-diverging Opisthokont, which uniquely retains a social amoeba lifestyle. *F. alba* grows as unicellular amoebae that aggregate to build multicellular, spore-generating fruiting bodies. We aim to establish *F. alba* as a new model organism to explore the evolutionary cell biology of fungi and animals. *F. alba* is a non-axenic cellular slime mold and only grows in co-culture with feeder bacteria. Greatly-improved culture methods for the organism revealed a unique bacterial age-dependent germination for *F. alba* and a cryptic
invasive social behavior distinct from fruit formation. The amoebae coalesced into dynamic collectives, which invaded virgin bacterial resources. Collectivity allowed groups of cells to migrate in a highly directional manner and thereby invade large distances more rapidly than isolated amoeba. Invasion collectivity emerges from amoeba cell density and is dependent on the bacterial environment. These features are undescribed for other cellular slime molds and offer clues into the changes that occurred during Opisthokont diversification. Specifically the surprising discovery of a new bacteria-dependent, animal-like invasion collectives in addition to spore-generating collectives reshapes our understanding of the origins of both Metazoan and fungal multicellularity.

M89
A non-canonical role for aminoacyl-tRNA synthetase links the actin cytoskeleton to Charcot-Marie-Tooth neuropathy

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Aminoacyl-tRNA synthetases are a family of ancient essential enzymes that catalyze the attachment of amino acids to their cognate tRNAs to continuously supply the protein-synthesizing ribosome. Mutations in tyrosyl-tRNA synthetase (YARS) and five other synthetases were identified in patients with Charcot-Marie-Tooth (CMT) neuropathy, raising a conundrum between the essential need for these enzymes in every cell with the tissue specificity of axonal degeneration and demyelination of the peripheral nerves in patients. Mutant synthetases with a wide spectrum of enzymatic activities all lead to peripheral neurodegeneration in the CMT. Non-canonical functions attributed to the CMT-causing synthetases suggest complex molecular mechanisms underlying the disease pathogenesis beyond the loss of catalytic activity alone. To explore the pathomechanism behind synthetase-induced CMT we performed an unbiased screen for interactors in a Drosophila model of YARS-induced neuropathy. We discovered the actin bundler Plastin as a genetic regulator of disease-relevant phenotypes. We also investigated the interactome of YARS in cellulo and found this synthetase enriched in protein complexes that contain actin and actin-binding proteins. Using in vitro studies, we demonstrate that YARS itself has an actin binding- and bundling functions, which are evolutionary conserved and contribute to the organization of actin cytoskeleton in Drosophila neurons and in patient-derived cells. CMT-causing mutant YARS induced stronger actin bundling in vitro, disorganization of actin bundle-rich stress fibres in patient-derived cells and impairs multiple actin-based steps of the synaptic vesicle cycle in fly neurons. Thus, we propose an actin-based mechanism behind the neuronal dysfunction in the mutant YARS Drosophila model, and in CMT neuropathy in humans. Several synthetases and other components of the protein translation machinery, including mRNAs, ribosomes, eukaryotic initiation- and elongation factors have been
previously associated with the actin cytoskeleton. Our findings suggest that actin cytoskeleton impairments might be a common denominator in the etiology of synthetase-induced CMT and provide a target for therapeutic intervention.

M90

*Naegleria* mitotic spindles are built from unique tubulins and highlight core spindle features

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The mitotic spindle is an essential component of cell division machinery throughout the eukaryotic tree of life, but the organization and dynamics of this microtubule-based structure vary significantly across species. The objective of this study is to understand the organization and function of the mitotic spindle in the unicellular eukaryote *Naegleria gruberi*, which is part of a lineage that diverged from animals and fungi over a billion years ago. *Naegleria* have several unusual properties which make them a unique model system for spindle biology. First, *Naegleria* amoebae lack centrosomes or any cytoplasmic microtubules, assembling microtubules within the nucleus exclusively for mitosis via an unknown, centrosome-independent mechanism. Second, *Naegleria*’s cell division can be synchronized using a heat shock, enabling identification of the sequence of events underlying spindle formation. Using quantitative light microscopy, we find that *Naegleria*’s mitotic spindle is a distinctive barrel-like structure built from a ring of microtubule bundles. Similar to those of other species, *Naegleria*’s spindle is twisted and its length increases during mitosis, suggesting that these aspects of mitosis are ancestral features. Because bundle numbers increase during metaphase, we hypothesize that the initial bundles represent kinetochore fibers, and later-forming bundles function as bridging fibers to facilitate spindle elongation. We have also investigated the evolutionary divergence in *Naegleria* tubulin genes. Previous studies showed that *Naegleria* amoebae express a divergent α-tubulin during mitosis, and we now show that *Naegleria* amoebae express a second mitotic α- and two mitotic β-tubulins. Sequence analysis of the mitotic tubulins reveals that they are at most 58% identical to human α- and β-tubulins, and contain residues that suggest distinct microtubule properties. Consistent with this, we find that standard microtubule inhibiting drugs do not affect the growth of *Naegleria*, suggesting that these inhibitors may not bind to the divergent mitotic tubulins. The conserved and divergent properties of *Naegleria* spindles suggest that *Naegleria* will be a promising system to uncover core principles underlying spindle assembly and function in diverse evolutionary contexts.
Intracellular Trafficking: From Mechanisms and Regulation to Disease

M91
Fluorogen Activating Proteins as a powerful new imaging tool for quantitative protein trafficking studies in yeast
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Recent advances in genetically encoded fluorescent probes have led to fluorogen-activating proteins (FAPs). This technology has two components: a non-fluorescent single chain antibody (SCA), which is fused to a protein of interest, and a fluorogen, which in this case is a malachite-green (MG) dye derivative that is non-fluorescent in solution. Binding of a fluorogen to the SCA results in a 20,000-fold increase in fluorescence, producing a signal in the far-red range (633 nm) that is similar in intensity to enhanced green fluorescent protein. Imaging using FAPs have distinct advantages over traditional fluorescent proteins that make them amenable to quantitative protein trafficking studies. Specifically, a cell impermeant MG-derivative, MG-B-Tau, selectively detects SCA-tagged proteins at the cell surface allowing for quantitative studies of the dynamics of protein internalization or recycling. Although developed in yeast, FAPs are surprisingly underutilized in this organism. To facilitate use of FAP technology in yeast we: 1) optimized the SCA sequence for expression in yeast, 2) created a series of FAP-tagging vectors that can be used to express your favorite protein fused to FAP under a range of promoters, 3) generated a suite of sub-cellular markers tagged with FAP for in vivo co-localization studies, and 4) demonstrated the usefulness of the FAP system by assessing the endocytic trafficking of yeast G-protein coupled receptor Ste3. We find that Ste3 endocytosis is regulated in both a constitutive and ligand-stimulated way by the α-arrestin protein trafficking adaptors. We use the FAP-tagged Ste3 to define α-arrestin contributions to each of these pathways. Thus, we develop a useful tool for the cell biology community and demonstrate the powerful capabilities of the FAP technology for quantitative protein trafficking studies.

M92
In vitro Reconstitution of Membrane Curvature Sensing in Autophagy
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Multiple membrane curvature sensors have been identified among known autophagy effector proteins, yet how they may work together to sense curvature remains unclear. In this study, we used an in vitro reconstitution approach to quantify how the interaction between curvature sensors and membrane could regulate autophagosome biogenesis. We assayed the curvature sensing activity of autophagy initiation proteins including ATG3, ATG12-5-16, and PI3KC3-C1 by pulling membrane nanotubes from giant unilamellar vesicles (GUVs) and compared protein enrichment on the highly curved membrane tube relative to the flat GUV surface using confocal fluorescence microscopy. Importantly, this approach enabled a quantitative dissection of the relative contributions of these proteins to sensing curvature on a phagophore-like membrane with contiguous regions of high and low curvature. We found that many
membrane binding proteins in autophagy sense membrane curvature, and that the LC3 lipidation complex ATG12-5-16 is a major curvature sensor.

M93

A cell-based GEF assay reveals new substrates for DENN domains and cooperativity between Rab and Rho GTPases in the repressor pathway of primary ciliogenesis

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Primary cilia are cellular antennae responding to extracellular stimuli to drive signaling cascades including the Hedgehog pathway (Hh), crucial for cell and organism development. Disruption of ciliogenesis leads to a diverse group of disorders called ciliopathies. Ciliogenesis involves membrane trafficking mediated by small GTPases including Rabs. Rabs are molecular switches in membrane trafficking and are activated by guanine nucleotide exchange factors (GEFs), the largest family being the DENN domain-bearing proteins. For many Rabs, the activating GEF remains unknown. Here we develop a cellular GEF assay based on DENN domain recruitment of Rab substrates to mitochondria, and comprehensively screen two major DENN domain families against all 60 Rabs, uncovering 19 novel DENN/Rab pairs. The screen reveals that Rab10, involved in the formation of primary cilia, is a substrate for DENND2B/ST5, previously implicated in a severe mental retardation and cancer, diseases involving primary cilia dysfunction. Depletion of DENND2B causes enhanced cilia formation and increased cilia length, and suppresses Hh signaling. The enhanced cilia formation is rescued by the expression of active Rab10, which we find controls recruitment of CP110, a known inhibitor of cilia formation to the distal end of the mother centriole, establishing a mechanism for DENND2B-mediated inhibition of cilia formation. Intriguingly, defects in ciliary length are not rescued by the expression of Rab10 but by active RhoA, and we discover that DENND2B is a GEF for RhoA, the first non-Rab substrate of DENN GEF activity. Thus, the cellular DENN/Rab screen identifies an unexpected diversity in DENN domain-mediated control of small GTPases and identifies a regulator of the repressor pathway in ciliogenesis.

M94

Rabip4 isoforms bind Arl8b and regulate retrograde transport of CI-M6PR to maintain lysosomal homeostasis

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Lysosomes receive and degrade cargo acquired via endocytic, autophagic, and phagocytic routes. One of the key players regulating lysosome function is the small Arf-like (Arl) GTP-binding (G) protein, Arl8b, which regulates lysosome positioning and fusion with other compartments. Previous studies have shown that RUN domain-containing proteins, PLEKHM1 and PLEKHM2 bind to Arl8b via their RUN domains to regulate cargo trafficking and lysosome motility, respectively. To gain insights into the Arl8b function, we screened for other endocytic RUN domain-containing proteins as potential Arl8b interaction partners. Here we report RUN and FYVE domain-containing protein (Rufy) 1 isoforms Rabip4 and Rabip4' interacts and colocalize with Arl8b on a subset of Rab14 and EEA1-positive early/recycling vesicles. Rabip4/4’ depletion impairs the CI-M6PR retrieval from endosomes to the trans-Golgi network
and consequently delays the trafficking of cargo procathepsin D to lysosomes. Accordingly, lysosomes were enlarged and less degradative in Rabip4/4’ depleted cells. Mass spectrometric-based identification of Rabip4/4’ interactome revealed dynein-dynactin complex as interaction partner. Consistent with this finding, CI-M6PR endosomes show reduced directional movement and run lengths upon Rabip4/4’ knockdown suggesting its role as a dynein adaptor. Our results suggest a novel upstream role of Arl8b in the endocytic transport pathway wherein it interacts with Rabip4/4’ to regulate cargo transport from endosomes to the TGN.

M95
Mice lacking the BORCS5 subunit of BORC show impaired axonal lysosome transport and neonatal lethality
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Lysosomes’ ability to move throughout the cytoplasm is critical for various cellular functions such as autophagy, cell migration and tumor invasion. This ability is particularly crucial in neurons because of their extreme asymmetry and length of axons and dendrites. Our lab discovered an eight-subunit complex named BORC that mediates sequential recruitment of the small GTPase ARL8, the adaptor protein SKIP, and kinesins-1 and -3 to lysosomes, promoting their movement toward the plus-end of microtubules in the periphery of non-polarized cells. Knock-out (KO) of any of the BORC subunits causes clustering of lysosomes in the pericentrosomal area of the cell. A recent study revealed the presence of a single-nucleotide, splice-acceptor variant of the BORCS5 gene, encoding the BORCS5 subunit of BORC, in a patient with global developmental delay, corpus callosum agenesis, seizures, polymicrogyria and abnormality of the cerebral cortex. Furthermore, single nucleotide polymorphisms causing altered expression of the BORCS7 subunit of BORC have been linked to schizophrenia, emphasizing the importance of BORC in neurons. To analyze the role of BORC for neuronal function, we used the CRISPR-Cas9 system to generate a BORCS5-KO mouse. We found that the resulting KO embryos grew to term, but the pups died of suffocation immediately after birth. To address the importance of BORC in neuronal organelle transport, we prepared cultures of hippocampal neurons from control and BORCS5-KO embryos. The neurons were transfected with plasmids encoding lysosomal and synaptic vesicle markers fused to fluorescent proteins. We found that BORCS5 KO prevented the transport of lysosomes into the axon but not the dendrites. In contrast, there was no effect in the transport of synaptic vesicle precursors (SVPs) into the axon. Analysis of brain tissues and neuromuscular junctions from WT and KO embryos confirmed that BORC is necessary for lysosomes, but not for SVPs, to enter the axon and to reach synaptic terminals. These findings indicate that in mouse neurons BORC plays a role in the transport of lysosomes but not SVPs into the axon. Furthermore, the neuropathogenesis caused by mutations in the BORC complex could result from impaired lysosome transport into the axon. Our findings thus provide a framework for future studies on the control of lysosome and SVPs trafficking as they move toward the axon terminal in mammalian neurons.
Huntingtin and Rab7 co-migrate retrogradely on a signaling late endosome during axonal injury: A novel role for Huntingtin.
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Huntingtin (HTT) is a 350kDa protein of unknown function. While HTT moves bi-directionally within axons and loss/reduction of HTT causes axonal transport defects, the identity of the cargo-containing vesicles that HTT helps move within axons remain elusive. Previously, we found an axonal retrogradely moving HTT-Rab7 vesicle complex; however, its biological relevance was unclear. Using Drosophila genetics, in vivo microscopy, biochemical isolation of membranes from mouse brains and human iPSC derived neurons, together with pharmacological inhibition, we identified that adaptor proteins huntingtin interacting protein HIP1 and Rab interacting protein RILP aids the retrograde motility of LAMP-1 containing HTT-Rab7 late endosomes, but not autophagosomes. Loss/reduction of Syntaxin17, the protein that mediates autophagosome-late endosome fusion to form amphisomes, and Chloroquine/BafilomycinA1-mediated pharmacological inhibition, which disrupts lysosome acidification, and autophagosome-lysosome fusion, perturbed the in vivo motility of these HTT-Rab7 vesicles. However, loss/reduction ATG5, the protein involved in the assembly of autophagosomes from phagophores had no effect. Further, since HTT-Rab7 vesicles co-localize with long-distance signaling components (the BMP-receptors TKV/WIT), and moves retrogradely after Drosophila nerve crush, we propose that these vesicles likely traffic damage signals following axonal injury. Together, our findings support a previously unknown role for HTT in the retrograde movement of a Rab7-LAMP1-containing signaling late endosome.

Mechanobiology of the Cell

Chromatin and lamins A, B, and C perturbations differentially affect nuclear shape, rupture, and function
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The nucleus is a mechanically stable compartment of the cell that contains the genome and performs many essential functions. Perturbations of the nuclear components chromatin or lamins have been shown to result in both abnormal shape and loss of compartmentalization via rupture of the nucleus, which impact nuclear function. However, the different effects of chromatin and lamin perturbations and the mechanisms of nuclear rupture have yet to be systematically investigated. We used MEF cells to compare and contrast wild type (WT), chromatin decompaction (VPA), genetic loss of lamin B1 (LMNB1-/-), joint loss of lamin A/C (LMNA/-/-), and individual depletion of lamin A and lamin C via specific knockdown. NLS-GFP concentrates in the nucleus where it provides both a measure of shape and
rupture when it is lost from the nucleus and spills into the cytoplasm. Blebbed nuclei and bleb-based nuclear ruptures, which lead to increased dysfunction via DNA damage, occur at low frequency in wild type and high frequency upon chromatin perturbation or lamin B1 loss, both dependent on histone modification state. However, joint lamin A/C loss via LMNA/- results in overall abnormal shape (decreased circularity) but no increase in nuclear blebbing, rupture frequency, or DNA damage associated with abnormal shape. Inhibition of actin-based contraction by Y27632 drastically decreases the frequencies of nuclear blebs, bleb-based ruptures, and associated DNA damage for WT, VPA, and LMNB1/- but not LMNA/-, which presents differently. Fine spatiotemporal imaging of bleb-based nuclear ruptures reveals the novel finding that nuclear blebs are the site of rupture and are effectively a separate compartment from the nuclear body. NLS-GFP loss rates are significantly different, with 90% of total loss occurring within 10 seconds for bleb versus 40-60 seconds for the body. To recapitulate actin contraction, we used micromanipulation to apply dynamic local compression via a pipette tip in live cells. Compression induced bleb-based nuclear rupture and faster loss of NLS-GFP in the bleb compared to the nuclear body. Finally, we aimed to identify the source of LMNA/- phenotype by specific knockdowns of lamin A and lamin C. Lamin C knockdown phenocopies LMNA/- non-bleb-based nuclear ruptures. Oppositely, lamin A depletion displays chromatin independent increased nuclear blebs and bleb-based nuclear rupture, revealing that LMNA/- is not equivalent to lamin A loss alone. Thus, we provide a clear systematic study of chromatin and lamin perturbations, which reveals differential shape and compartmentalization behavior that impact nuclear function through DNA damage.

M98

Upregulation of Lamin B Receptor Drives Nuclear Envelope Fragility in Metastatic Melanoma

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Malignant melanoma is characterized by its mutational heterogeneity and aggressive metastatic spread. During metastasis, melanoma cells migrate through diverse microenvironments, including regions of dense tissue confinement to reach the vasculature. Microenvironmental confinement of tumor cells causes nuclear deformation, which can lead to loss of nuclear envelope (NE) integrity and DNA damage, improper repair of which leads to genomic aberrations and heterogeneity. We hypothesize that during metastatic progression, expression levels of NE genes are altered, facilitating nuclear deformability and NE fragility, mediating an increase in genetic heterogeneity within the population. To test this hypothesis, we performed bioinformatic analysis of RNA-seq data sets from patient samples of metastatic melanoma and benign nevi, which revealed several NE proteins upregulated in metastatic disease. Performing a targeted siRNA-based screen using a PDMS confinement device to assay for nuclear fragility, we found reduction of lamin B receptor (LBR) dramatically reduced NE fragility in melanoma cells, and ectopic overexpression of LBR was sufficient to increase NE fragility in benign melanocytes. Utilizing functional protein domain truncations and point mutations in LBR, we found the cholesterol synthase activity of LBR was specifically required for increased NE fragility, independent of LBRs additional roles tethering heterochromatin and lamin B to the NE. Additionally, we found that reduction of LBR in melanoma cells results in a reorganization of cholesterol in the NE. Thus, LBR generated cholesterol in the NE promotes NE fragility. To determine if LBR-mediated NE fragility correlated with increased nuclear deformability, we assayed NE mechanics with atomic force...
in microscopy. In melanoma cells, we find reduction of LBR results in an increase in nuclear stiffness and decreased deformability, while LBR overexpression in benign melanocytes results in decreased nuclear stiffness and increased deformability. Altogether, these results show for the first time that upregulation of LBR in metastatic melanoma plays dual roles in reducing nuclear deformability and increasing NE rupture, specifically through alterations in cholesterol organization in the NE. Ongoing work focuses on LBRs role promoting heritable genetic heterogeneity in melanoma during migration in confined microenvironments in vivo.

M99  
Age-dependent Nuclear Lamina Remodeling Induces Cardiac Dysfunction via Misregulation of Cardiac Transcriptional Programs  
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Cellular mechanics play a pivotal role in regulating heart function. Cytoskeletal and extracellular matrix properties regulate cardiomyocyte contractility, while nuclear mechanics govern the sensitivity of force transduction to the nuclear interior to modulate gene expression. These structures have been observed to remodel with age, yet the mechanisms contributing to age-dependent remodeling and how tissue function is affected is unclear. Using the rapidly aging model organism, the fruit fly, we sought to assess how nuclear mechanics are altered upon aging in the heart and their role in regulating heart function. We observed that cardiomyocyte nuclei decrease in size and increase in stiffness upon aging, dependent on an age-associated reduction of the nuclear lamina intermediate filament, Lamin C, homologue of mammalian Lamin A/C. Conversely, skeletal muscle nuclei undergo age-dependent hypertrophy, suggesting lamins may have cardiac-specific functions with age. We found that nuclear shrinkage and stiffening, induced by premature loss of Lamin C, is associated with decreased heart contractility, sarcomere disorganization and shortened lifespan. The age-associated Lamin C reduction also leads to downregulation of cardiomyocyte transcription factors and cytoskeletal regulators, likely via reduced chromatin accessibility at key cardiac programeing loci. Subsequently, we demonstrated there is a unique, adult-specific role of the cardiac transcription factors tinman/Nkx2.5; H15/Tbx20 and Hand/HAND1/2. We found that maintenance of Lamin C expression could sustain their expression, thereby preventing age-dependent cardiac decline. With evidence of conservation in mammals, our findings suggest age-dependent nuclear remodeling is a major mechanism contributing to cardiac dysfunction.

M100  
Non-local mechanical feedback functions as an important input for myosin recruitment during Drosophila germ-band extension  
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Morphogenesis is the process by which DNA is decoded to produce form, yet bridging genes and shape change requires an understanding of tissue mechanics. While gene expression profiles can pattern components that determine a cell’s mechanical properties, post-translational feedback mechanisms could offer additional input to these properties, particularly in response to extrinsic factors. We address
this possibility using *Drosophila* gastrulation as a system to ask whether such mechanical feedback mechanisms exist and if so, what relevance they have for normal morphogenetic processes. By employing quantitative approaches to analyze full embryo imaging data acquired by multiview light-sheet microscopy, we characterize the myosin and strain patterns in early germ-band extension and observe a significant correlation between strain rate and myosin rate with a clear gradient along the dorsal-ventral (D-V) axis. We utilize an optogenetic construct to locally pattern actomyosin contractility, therefore generating ectopic strains, and measure strong non-local effects in myosin recruitment in response to induced deformations. We further demonstrate that this response acts isotropically within a cell but that the strength of the response is modulated along the D-V axis. Higher temporal and spatial resolution data from confocal microscopy reveals single edge dynamics of strain rate and myosin rate and allows us to accurately capture the observations with a concentration oscillator model incorporating a mechanical feedback mechanism for myosin recruitment. Finally, we analyze mutant embryos that fail to form a ventral furrow, and therefore have greatly reduced strain rates, and measure a proportional reduction in myosin accumulation that leads to slower rates of germ-band extension. Taken together, our work clearly demonstrates that a strain rate dependent mechanical feedback mechanism for myosin recruitment can account for much of the observed myosin distribution during *Drosophila* germ-band extension, highlighting the relevance of this mechanism in development.

M101

**Tuning cellular contractility by assembly of subcellular actomyosin structures**

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Cells require mechanical forces to perform dynamic processes, such as cell migration or cell division. Mechanical output of the cell is tuned by the actomyosin cytoskeleton, where myosin motors pull on F-actin networks in cells to generate contractile forces. However, it is unclear how forces at the cellular scale can be regulated by the subcellular arrangement of actomyosin structures. To this end, we aim to quantitatively characterize how actomyosin assemblies remodel incells leading to varying levels of contractility. Using quantitative microscopy, we observe that myosin appears as punctate structures in cells, suggesting that myosin locally accumulates in clusters. The number of myosin in these clusters shows a heavy-tailed distribution, with larger clusters located on thick F-actin bundles. In more contractile cells, for example by activating RhoA, both the number and the size of myosin clusters increase. This reflects an increase in the density of myosin motors at the subcellular scale, which correlates well with the measured mechanical energy density produced by the cell. With a simple simulation, we further show that the distribution of myosin clusters can be altered by the F-actin network. Our results can provide insight on how actomyosin assemblies tune cellular contractility.
**ORAL PRESENTATIONS**

**M102**

**Building Membraneless Organelles to Control Cell Behavior**

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Liquid-liquid phase separation (LLPS) of intrinsically disordered proteins (IDPs) into biomolecular condensates is a fundamental organizing principle in cell biology. Subcellular partitioning and compartmentalization of proteins and other macromolecules is used to insulate components or to enhance reaction rates. Inspired by these functions, we sought to leverage the principles of IDP self-assembly to build intracellular compartments de novo capable of functioning as hubs to regulate the flow of information through cellular control systems. Using the disordered RGG domain of the germ granule protein Laf-1, we designed a membraneless organelle platform to regulate endogenous cellular activities through targeted insulation of endogenous proteins. By encoding and expressing a disordered protein scaffold, we assembled micron size condensates and demonstrated selective recruitment of endogenous enzymes via genomic tagging with small, high-affinity dimerization motifs in both yeast and mammalian systems. By relocalizing up to ninety percent of a targeted enzymes to our membraneless organelles, thereby depleting them from the rest of the cell, we modulate cell functions and behaviors, including proliferation, cell division, and cytoskeletal organization. By embedding multiple strategies for controlled cargo release from condensates, we rapidly control pathway activity and switch cells between functional states. Collectively, this new condensate platform offers a powerful and generalizable approach to dissect and perturb protein networks for cell and developmental biology.

**Nuclear Organization in Space and Time**

**M103**

**Nucleolar homeostasis connects with nuclear organization**

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Beyond its classically established role in ribosome synthesis, the nucleolus is now known to be a multifunctional nuclear organelle besides being sites of ribosome synthesis. Although certain stressors and genotoxic encounters disrupt nucleolar integrity and ribosome synthesis, they can influence much more than just the nucleolus, making it difficult to selectively address the nucleolar function. For example, classical studies use low concentrations of acintomycin D to inhibit rDNA transcription and perturb the nucleolus. But the drug also inhibits topoisomerases I and II and intercalates DNA with selective affinity for G+C-rich sequences that abound throughout the genome. To more selectively tease out the potential unrecognized roles of the nucleolus, we disrupted rDNA transcription by siRNA knockdown of the RNA polymerase I largest subunit, RPA194, in HeLa cells, which reduced pre-rRNA synthesis and induced
nucleolar segregation, similar to that observed in actinomycin D-treated cells. Nucleolar segregation induced by RP194 knockdown led to a repositioning the centromeric regions of chromosomes situated in the nucleolar periphery. In addition, spatially distal Cajal bodies, lying 0.1 - more than one micron away from the nucleoli, underwent morphological alterations and loss of certain components. Furthermore, certain genomic loci situated far from nucleoli displayed extensive repositioning. These widespread effects throughout the 3-D nucleome were not observed when the pre-ribosomal RNA processing factor UTP4 was knocked down, which also reduced ribosome synthesis, but does not induce nucleolar reorganization, establishing that the RPA194 effects throughout the nucleus are not due to an inhibition of ribosome synthesis but rather a nucleolar reorganization. These findings point to an intranuclear commutative system that links the homeostasis of the nucleolus to the maintenance and localization of certain proximal and distal nuclear bodies and gene loci.

M104
Cenp-b-mediated DNA loops regulate activity and stability of human centromeres
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Chromosome inheritance depends on centromeres, epigenetically specified regions of chromosomes. While conventional human centromeres are known to be built of long tandem DNA repeats, much of their architecture remains unknown. Using single-molecule techniques such as AFM, nanopores, and optical tweezers, we find that human centromeric DNA exhibits complex DNA folds such as local hairpins. Furthermore, upon binding to a specific sequence within centromeric regions, the DNA-binding protein CENP-B compacts centromeres by forming pronounced ~500 bp loops between the DNA repeats, which favours inter-chromosome centromere clustering as observed by 3D-SIM cell imaging. This DNA loop-mediated organization of centromeric chromatin participates in maintaining centromere position and integrity upon microtubule pulling during mitosis. Our findings emphasize the importance of DNA topology in centromeric regulation and stability.

M105
How does the C-terminus of the Synaptonemal Complex Protein SYP-4 regulate Crossover Formation during C. elegans Meiosis?
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Meiosis is a specialised cell division process consisting of two consecutive cell divisions to produce gametes. In meiosis I, the parental homologous chromosomes must segregate to different cells. This depends on both, accurate homologous chromosome pairing and the formation of crossovers between the homologs. The latter is regulated by two mechanisms: crossover assurance, which ensures that at least one crossover occurs between each pair of homologs, and crossover interference, which ensures a non-random distribution of crossovers along the chromosomes. In wild-type C. elegans animals, this gives rise to the formation of a single crossover event per pair of homologous chromosomes. In most sexually reproducing organisms, the assembly of the synaptonemal complex stabilises homologous chromosome pairing and is thus essential for their accurate segregation. In recent years, several studies
have identified an additional function of the synaptonemal complex in crossover regulation. However, the question of how the synaptonemal complex regulates crossover formation still remains unsolved. Our data combining genome editing, fluorescence microscopy, and automated image analysis now reveals that the C-terminus of SYP-4 is a critical regulator of crossover formation. We show that deletion of the C-terminus of SYP-4 does not affect the assembly of the synaptonemal complex but severely impairs the regulation of crossover events along chromosomes and diminishes the viability of the progeny. We therefore hypothesised that the C-terminus of SYP-4 is a central hub for crossover regulation within the synaptonemal complex that may be regulated by post-translational modifications. Indeed, our results using mass-spectrometry to identify such modifications suggest that the C-terminus of SYP-4 carries several phosphorylation sites. Using genome editing to generate phospho-null and phospho-mimetic SYP-4 mutants, we now show that inhibiting or mimicking phosphorylation of these sites indeed impairs crossover interference while crossover assurance and synaptonemal complex assembly remain unaffected. Thus, our results suggest that the number of crossover events along individual chromosomes is limited by post-translational modifications within the C-terminus of SYP-4 which may shed light on how C. elegans can tightly regulate the number and distribution of crossover events.

M106

**Nuclear accumulation of CHMP7 initiates nuclear pore complex injury in familial and sporadic ALS**

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Nuclear pore complex (NPC) injury has recently emerged as an early and significant contributor to familial and sporadic Amyotrophic Lateral Sclerosis (ALS) disease pathogenesis. However, the molecular events leading to this pathological phenomenon characterized by the reduction of specific nucleoporins (Nups) from the NPC remains largely unknown. This is due in part to a lack of knowledge regarding the biological pathways and proteins underlying NPC homeostasis specifically in human neurons. Using induced pluripotent stem cell (iPSC) derived neurons (iPSNs) and postmortem human CNS tissues, we have recently uncovered that aberrant nuclear accumulation of the ESCRT-III protein CHMP7 initiates NPC injury in familial and sporadic ALS neurons. Critically, knockdown of CHMP7 alleviates disease associated Nup alterations, deficits in Ran GTPase localization, defects in TDP-43 associated mRNA expression, and impaired neuronal survival in response to glutamate stress. Using cutting edge genome wide Crispr screening, mass spectrometry, biochemistry, and confocal and super resolution microscopy technologies, we are investigating the molecular mechanisms by which 1. CHMP7 is recruited to the nuclear envelope and/or nucleoplasm in human neurons in health and disease, 2. CHMP7 aberrantly accumulates within human ALS neuronal nuclei, and 3. CHMP7 initiates Nup removal and degradation from the NPC in human neurons in health and disease. In yeast and non-neuronal mammalian cells, nuclear relocalization of CHMP7 has been shown to recruit the ESCRT-III proteins CHMP4B, CHMP2B, and VPS4 to facilitate NPC and nuclear envelope (NE) repair and homeostasis. Intriguingly, neither CHMP4B nor CHMP2B pathology is observed in familial and sporadic ALS neurons. In contrast, VPS4 expression is significantly increased in a CHMP7 dependent manner in ALS neuronal nuclei prior to the emergence of nuclear pore injury. However, unlike CHMP7 knockdown, impaired VPS4 function does not mitigate NPC injury and instead results in the emergence of intranuclear POM121 “degradation intermediates”. Collectively, these data support a role for altered CHMP7 mediated Nup homeostasis as a prominent initiating pathomechanism for familial and sporadic ALS and highlights the potential for
CHMP7 as therapeutic target. Moreover, this work sheds light on the cell biological mechanisms that regulate CHMP7 localization and activity in human neurons in health and disease.

M107
Identifying the role of centromeric island in centromere function using genome editing
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The centromere is essential for accurate chromosome segregation during cell division. Centromeric chromatin is unique due to the presence of a histone H3 variant protein called CENP-A. Multiple studies support the model that CENP-A is the major determinant for centromere identity and propagation. In contrast, the function of highly repetitive centromere DNA (cenDNA) remains elusive due to limited tools available for studying them in multicellular organisms. We recently reported the sequence and organization of all the centromeres of D. melanogaster obtained using ChIP and long-read sequencing. The functional centromere occupies primarily islands of complex DNA enriched in retroelements that are flanked by blocks of simple satellites. To determine if these centromeric islands are essential for centromere identity, we have engineered an in vivo CRISPR system to delete the centromere 3 island, called Giglio. Transgenic flies were constructed such that two unique gRNAs each complexed with Fok1-dCas9 protein would target either side of Giglio and induce double-stranded breaks that may be repaired by non-homologous end joining resulting in a complete deletion. CRISPR activity was inferred by visualizing double stranded breaks at the centromere. Successful Giglio deletion was observed in either or both chromosome 3 homologs. However, centromere remained at the endogenous locus in the Giglio-deleted population; this indicates formation of a neocentromere with new underlying sequence. In a few cases, acentric chromosomes 3 were also observed, which suggests that deleting cenDNA might impact the centromere. Furthermore, a slight increase in aneuploidy and chromosome 3 breaks were observed in CR+ larval brains. The relation between aneuploidy and Giglio deletion will be determined by visualizing and quantifying chromosome segregation defects in anaphase. Broken chromosome 3s were observed at the centromere, implying CRISPR activity. Ongoing work is identifying new underlying sequence of the neocentromere using chromatin fiber imaging and CUT&Tag combined with long-read sequencing. The sequence information might reveal if the centromere is biased to certain type of sequence. Overall, this work suggests that cenDNA is not critical for centromere in mitosis. However, given the low frequency of island deletion in this system, future work will be to develop a second system where we are introducing attB/attP recombination cassettes on either side of Giglio to induce its excision upon somatic expression of the phiC31 recombinase. Furthermore, we will also induce germline expression of the Giglio deletion to understand the role of cenDNA in meiosis.

M108
Ewsr1 (ewing sarcoma breakpoint region 1) facilitates cenp-a maintenance at the centromere
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The centromere is essential for ensuring high-fidelity chromosome transmission. CENP-A, the centromeric histone H3 variant, is thought to be the epigenetic mark of centromere identity. The molecular mechanism of CENP-A deposition at the centromere is crucial for proper centromere inheritance and function. Here, we report that CENP-A interacts with EWSR1 (Ewing Sarcoma Breakpoint Region 1) and EWSR1-FLI1 (the oncogenic fusion protein in Ewing sarcoma). We found that EWSR1 is
required for maintaining CENP-A at the centromere by binding to centromeric RNA and CENP-A in interphase cells. EWSR1 and EWSR1-FLI1 bind to CENP-A through the SYGQ2 region within the prion-like domain, which is important for phase separation. EWSR1 binds to R-loops through its RNA binding domain \textit{in vitro}. These results suggest that EWSR1 guards CENP-A in centromeric chromatins, presumably by creating phase separation and by binding to centromeric RNA. EWSR1-FLI1 causes chromosome instability by inhibiting the kinetochore function of EWSR1 in Ewing sarcoma cells.

\textbf{Organelle Biophysics and Phase Separation}

\textbf{M109}
\textbf{Identifying the role of septins in mitochondrial fission}
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Mitochondria maintain a dynamic network that extends throughout the cell by continually undergoing processes of fission (mitochondrial division) and fusion (the joining of mitochondria). Fission and fusion are collectively referred to as mitochondrial dynamics, and are essential for maintaining mitochondrial health and for mitochondria to respond to different cellular conditions. Fission is accomplished by a multi-step pathway, and recently, a role for septins has emerged. Septins are a family of proteins that form complex structures like filaments throughout the cytosol, and are considered a major component of the cytoskeleton. The objective of this study is to identify the mechanistic contribution of septins to the mitochondrial fission pathway. We find that the depletion of Septin9 in human epithelial cells causes hyper-elongated mitochondria because of decreased rates of fission. By live super-resolution microscopy, we find that Septin9 is on mitochondria at sites that undergo fission, and that Septin9 most often arrives on these sites prior to another fission component- dynamin-related protein (Drp1). This suggests that septins may take part in early steps in fission, such as those initiated by the endoplasmic reticulum (ER). The ER is a critical component of mitochondrial fission, as it is thought to mediate actin polymerization to generate localized actomyosin constrictional force onto mitochondria to initiate the fission process. Although myosin activation is crucial for this initial constriction of the mitochondrial diameter, it is unknown how myosin activity is regulated at fission sites. We propose a mechanistic role for septins in mitochondrial fission in mediating myosin activation through a Septin9-specific guanine nucleotide exchange factor- ARHGEF18. Notably, ARHGEF18 has previously been identified to influence myosin activity through Rho and ROCK signaling. Since we find that depletion of ARHGEF18 impairs mitochondrial fission, and that Septin9 arrives at mitochondrial fission sites prior to the fission mediator Drp1, septin octamers may contribute to fission by regionally activating myosin, which then generates a constrictional, pinching force necessary to locally reduce the mitochondrial diameter and recruit Drp1 to future fission sites.
Ndc1 is a dynamic membrane adaptor necessary for assembly of nuclear pores after mitosis in *C. elegans*

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Nuclear pore complexes (NPCs) are large protein assemblies that facilitate transport of macromolecules across the nuclear envelope (NE). How thousands of NPCs rapidly assemble after open mitosis to form a functional NE is not known. Recruitment of the Nup107-160 outer ring scaffold to chromatin initiates NPC assembly. The Nup53/93 complex bridges the outer ring to the central channel to form a functional pore. Nup53 interacts with the conserved transmembrane nucleoporin Ndc1; however, how Ndc1 contributes to post-mitotic NPC assembly is unclear. Here, we use *C. elegans* embryos to show that the timely formation of a functional NE after mitosis depends on Ndc1. Endogenously tagged Ndc1 is recruited early to the reforming NE and is highly mobile in the nuclear rim. 3D analysis of post-mitotic NE formation revealed a decreased NPC density in NEs of *ndc1* deleted embryos - continuous nuclear membranes contained few holes where assembling NPCs are normally located. Nup160 is highly mobile in NEs depleted of Ndc1 and outer ring scaffold components are less enriched at the rim. When both *ndc1* and *nup53* are absent, nuclear assembly fails. Together, these data show that Ndc1 dynamically associates with the NE and promotes stable association of the outer ring scaffold in the NE to facilitate NPC assembly after open mitosis. Furthermore, Ndc1 and Nup53 function in parallel to drive nuclear assembly. We propose that Ndc1 is a dynamic membrane adaptor that helps recruit and promote the self-assembly of the nuclear pore scaffold to drive post-mitotic NPC assembly.

Mapping and quantifying inter-organelle interactions using Soft X-ray Tomography

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Inter-organelle communication is required for the coordination and control of the function of individual sub-cellular compartments. The maintenance of the cell well-being is usually accompanied by rearrangements in the cellular ultrastructure, which alters the connectivity between organelles controlling the cell health condition\(^1,2\). High-resolution technologies, such as fluorescent and electron microscopy, have been demonstrated to be able to characterize inter-organelle interaction in the entire cell; however, both methodologies require extensive cell manipulation\(^2,3\). We used Soft X-ray Tomography (SXT)\(^4\) to rapidly map the spatio-temporal evolution of insulin vesicles and their co-localization and interaction with mitochondria in pancreatic β-cells during the insulin secretory process. We captured the cell ultrastructural reorganization without staining and slicing, preserving high spatial resolution (60 nm) and short time of collection (<10 min)\(^5\). We mapped the systemic interaction between mitochondria and insulin vesicles, as a response to the stimulation with glucose and the incretin exendin-4. We tracked the alteration of the interaction during the secretory process, showing that exendin-4 induces more frequent association between insulin vesicles and mitochondria in vicinity.

M112
A mitotic chromatin phase transition renders chromosomes microtubule-impermeable
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Dividing eukaryotic cells package extremely long chromosomal DNA molecules into discrete bodies to enable microtubule-mediated transport of one genome copy to each of the newly forming daughter cells. Mitotic chromosome assembly involves DNA looping by condensin complexes and chromatin volume compaction by affinity-based interactions between nucleosomes that are regulated by histone modifications. While DNA looping by condensin confers resistance of chromosomes towards spindle pulling forces, it is not known how chromatin compaction by mitosis-specific changes in histone modifications contributes to the material properties and mechanical function of mitotic chromosomes. Using a combination of light microscopy, single-cell micromanipulations, and electron tomography, we show that a global deacetylation during mitotic entry renders chromatin insoluble in the mitotic cytoplasm. This phase transition results in the formation of a microtubule-impermeable surface on mitotic chromosomes, enabling astral microtubules to push on chromosome arms upon end-on contact, rather than growing through chromatin fiber loops. Based on cell biological and biochemical reconstitution experiments, we found that chromatin condensates exclude soluble tubulin dimers and other negatively charged macromolecules, suggesting that electrostatic repulsion keeps microtubules out of mitotic chromosomes. Hyperacetylated chromosomes are frequently perforated by microtubules and fail to properly congress and segregate. Our work provides insights into how the coordinated action
of DNA looping and acetylation-regulated chromatin compaction forms mitotic chromosomes that can be faithfully transported by the mitotic spindle.

**M113**

**Liquid-crystalline phase transitions within lipid droplets can selectively remodel the LD proteome**

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Lipid droplets (LDs) serve as reservoirs for neutral lipids including triglyceride (TG) and sterol-ester (SE) that can be mobilized to promote growth or metabolic remodeling. How these lipids are organized within the LD interior, and how they influence the LD surface proteome, remains poorly understood. Here, we show that in response to glucose starvation, yeast LDs exhibit lipid phase transitions that promote the formation of liquid-crystalline layers (LCL) composed of SEs within the LD core. Importantly, we find that LCL formation requires TG lipolysis, and can be reversed by briefly heating yeast above the phase transition temperature of SEs. Using unbiased LC-MS/MS proteomics of isolated LCL-LDs combined with fluorescence imaging, we find that specific proteins are delocalized from LCL-LDs, whereas others are retained. In line with this, canonical LD protein Erg6 delocalizes from LCL-LDs, whereas the perilipin-like Plin1/Pet10 remains LD associated. Brief temperature elevation induces rapid Erg6 LD re-targeting, suggesting its delocalization is a biophysical consequence of LCL formation. Mechanistic analysis of LD targeting with model Type I LD protein LiveDrop indicates that many Type I LD proteins are re-targeted from LDs to the ER network in response to liquid-crystalline phase transitions. In contrast, TG lipases remain bound to LCL-LDs to drive energetic mobilization. Surprisingly, we also find that LCL-LDs are decorated by membrane trafficking proteins not normally localized to LDs, including some BAR domain-containing proteins, suggesting LD lipid phase transitions can promote the recruitment of non-canonical machinery to LDs. Collectively, we propose that TG lipolysis promotes lipid phase transitions within LDs that restructures the LD sub-architecture and selectively remodels the LD surface proteome.

**M114**

**The *C. elegans* homolog of Nucleolin, NUCL-1, contributes to nucleolar organization through its intrinsically disordered RG/RGG repeat domain**

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Intrinsically disordered domains influence liquid-liquid phase separation (LLPS) of membraneless organelles (MLOs). One such domain, consisting of Arginine and Glycine (RG/RGG) repeats, is critical for both P-granule and nucleolar function in *C. elegans*. Using the command-line search tool, “grep,” we have identified the 50 longest RG/RGG repeat sequences in the *C. elegans* proteome, ranging in length from 321 to 21 amino acids. Two RG/RGG motifs emerged from MEME analysis, a phenylalanine-rich motif typical of nucleolar proteins and a tyrosine-rich motif typical of P-granule proteins. We discovered that one of the longest nucleolar-like RG/RGG motifs (176 aa) was present in the highly abundant but uncharacterized K07H8.10 protein. In addition to its N terminal RG/RGG repeat domain, K07H8.10 contains a coiled-coil acidic domain and two C terminal RNA recognition motifs (RRMs). These domains are present in Nucleolin, a protein conserved across animals, plants, and fungi, but thought to be absent in nematodes. We show that in nematodes, the order of Nucleolin’s three domains is rearranged, evading its detection, and that once this is accounted for, K07H8.10 (now called NUCL-1) shows
extensive sequence homology to Nucleolin. Using a split-GFP tagging strategy and super-resolution microscopy, we show that NUCL-1 in the germline of living worms localizes to GC and FC substructures, contrasting with the DFC localization of Fibrillarin (FIB-1), another nucleolar RG/RGG domain protein. Worms are still fertile when NUCL-1 or its RG/RGG domain are removed. Interestingly, deleting the RG/RGG domain of NUCL-1 does not impair its nucleolar localization; however, super-resolution imaging reveals that sub-nucleolar compartmentalization of both NUCL-1 and FIB-1 are disrupted. Deletion of FIB-1’s 107 amino acid-long RG/RGG domain on the NUCL-1 RG/RGG deletion background suggests functional redundancy between the two nucleolar RG/RGG domains, as double mutant worms are subviable compared to either single mutant. Finally, deletion of the RG/RGG domains from two other nucleolar proteins, LPD-6 and Y66H1A.4 (GAR1), again demonstrate that these domains are not necessary for protein localization to the nucleolus. Our work in C. elegans shows that unique motifs in RG/RGG domains predict MLO localization, despite the surprising result that they are completely dispensable for this localization in vivo.

Stem Cell Renewal, Quiescence, and Differentiation

M115
Syndecan-2 expression marks hematopoietic stem cells and controls stem cell repopulating capacity
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Syndecans are heparan sulfate proteoglycans containing a transmembrane core protein decorated with heparan sulfate glycosaminoglycan chains, which interact with growth factors to regulate downstream proliferative, adhesive, and survival signaling. Hematopoietic stem cells (HSCs) coordinate proliferative signaling to preserve the HSC pool throughout the lifetime of an organism. Previous studies detected increased expression of Sdc2, which encodes for Syndecan-2, in murine HSCs compared to hematopoietic multipotent progenitors (MPPs), but the function of Syndecan-2 in HSCs has yet to be elucidated. Based on the role of Syndecan-2 in cellular proliferation, we hypothesized Syndecan-2 marks HSCs and controls HSC proliferation. We first quantified Syndecan-2 expression on murine bone marrow HSCs and MPPs. Using flow cytometry, we determined Syndecan-2 is tenfold enriched on the surface of HSCs compared to MPPs. We next quantified how Syndecan-2 expression marks HSCs with distinct functional properties by using in vivo competitive repopulation assays to quantify the ability for transplanted HSCs to regenerate the hematopoietic system of lethally irradiated mice. We transplanted CD34-c-Kit+Sca-1-Lineage (34 KSL) Syndecan-2+ (Sdc2+ HSCs) or Syndecan-2− (Sdc2− HSCs) and determined Sdc2+ HSCs exhibit significantly elevated hematopoietic repopulation and self-renewal potential compared to Sdc2− HSCs. These data suggest Syndecan-2 expression marks HSCs with enhanced functional ability. We next analyzed the regulatory role of Syndecan-2 in HSC function using a lentiviral shRNA knockdown approach. We determined Syndecan-2 knockdown significantly reduced HSC self-renewal upon transplantation. Given the defined function of Syndecan-2 in cellular proliferation, we tested how Syndecan-2 regulates HSC cell cycling. We detected significantly decreased HSCs in the G0 cell cycle phase upon Syndecan-2 knockdown compared to control conditions, suggesting Syndecan-2 controls HSC quiescence. As Cdkn1c is a well-defined regulator of HSC cell cycle entry, we quantified Cdkn1c expression upon Syndecan-2 knockdown and detected significantly decreased Cdkn1c compared to control cells. We next used a double knockdown system to reduce Sdc2 and Cdkn1c expression and
determined knockdown of both Sdc2 and Cdkn1c does not further reduce the proportion of G0 quiescent HSCs compared to Sdc2 knockdown alone. Functional analysis of hematopoietic colony formation showed knockdown of Sdc2, Cdkn1c, or both similarly diminishes colony formation compared to control conditions, underscoring the importance of Syndecan-2 in hematopoietic differentiation. Our study reveals Syndecan-2 is a novel marker of HSCs that controls HSC function through cell cycle regulation by Cdkn1c.

M116
Tetraspanin CD82 Regulates Hematopoietic Stem and Progenitor Cell Quiescence and Regeneration
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The significant cellular demand of the hematopoietic system is maintained by a rare pool of tissue-specific, hematopoietic stem and progenitor cells (HSPCs) that are primarily found in a quiescent state. Upon hematopoietic stress, HSPCs undergo rapid cell cycle activation, but ultimately must return to quiescence to prevent hematopoietic exhaustion. Evidence from our laboratory demonstrates that the tetraspanin CD82 plays a critical role in the regulation of HSPC quiescence and activation. Tetraspanins are membrane scaffold proteins with the ability to modulate signaling through the organization of membrane receptors and intracellular signaling molecules. Previous work from our laboratory identified a role for CD82 in HSPC quiescence, where we find a reduction in long term-HSCs in global CD82KO mice, resulting from increased HSPC activation and a reduction of quiescent G0 cells. In the present study, we test the hypothesis that CD82 expression promotes HSPC return to quiescence following hematopoietic stress, by regulating the activation of TGFβ signaling. To investigate the impact of CD82 expression on hematopoietic regeneration under stress, we treated WT and CD82KO mice with the chemotherapy agent 5-FU and measured overall survival. We find that CD82KO mice have longer overall survival compared to WT counterparts. Moreover, we find an increase in peripheral blood HSPCs in CD82KO mice during the early recovery period from 5FU treatment, suggestive of enhanced cell activation upon stress and effective regeneration post injury. Mechanistically, the cytokine TGFβ plays an essential role in supporting HSPC quiescence and activation, as one of the most potent inhibitors of HSPC growth. To investigate if CD82 plays a role in TGFβ signaling, we stimulated WT and CD82KO HSPCs with TGFβ and measured nuclear translocation of SMAD2/3, a downstream effector of TGFβ activation. Confocal imaging demonstrates that CD82KO HSPCs have decreased nuclear translocation of SMAD2/3 upon TGFβ activation, consistent with reduced TGFβ signaling. Similarly, human CD34+ HSPCs sorted for low and high CD82 expression have decreased and increased SMAD2/3 nuclear translocation, respectively. Finally, to determine how CD82 impacts TGFβ signaling within the niche, we analyzed bone marrow sections from mice injected with HSPC-like cells differentially expressing CD82. Confocal imaging finds an increase in active TGFβ expression surrounding HSPCs with increased CD82 expression, whereas reduced active TGFβ is detected around control cells. Together, these data suggest that CD82 regulates HSPC quiescence and activation through modulation of TGFβ signaling. Future studies will focus on elucidating the mechanism by which CD82 modulates local TGFβ signaling within the niche.
Differentiation-dependent changes in Lamin B Receptor (LBR) localization influence lamin B1 dynamics and expression of differentiation markers

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The nuclear lamina is a complex network of proteins found just within the inner nuclear membrane of metazoan nuclei. This structure serves various roles including chromatin organization and structural support. Genetic disruptions to this structure are known to cause a variety of diseases in humans, collectively termed laminopathies. The symptoms of these conditions vary significantly from muscular dystrophies to premature aging, suggesting nuclear lamina structure is important in development and cellular function. While recent research has advanced our understanding of the role of the nuclear lamina in its numerous functions, less is known about how nuclear lamina structure changes during cellular differentiation, changes that may influence gene regulation. Here we examine the structure of the nuclear lamina in human induced pluripotent stem cells (iPSCs) and differentiated germ layer cells. Specifically, we measured the dynamics of a ubiquitous nuclear lamina protein: lamin B1. Fluorescence recovery after photobleaching (FRAP) studies revealed that lamin B1 dynamics generally increase as iPSCs differentiate, especially in mesoderm and ectoderm cells. Screening a panel of nuclear envelope proteins for differentiation-dependent changes in expression levels and localization, we found that Lamin B Receptor (LBR) partially redistributes from the nucleus to cytoplasmic puncta in mesoderm cells. To test if this change in localization might contribute to increased lamin B1 dynamics, we knocked down LBR in iPSCs and observed an increase in the lamin B1 mobile fraction, a change that was not observed for ELYS, emerin, or lamin B2 knockdown. LBR knockdown in mesoderm cells did not affect lamin B1 dynamics, perhaps because lamin B1 dynamics have reached a threshold in that cell type. We also found that LBR affects expression of differentiation markers. For example, LBR knockdown in iPSCs led to a 49% and 70% increase in Nanog and HNF-3β expression, respectively, while expression of Oct4 and Brachyury was reduced by 13% and 39% respectively. These data suggest that LBR may act as a differentiation-dependent tether for lamin B1 that impacts the differentiation process.

Using the epithelial to mesenchymal transition (EMT) in human induced pluripotent stem cells (hiPSC) as a model to study cell states and state transitions by combining cell behavior, cell organization, and cell identity.

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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how cells transition between states during differentiation and disease. The epithelial to mesenchymal transition (EMT) is a state change that occurs normally during development and wound healing and pathologically in cancer metastasis and fibrosis. We are using 3D, live-cell imaging across several spatial scales to capture the dynamic behavior of cells, including shape, migration analysis, and organelle localization in a model of EMT induced in human induced pluripotent stem cells (hiPSC). We are integrating this information with the expression of canonical and novel markers of cell identity, using fixed-cell antibody labeling and scRNAseq-informed RNA FISH. Early migrating cells have different patterns of marker expression, suggesting they may be in different states. Additionally, we see spatial patterning of structure organization and cell identity markers within colonies before cells begin to...
migrate, which suggests that different cells in the same population may either take different trajectories or take the same trajectory with different timings as they differentiate. We believe that this multi-modal, multi-scale approach might serve as a framework for studying EMT in other contexts and in understanding cell states and transitions more generally.

M119
**Asymmetric nucleosome density and differential condensation of sister chromatids coordinates with Cdc6 to ensure distinct cell fates**

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Stem cells undergo asymmetric division to produce both a self-renewing stem cell and a differentiating daughter cell. During *Drosophila* male germline stem cell (GSC) asymmetric division, preexisting old histones H3 and H4 are enriched in the self-renewed stem daughter cell, whereas the newly synthesized H3 and H4 are enriched in the differentiating daughter cell. However, the biological consequences in the two daughter cells resulting from asymmetric histone inheritance remained to be elucidated. In this work, we track both old and new histones throughout GSC cell cycle using high spatial and temporal resolution microscopy. We find several unique features differentiating old versus new histone-enriched sister chromatids, including nucleosome density, chromosomal condensation, and H3 Ser10 phosphorylation. These distinct chromosomal features lead to their differential association with Cdc6, an essential component of the pre-replication complex, which subsequently contributes to asynchronous initiation of DNA replication in the two resulting daughter cells. Disruption of asymmetric histone inheritance abolishes both differential Cdc6 association and asynchronous S-phase entry, demonstrating that asymmetric histone acts upstream of these critical events during cell cycle progression. Furthermore, GSC defects are detected under these conditions, indicating a connection between histone inheritance, cell cycle progression and cell fate decision. Together, these studies reveal that cell cycle remodeling as a crucial biological ‘readout’ of asymmetric histone inheritance, which precedes and could lead to other well-known readouts such as differential gene expression. This work also enhances our understanding of asymmetric histone inheritance and epigenetic regulation in other stem cells or asymmetrically dividing cells in multicellular organisms.

M120
**X chromosome associated histone demethylase UTX reshapes neuronal transcriptomics landscape to regulate optic nerve regeneration**

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Neurons in the mature mammalian central nervous system (CNS) cannot regenerate their axons after various injuries or degenerative diseases. During maturation, CNS neurons gradually lose their intrinsic regenerative ability due to changed chromatin and transcriptomics landscapes. Recent studies using advanced multomics analyses discovered that after neural injury mature neurons in either peripheral nervous system (PNS) or CNS somehow reshaped their transcriptional profiles back to a younger state favoring axon regeneration. It is well known that epigenetic modifications of histones or DNAs play key roles in regulation of chromatin structure and gene transcription. X chromosome gene *UTX*, also named *KDM6A*, encodes a chromatin modifier that specifically erases trimethyl groups of H3 histone at lysine
27 (H3K27me3), thereby generating transcriptionally permissive chromatin. UTX has been shown to play important roles in development, aging, and cell reprogramming. Here we report that UTX is a novel and key epigenetic regulator of PNS and CNS axon regeneration in vivo. Specifically, UTX expression was markedly reduced in sensory neurons after peripheral nerve regeneration, indicating UTX as a suppressor for axon regeneration. Indeed, knocking down UTX in sensory neurons enhanced sensory axon regeneration. In contrast, after optic nerve injury the level of UTX in retinal ganglion cells (RGCs) was not changed. More importantly, knocking out UTX in RGCs led to significantly enhanced RGC survival and optic nerve regeneration. In addition, in a genetic knock-in mouse line, in which wild type UTX is replaced with a mutant UTX lacking the histone demethylase activity, optic nerve regeneration was significantly improved as well. Furthermore, UTX and PTEN regulate two independent pathways that act synergistically to enhance optic nerve regeneration after axonal injury. Mechanistically, RNA-seq and ATAC-seq, either at bulk or single cell level, revealed that UTX-mediated regeneration state is triggered by reactivating developmental-like transcriptional programs. Lastly, advanced bioinformatics analysis identified microRNA-124 as a downstream gene repressed by UTX-regulated H3K27me3. Functionally, inhibiting microRNA-124 in RGCs also resulted in markedly increased optic nerve regeneration. Collectively, our findings revealed that UTX and H3K27me3 were novel and key epigenetic regulator of mammalian axon regeneration by remodeling neuronal chromatin structure and transcriptomics landscape. Furthermore, because UTX has higher expression level in female cells, our results also provided potential underlying reasons for sexual difference in certain type of glaucoma.

Tumor Progression and Therapy 2

M121
New features and functions of cancer lysosomes
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Pancreatic ductal adenocarcinoma (PDA) cells and tumors constitutively activate nutrient scavenging pathways namely autophagy (cellular self-catabolism) and macropinocytosis (bulk uptake of extracellular material) in order to maintain metabolic homeostasis. Importantly, both of these pathways converge on the lysosome, an acidic organelle that harbors within its lumen a complex series of enzymatic reactions that enable degradation of in-coming cargo material. Our prior work has shown that the lysosome is greatly expanded and qualitatively different in PDA cells compared to normal tissue. PDA tumors are effectively “addicted” to enhanced catabolic activity as blocking lysosome function specifically inhibits in vitro and in vivo growth of PDA cell lines and tumor xenografts with minimal effects on normal cells. How key changes in lysosome composition and content contribute to the aggressive nature of PDA remains an open question. Using comparative proteomic-based profiling of intact lysosomes isolated from PDA and normal pancreatic ductal epithelial cells, we have identified both resident and substrate proteins that are uniquely associated with PDA lysosomes. Several cancer-specific lysosomal membrane proteins emerged from this analysis that may endow cancer lysosomes with enhanced functionality including rapid membrane repair in the face of sustained mechanical and chemical insults. Similarly, we find that PDA lysosomes degrade select cellular proteins to enable dynamic remodeling of the cellular proteome in a manner that favors tumor growth and progression. Collectively, our studies support a central role for the lysosome in cellular adaptation to stress and mediator of tumorigenesis.
M122

Egf-targeted toxin as efficient anti-bladder cancer agent able to circumvent the presence of egfr endocytosis-impairing mutations and her2 in vitro and in vivo
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Bladder cancer is the 4th most prevalent cancer in men, 11th in women and presents one of the highest rates of post-treatment recurrence (70%) among malignancies. Despite its impact on human health, therapeutic approaches against bladder cancer are of limited efficacy. We previously developed a strategy based on receptor micro-clustering (µC) to induce agent fast internalization by bladder tumor cells. Importantly, µC effects can be elicited by different multivalent agents including anthrax toxin that (contrary to others like diphtheria toxin) clusters as heptamers at the plasma membrane inducing its own uptake. Further, and considering that bladder tumor cells express high levels of Epidermal Growth Factor (EGF) Receptor (EGFR), we used EGF to target this lethal toxin against cancer cells. Here we show that the EGF-toxin induces its own internalization and delivery into the cytosol of targeted cells within min of exposure. This agent was up to 1000 times more efficient that current anti-bladder cancer agents such as mitomycin C and Gemcitabine at eliminating bladder cancer cells. Further we also found that this agent was capable of eliminating Cancer Stem Cells of bladder and prostate origin. We established that under bladder instillation conditions, the toxin eliminated human, canine and mouse bladder cancer cells, including Her2-positive cells, with a LC50<1nM (<<intoxication dose ~1μM) and exposure time<3min. Since µC enhances internalization of cargoes independently of receptor dimer formation, we found that this strategy was insensitive to known efficacy-decreasing factors to EGF-based approaches such as internalization interference by presence of Her2 or by endocytosis-limiting mutations. Further, dogs with spontaneous, treatment-resistant Her2-positive bladder cancer (closely mimicking the human condition) showed marked reduction in tumor mass when treated with EGF-toxin. Safety studies indicated no deleterious effects in normal and patient dogs. These studies provide the foundation for an innovative and transformative anti-bladder cancer therapy. The EGF-toxin is a superior agent due to its high efficacy, fast action (minutes vs. current treatments requiring more than 2h retention of therapeutics in the bladder) and enhanced safety, but also due to its ability to target cancer stem cells, non-muscle invasive bladder cancers and Her2-positive tumors.

M123

Cancer-associated somatic mutations alter allosteric regulation of human phosphofructokinase-1
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Metabolic reprogramming, including increased uptake of glucose and excretion of lactic acid, is a hallmark of cancer. However, the molecular mechanisms regulating this process are incompletely understood. The enzyme phosphofructokinase-1 (PFK1), which catalyzes the initial step committing glucose to breakdown, is one of the most important rate-limiting glycolytic enzymes and is known as the “gatekeeper” of glycolysis. While increased activity and expression of PFK1 in cancers have been demonstrated, little is known about the effects of cancer-associated somatic mutations. We characterized one of cancer-associated somatic mutations in the platelet isoform of PFK1 (PFKP), PFKP-D564N, to gain insight into how PFK1 mutations can alter allosteric regulation of the enzyme and ultimately contribute to metabolic adaptation of cancer cells. Cellular glycolytic flux was evaluated in
human breast cancer cells expressing FLAG-tagged wild type or mutant PFKP using the Seahorse glycolytic stress assay. Cells expressing PFKP-D564N demonstrated a decreased rate of glycolysis in a basal state, while their ability to induce glycolytic flux under conditions of low cellular energy was enhanced compared to cells expressing wild-type PFKP. This mutation is located at the interface of PFKP’s catalytic and regulatory domains and was predicted to disrupt an evolutionarily conserved salt bridge between aspartic acid 564 and arginine 319. Moreover, cancer-associated mutations of the Asp-Arg pair were also identified in the liver PFK1 isoform, PFKL. Using purified recombinant proteins, we showed that disruption of the Asp-Arg pair in two PFK1 isoforms decreased enzyme activity, decreased allosteric communication between subunits, and altered allosteric regulation. ATP inhibition was not changed, and AMP was able to increase the affinity for F6P to a similar degree as in the wild type enzyme. Conversely, F1,6bP could not stabilize or activate mutant PFK1. Next, we determined the crystal structure to 3.2Å resolution and used molecular dynamic simulations to understand molecular mechanisms of altered allosteric regulation by F1,6bP. We showed that PFKP-D564N had a decreased total system energy and changes in the electrostatic surface potential of the allosteric effector site. Taken together, these results suggest that mutations in Arg-Asp pair at the interface of the catalytic-regulatory domains disrupts transmission of allosteric signals to the active site. This finding provides novel information on PFK1 structure and function that will allow for design of targeted therapeutics.

M124
Arhgap17, a Cdc42-specific gap, localizes to invadopodia and regulates their turnover as part of an Arhgap17/Cdc42/CIP4 complex
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One of the hallmarks of aggressive cancer is its ability to invade into surrounding tissues and metastasize. Many invasive cancers form invadopodia, which are protrusive, degradative structures that are regulated by the Rho family of GTPases. Rho GTPases commonly have aberrant activity in invasive cancers caused by changes in their upstream regulators: RhoGEFs and RhoGAPs. We have identified the RhoGAP ARHGAP17 (RICH1/NADRIN) as a negative regulator of invadopodia in triple negative breast cancer. Using confocal and STORM super-resolution microscopy techniques, we show that ARHGAP17 localizes to the regulatory ring surrounding invadopodia where it acts to promote disassembly by decreasing invadopodia lifetime through the inactivation of the Rho GTPase Cdc42. We further support these results in live cells by using a new localization-based Cdc42 biosensor, which reveals that ARHGAP17 suppresses Cdc42 activity specifically at the invadopodia ring. We have also identified the Cdc42 effector CIP4 as part of a complex with ARHGAP17 and Cdc42 that we propose is key in the regulation of invadopodia. Finally, using spheroid and inverse invasion assays, we show that the invadopodia phenotype correlates with increased invasive properties. In conclusion, we have identified ARHGAP17 as a key regulator of invadopodia and invasion in triple negative breast cancer by localizing to invadopodia and inactivating Cdc42 as part of a complex with CIP4. In future studies, we will further characterize the molecular mechanisms that regulate the ARHGAP17/Cdc42/CIP4 complex and their function during invadopodia formation.
Tumor microenvironment remodeling by cancer cells-released tracks on type I collagen


Metastasis is the leading cause of cancer-related deaths. During this process, tumor cells acquire invasive and migratory capacities in order to invade the surrounding tissues. To achieve this, the tumor microenvironment is modified to facilitate cancer cells proliferation and dissemination. Multiple mechanisms are involved in this evolution, including cell-cell communications through the tumor microenvironment and the extracellular matrix modifications. Indeed, extracellular vesicles such as exosomes or migrasomes are already known to induce protumor features such as migration, promoting tumor development and metastasis formation. Here we describe a new type of extracellular vesicles (referred as tracks) specifically released by cancer cells along type I collagen fibers during cell migration. We could characterize these tracks, their structure as well as their composition in terms of proteins and nucleic acids, and could show that they are different from classical extracellular vesicles known so far. These tracks are characterized by a discoidin domain receptor 1 (DDR1) staining in the extracellular space. Moreover, these tracks are very stable structures and can be internalized by neighboring cells. After internalization, they can modify the differentiation status of cells able to internalize these tracks. These data suggest that these collagen-associated tracks have a role in cell-cell communication and participate in the remodeling of the tumor microenvironment. Even if their function needs to be fully elucidated, their protein and RNA compositions could suggest that these tracks could help to promote cell proliferation and invasion. These tracks seem to be a new player in the tumor invasion process and could provide a better understanding underlying this process.

Introducing genetically modified fibroblasts to tumor microenvironment for stalling cancer progression

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The role of stromal fibroblasts associated with carcinogenesis and tumor progression is dynamic and context-dependent. While it has been shown some stromal fibroblasts can stall neoplasia from progressing into malignancy, a vast body of evidences demonstrate that most cancer-associated fibroblasts (CAFs), which are induced by cytokines secreted by cancer cells, contribute to the cancer invasion, proliferation, and forming hindrance of anti-cancer drug delivery. We hypothesize that introducing fibroblasts that are not sensitive to CAF-inducing cytokines in tumor microenvironment (TME) can suppress tumor progression, by antagonizing cancer cells and pro-cancer CAFs, and potentially be therapeutically beneficial. In this study, we established an engineered fibroblast line by permanently transfecting the normal fibroblasts with a dominant-negative form of RohA (RhoA-DN), an essential regulator for CAF induction, impairing CAF induction in these cells. We used a 3D co-culture system to evaluate whether the desensitized fibroblasts could suppress lung cancer progression. To recapitulate the key components in tumor microenvironment, a single tumor spheroid made of lung cancer cells was embedded in collagen matrix, to be surrounded by either control or RohA-DN-transfected fibroblasts. After two days of coculture, we found that the tumor spheroid was shrunk by
16% when co-cultured with RhoA-DN transfected fibroblasts. The expression level of Ki-67, a cell proliferation marker, in these cancer cells was 46% less compared with the control. Furthermore, we found paclitaxel, a widely used drug for lung cancer treatment, was more effective against cancer cells. In particular, we observed a 1.46-fold increase in cell death in tumor spheroids co-cultured with RhoA-DN-transfected fibroblasts. Taken together, our results demonstrate that introducing engineered fibroblasts with impaired CAF inductivity to TME can stall tumor progression and sensitize cancer cells to achieve more effective paclitaxel cytotoxicity. Our study results provide a conceptual foundation to a new therapeutic strategy for stalling cancer progression.

FRIDAY, DECEMBER 10, 2021

Actin Dynamics

M127

Tunneling Nanotubes Depend Upon Network Shifts Away from Branched Actin Formation to Promote Their Uniquely Long Growth


Actin-based cell protrusions known as tunneling nanotubes (TNTs) are able to connect remote cells and mediate the transfer of small to large (e.g., organelles, pathogens) components for intercellular communication in both normal physiological and pathological contexts. Although filopodia and TNTs share morphological features and are sustained by actin bundles, they exhibit clear differences: canonical filopodia are characteristically <5 µm and exhibit dynamic cycles of extension and retraction, while TNTs can reach far longer distances and remain stable for long periods of time to connect two cells. How the cell controls a common actin toolbox to generate one structure over the other remains unknown. Utilizing surface micropatterning to control intercellular distances, we first characterized the distances over which actin-dependent processes leads to the formation of TNTs in a mouse neuronal cell model. Our data shows that TNT formation between micropatterned cells occurs most frequently at distances of 15 to 20 µm, while TNT occurrence becomes rarer at 30 µm and non-existent at distances ≥40 µm. TNT-connected cells characteristically lacked lamellipodial morphologies, suggesting an inhibitory role for Arp2/3-mediated actin assembly. Upon Arp2/3 inhibition using CK-666, more TNT connections were observed and the distribution of TNTs at longer micropattern distances was promoted, signifying longer actin structures were formed under this condition. To confirm, optically pulled membrane nanotubes approximately 15-20 µm in length were used to mimic the tubular geometry of TNTs and characterize actin growth within; contrary to mock treated cells, CK-666 treated cells showed robust actin growth reaching the end of the pulled nanotubes. Finally, proteomic analysis using Eps8 (Epidermal growth factor receptor kinase substrate 8) as a previously identified, positive effector of functional TNTs revealed a consequence on key actin-related proteins relevant for the long growth of TNTs: Arp2/3 inhibition showed a loss of proteins enhancing filament turnover and
depolymerization and instead showed heightened interactions with accessory proteins such as the inverted Bin/Amphiphysin/Rvs (I-BAR) domain protein IRSp53, a key membrane deforming and curvature-sensitive protein involved in outward protrusion growth. Together, our data suggests that for a long TNT structure to form, the cell must control the homeostatic actin pool and inhibit the overutilization of actin in branched Arp2/3 networks, thereby freeing actin for utilization in cellular mechanisms that drive outward actin extension.

M128
**Protrusion growth driven by myosin-generated force**
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Actin-based membrane protrusions such as filopodia, stereocilia, and microvilli are defining morphological features of animal cells, and support a range of biological functions including cell motility, adhesion, mechanosensation, and nutrient absorption. A central model of protrusion growth, derived from decades of investigation, indicates that actin polymerization produces the primary force to bend the membrane outward. However, force-generating myosins are also abundant residents of all types of protrusions and can interact directly and indirectly with the membrane. Whether myosin-generated forces contribute to protrusion growth through these membrane interactions remains an unanswered question. To test this concept, we devised a drug-inducible system that gives us switchable control over the interaction between myosin motor domains and the plasma membrane. Strikingly, we found that the application of myosin-generated forces to the membrane is sufficient to drive robust protrusion growth. Using this system, our data reveal a mechanism for protrusion growth that is independent of both the cargo-carrying function of myosin and supporting actin-nucleating machinery. Additionally, we observed that different modes of membrane interactions (i.e. integral vs. peripheral) support protrusion growth albeit with varying efficiencies. Finally, we showed that multiple classes of myosin motor domains are able to drive protrusion growth in various cell types. Here, we present a novel system that provides temporal control over protrusion growth, and in turn uncover a mechanism for protrusion growth dependent on myosin-generated force.

M129
**Macrophage cortical actin counteracts tissue resistance**
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The infiltration of immune cells into tissues underlies the establishment of tissue resident macrophages, and responses to infections and tumors. Yet the mechanisms immune cells utilize to negotiate tissue barriers in living organisms are not well understood. A role for cortical actin has also not been previously investigated. Here we find that the tissue invasion of *Drosophila* macrophages, also known as plasmatocytes or hemocytes, utilizes enhanced cortical F-actin levels stimulated by the *Drosophila* member of the fos proto oncogene transcription factor family (Dfos, Kayak). RNA sequencing analysis and live imaging show that Dfos enhances F-actin levels around the entire macrophage surface by increasing mRNA levels of the membrane spanning molecular scaffold tetraspanin TM4SF, and the actin cross-linking filamin Cheerio. Both the filamin and the tetraspanin are themselves required for invasion and enhance the cortical localization of the formin Diaphanos, arguing that these may be previously
unrecognized regulators of formin activity. This effect on Dia to increase cortical actin is a critical function as expressing a dominant active form of Diaphanous can rescue the Dfos Dominant Negative macrophage invasion defect. In vivo imaging shows that Dfos enhances the efficiency of the initial phases of macrophage tissue entry. Genetic evidence argues that this Dfos-induced program in macrophages counteracts the constraint produced by the tension of surrounding tissues and buffers the mechanical properties of the macrophage nucleus from affecting tissue entry. We thus identify strengthening the cortical actin cytoskeleton through Dfos as a key process allowing efficient forward movement of an immune cell into surrounding tissues.

M130
A Local Ca²⁺ and RhoA Signaling Crosstalk Facilitates Cell Migration by Reinforcing Actin Network in the Lamellipodia
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Cell migration underlies a wide range of physiological processes from embryonic development to tissue regeneration. During migration, cells integrate different signaling networks, such as small GTPases and Ca²⁺-dependent signaling, to organize the actin cytoskeleton and coordinate the cell front and back. In the cell front, the actin cytoskeleton assembles into a dense meshwork, known as a lamellipodium, which is imperative for environment sensing and fast migration. Although lamellipodia assembly is known to require Ca²⁺-signaling, the underlying mechanisms remain elusive. We first identified a mechanosensitive Ca²⁺-permeant channel, TRPV4, as a positive regulator of lamellipodia protrusion by screening a panel of siRNAs targeting ion channels in cell spreading assays. We found that TRPV4 is responsible for the Ca²⁺ influx in protruding lamellipodia. Interestingly, we also found that the C-terminal actin binding domain of TRPV4 is essential for mediating the Ca²⁺ influx in lamellipodia. Suppression of TRPV4 activity decreased the density of filamentous actin (F-actin) in lamellipodia, indicating that Ca³⁺ influx via TRPV4 promotes actin assembly and such Ca²⁺ influx is mediated by the TRPV4/actin interaction. To understand how TRPV4-mediated Ca²⁺ signaling facilitates F-actin assembly, we interrogated the involvement of small GTPases, the key regulators of actin dynamics, by using FRET biosensors. We found that RhoA activity peaked in protruding lamellipodia, which coincided spatially with the Ca²⁺ influx. The local upregulation of RhoA activity was dampened upon TRPV4 inhibition. Suppressing RhoA or formins also decreased F-actin density to a similar level as TRPV4 inhibition, suggesting that TRPV4-mediated Ca²⁺ signaling promotes actin assembly by maintaining a high RhoA activity. By immunostaining we showed that suppression of TRPV4 decreased the amount of active Ca²⁺-calmodulin-dependent protein kinase II (CaMKII), a key Ca²⁺ signaling hub, in lamellipodia. Furthermore, we showed that local inhibition of CaMKII halted cell protrusions, suppressed RhoA activity and decreased F-actin density in lamellipodia, suggesting that CaMKII transduces local Ca²⁺ signals from TRPV4 to RhoA. Finally, we showed that suppression of this new signaling axis suppresses cell protrusive activity and migration in a tissue-mimicking microenvironment. Together these data elucidate a novel Ca²⁺ signaling axis composed of TRPV4/CaMKII/RhoA that reinforces lamellipodial actin network, facilitates lamellipodial protrusions, and promotes cell migration.
M131
Actomyosin ring architecture during cell wound repair requires the coordinated action of linear and branched actin nucleation factors
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During cellular wound repair, actin and other cytoskeletal proteins act in concert to coordinate wound closure in a timely fashion. A critical step is to form an actomyosin ring that contracts to close the wound. This process is dynamically regulated in part by Rho family GTPases, which through their different effectors can regulate both linear and branched actin nucleators to facilitate cytoskeletal dynamics. We have previously shown that knockdown of the formin Diaphanous, a linear actin nucleator and downstream effector of Rho, results in prolonged wound closure and the inability to form a proper actomyosin ring. However, when all linear nucleation factors are removed, some actin organization persists. We investigated the contribution of branched nucleation factors to cell wound repair. We show that the Wiskott-Aldrich Syndrome (WAS) protein family members (WASp, Wash, and SCAR), and their cofactor Arp2/3, play non-redundant roles in regulating actin organization and architecture of the contractile actomyosin ring. Notably, we observe stark differences in the spatial and temporal recruitment patterns to wounds among the WAS proteins during the wound repair process. In particular, WASp is recruited early, SCAR is recruited later, and Wash is present throughout the process. Further, individual knockdown of the WAS proteins resulted in different actomyosin ring architectures, including differences in mesh density and filament orientation. Dynamically, WASp knockdown exhibits slower contraction whereas SCAR contracts faster and Wash contracts similarly to wildtype. Interestingly, in the absence of branched actin, wounds exhibited an abundance of elongated linear filaments, as well as other unique actin structures, which colocalize with Diaphanous. When both linear and branched nucleation factors are knocked down, we observe a significant decrease of these elongated filaments and the absence of previously seen actin structures. We also inhibited myosin activity in an Arp3 knockdown and observed a surprising wound repair phenotype consisting of spiraling linear filaments and excessive actin bundling. Our results emphasize the strong requirement for balance and crosstalk among linear and branched actin nucleators, as well as myosin, to facilitate proper actin filament architecture, organization, and dynamic contractile closure of the actomyosin ring during cell wound repair.

M132
Rho GTPase Signaling Dynamics in Migrating and Stationary Cells
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Cell migration plays a critical role in processes such as angiogenesis, wound healing and morphogenesis. External cues, such as chemical and mechanical signals from the environment or from neighbouring cells, direct cell migration. Rho GTPases are signal transducers that mediate the cytoskeletal reorganization necessary for morphological polarization and protrusive activity at the leading edge of migrating cells. How patterns of Rho GTPase activities result in the various shape and movement phenotypes adherent cells display remains incompletely understood, in part due to the highly heterogeneous behaviors of cells plated on uniform 2D substrates. To contrast the dynamics of Rho GTPases activities in migrating and stationary cells, we used endothelial cells expressing Förster Resonance Energy Transfer (FRET)-based biosensors for Rho GTPases plated on micropatterned extracellular matrix (ECM) substrates. By restricting, enhancing, or controlling cell motile behavior
through defined substrate geometries, we overcome the challenge of heterogeneous cell behavior. This enabled parallel measurements of both local Rho GTPase and cell edge dynamics during reproducible cell motility behaviors. We found that the patterns of local edge velocity and Rho GTPase activities were strikingly different for migrating and stationary cells. While stationary cells showed pulsatile Rho GTPase and cell edge dynamics, migrating cells had a relatively stable protrusion at the front and retraction at the rear, as well as stable gradients of different Rho GTPases. Further, we found that cell spreading area and geometry are intimately linked to cell motile behaviour. Cells which migrate on patterns of a given width tend to have significantly smaller aspect ratios than those that fail to polarize and migrate. Finally, we found that confining cells on circular micropatterns with different areas lead to them exhibiting different types of protrusive activities, including lamellipodia and membrane blebs. Thus, the use of FRET-based biosensors in conjunction with micropatterned ECM allows us to characterize the activity of Rho GTPases required for cell migration with high spatio-temporal resolution. Elucidating the activity of Rho GTPases and their downstream effectors in cell migration will provide important insight into the molecular events controlling cell polarization and migration.

In the Neuron

M133

Neuronal polarity requires an endocytic clearance mechanism in the axon initial segment

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Neurons are highly polarized cells that face a fundamental challenge of compartmentalizing a vast and diverse repertoire of proteins to function properly. The axon initial segment (AIS) is a critical region between the axon and dendrites that acts as a selective filter for intracellular vesicles and a diffusion barrier to establish and maintain neuronal polarity. However, it remains unclear how the AIS maintains the stringent polarity between the connected axonal and dendritic compartments over the decades-long lifetime of the neuron. We uncovered a conserved and widespread endocytic clearance mechanism in the AIS that is essential for maintaining receptor compartmentalization and neuronal polarity. We find that axonally and dendritically polarized transmembrane receptors that diffuse into the AIS are recognized by clathrin-mediated endocytic machinery, endocytosed, and targeted to late endosomes for degradation. The AIS is a specialized region previously thought to lack significant endocytosis due to its dense submembranous cytoskeletal network and the study of AIS-resident proteins that accumulate in the AIS through inhibited endocytosis. However, we find that axonally and dendritically polarized receptors colocalize with clathrin-coated pits in the AIS and display characteristic features of endocytic structures. Inhibiting endocytosis causes polarized receptor mislocalization as well as subsequent morphological and behavioral deficits. Thus, endocytosis is an active mechanism to capture and remove axonal and dendritic proteins from the AIS to maintain their compartmentalization. Forcing receptor interaction with the AIS master organizer, ankyrinG, prevents receptor endocytosis, causes receptor accumulation in the AIS, and weakens receptor compartmentalization. Therefore, specificity of this pathway is controlled by a balance between interactions with the endocytic machinery and AIS architecture: receptor clearance is promoted by endocytic machinery interaction and inhibited by ankyrinG interaction. Our results elucidate a previously unrecognized endocytic clearance mechanism in the AIS that is essential for neuronal polarity and define a framework for understanding AIS endocytosis. This endocytic clearance mechanism works in concert with known polarity mechanisms of the AIS, such as the cytoplastic vesicle filter and diffusion barrier, to maintain polarity over the long lifespan of the
neuron. Through the study of one of the most polarized cell types, the present results reveal a mechanism by which cells can achieve strict compartmentalization even along a contiguous membrane region.

M134
Chemico-genetic Discovery of Inhibitory Synaptic Protein Phosphorylation In Vivo
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Phosphorylation has been studied for decades as one of the most efficient mechanisms to transduce temporally and spatially relevant activity-dependent information. Most studies show phosphorylation is an essential component of signal transduction at glutamatergic excitatory post-synapses in the brain, especially during plasticity/learning and memory. However, it was only recently appreciated that inhibitory GABAergic post-synapses (iPSDs) on principle cells also undergo activity-dependent plasticity like their excitatory counterparts. As such, limited studies have shown a handful of canonical iPSD proteins are phosphorylated during plasticity. While this supports phosphorylation as a likely mechanism underlying signaling during inhibitory synaptic plasticity, an in-depth analysis of how phosphorylation is coupled to inhibitory synapse structure and function has not been attempted or even possible. Moreover, it is still unclear which kinases are present and therefore act at iPSDs. Thus, the inhibitory synaptic phospho-proteome remains elusive, which is a significant barrier to understanding how activity modifies inhibition important for shaping experience-dependent brain function. We will present novel methodology combining Cre-dependent in vivo proximity biotinylation (iBioID) with phospho-proteomics to isolate forebrain principle cell iPSDs. This is, to our knowledge, the first combination of both cell-type specific and proximity-based phospho-proteomics. Our approach enabled us to identify ~6,000 phospho-peptides corresponding to ~1,400 phospho-proteins. Among these phospho-proteins, a subset of phospho-proteins with ~100 phospho-sites is enriched at iPSDs. Our identified inhibitory phospho-proteome forms a dense and highly interconnected network, enriched for synaptic scaffolds, receptors, cell adhesion complexes, kinases, and signaling molecules. We will present ongoing work that incorporates the discovery of activity-dependent phosphorylation and exploration of iPSD kinase-substrate relationships. Overall, the combination of these conceptually and technically innovative approaches have revealed and continue to provide promising new mechanisms to understand iPSD function and organization, especially during plasticity.

M135
Neurexin localization and presynaptic assembly functions are mediated by intracellular interactions
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Synaptic cell-adhesion molecules (sCAMs) are thought to mediate the formation of neuronal circuits by recognizing appropriate trans-synaptic partners and initiating the assembly of pre- and post-synaptic specializations. Of the sCAMs, neurexins are among the most extensively studied, and all neurexin family members have been identified as risk factors for autism and other neurodevelopmental disorders. The human genome contains three neurexin genes which, through alternative splicing, encode ~4000 long (α), medium (β) and short (γ) isoforms that differ in their extracellular domain but retain an identical
intracellular domain. The *C. elegans* genome contains a single neurexin gene nrx-1, which is expressed only as several long (α) and short (γ) isoforms that also share a common intracellular domain. We have previously shown that in *C. elegans* the short (γ) isoform of NRX-1 lacking any canonical extracellular binding domains nonetheless localizes to presynaptic active zones and regulates presynaptic maturation and stability (Kurshan, 2018). We now find that expression of the intracellular domain alone, targeted to the membrane with a myristoylation sequence, can rescue the nrx-1 null phenotype (although a cytosolic version does not), indicating that the membrane-bound NRX-1 intracellular domain is sufficient for mediating its presynaptic assembly functions. To evaluate the purpose of the short nrx-1 isoform, and whether nrx-1 isoforms are differentially expressed across the *C. elegans* nervous system, we generated promoter reporters for the short (γ) and long (α) isoforms, and found differential expression patterns. We used the cell identification tool NeuroPal to determine the specific neurons in which these isoforms are expressed. In addition, we have generated long and short isoform-specific knockouts of nrx-1 to reveal the contribution of each isoform to presynaptic assembly. To identify mediators of NRX-1 localization at synapses, we performed a candidate screen of potential intracellular interactors using endogenously-tagged NRX-1 and identified the synaptic vesicle kinesin UNC-104, as well as active zone scaffold molecules SYD-1 and SYD-2. A deletion of the intrinsically disordered domain of SYD-2, shown previously to mediate its ability to undergo phase separation and thereby recruit additional active zone molecules, affected NRX-1 localization, implicating phase separation as a potential mechanism for NRX-1 recruitment to synapses. Together these results identify a hitherto unappreciated contribution of neurexin’s intracellular domain to its synaptic localization as well as to its function in presynaptic assembly, with implications for understanding neurexin-associated neurodevelopmental disorders.

M136

**The role of βII-spectrin in cerebellar circuitry**

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We recently reported de novo variants in *SPTBN1*, which encodes the cytoskeleton-associated protein βII-spectrin, as the genetic cause of a developmental delay syndrome that is comorbid with seizures, intellectual disability, ADHD, and autism spectrum disorder. Mice lacking βII-spectrin in the brain exhibit significant changes in cortical and cerebellar connectivity. However, the neuronal types and brain circuits that are most vulnerable to deficits in βII-spectrin function have been poorly studied. Given the emerging evidence of the involvement of the cerebellum in cognitive and social functions and epilepsy, we aimed to discern the cell-type specific functional and behavioral consequences of deficits in cerebellar βII-spectrin. Using novel, cerebellar cell type-specific βII-spectrin knockout mouse models, we found that βII-spectrin is highly enriched in the axons of cerebellar granule cells (GCs). Loss of βII-spectrin exclusively in these neurons resulted in hyperactivity, episodes of absence-like and tonic-clonic seizures, and deficits in motor function that worsened with age. These behavioral phenotypes were accompanied by progressive changes in cerebellar architecture, including thinning of the GC layer and reduced dendritic arborization of Purkinje neurons. Through electrophysiological, biochemical, and imaging studies conducted in cerebellar preparations and primary GC cultures, we determined that βII-spectrin loss alters the firing properties of GCs. Mechanistically, βII-spectrin deficiency in GC disrupted
the organization and macromolecular composition of axonal domains required for proper synaptic excitability and caused deficits in synaptic vesicle dynamics. Together, our data suggest a novel role for βII-spectrin in cerebellar circuitry, connecting potential disruption of GC excitability with cerebellar dysfunction and the clinical manifestations of the SPTBN1 syndrome. We will discuss insights into the processes through which βII-spectrin contributes to GC excitability and cerebellar function.

M137

Role of ER architecture in regulating synaptic properties in Drosophila motor neurons

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Axons contain a network of mainly smooth tubular ER. Several proteins with intramembrane hairpins that model ER membranes, of spastin, atlastin, REEP and reticulon families, help form this network; and mutations in them cause the axon degenerative diseases, hereditary spastic paraplegia (HSP). Axonal ER shows striking continuity over great subcellular distances, making it potentially a channel for long-range communication, and earning it the term "a neuron within a neuron". We want to understand how the specialized axonal ER architecture is formed, and influences axon maintenance and degeneration. In Drosophila we find an axonal ER network that is highly dynamic, whose amount, continuity, and tubule diameter, is determined by ER-shaping HSP proteins. Some mutants show sporadic disruption of continuity; this is a plausible pathomechanism of HSP, that could explain preferential susceptibility of long axons. We propose homeostatic mechanisms to maintain optimal continuity, density and connectivity of axonal ER. To dissect the processes that form and maintain the axonal ER network, we have developed a strategy for forward genetic screening in Drosophila. To understand the physiological roles of ER architecture and how these might influence neurodegeneration, we have asked how alterations in this architecture affect presynaptic physiology at the larval neuromuscular junction. We find that Rtnl1 mutants show loss of tubular but not cisternal ER in presynaptic motor terminals, and generally lower ER luminal, cytosolic, and mitochondrial calcium fluxes during repetitive stimulation, in ways that appear mostly independent of bulk effects of ER calcium flux. Presynaptic function could therefore also be a potential target of HSP, perhaps via effects of Ca²⁺ such as regulation of ATP synthesis. We have also investigated the implications of another feature of axonal ER, its tiny diameter: using mutations that increase ER tubule diameter, we find that their tiny wild type diameter is limiting for luminal protein diffusion; this constrained lumen continuity appears paradoxical given the striking continuity of its membrane. The tools and approaches that we have developed will allow further dissection of the formation and roles of axonal and presynaptic ER architecture in an in vivo system.

M138

Inpp5k and Atlastin-1 maintain the non-uniform distribution of endoplasmic reticulum-plasma membrane contacts in neuron

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In neurons, the endoplasmic reticulum (ER) extends throughout all cellular processes, forming multiple contacts with the plasma membrane (PM). Growing evidence suggests that ER-PM contacts contribute to general cell physiology, as well as to the unique functional properties of neurons, thereby fine-tuning neuronal physiology. However, the mechanisms that regulate the distribution of neuronal ER-PM
contacts remain elusive. In this study, we used the *C. elegans* DA9 motor neuron as our model system to explore the molecular mechanisms that maintain the distribution of neuronal ER-PM contacts. We developed a novel strategy for visualizing ER-PM contacts in live *C. elegans* neurons using a split GFP approach, and found that neuronal ER-PM contacts are highly enriched in the somatodendritic region and generally absent from the axon. Using a forward genetic screen, we identified that two proteins involved in ER shaping, namely the dynamin-like GTPase ATLN-1 (human Atlastin-1) and the inositol 5-phosphatase CIL-1 (human INPP5K), help to maintain the non-uniform, somatodendritic enrichment of neuronal ER-PM contacts. Genetic and cell biological assays revealed that CIL-1 acts upstream of ATLN-1 to maintain the balance between tubules and sheets of the ER at cell cortex and restrict the distribution of ER sheets to somatodendrites. In mutants with reduced activities of CIL-1 or ATLN-1, ER sheets expand and invade into the axon. This was accompanied by the ectopic formation of axonal ER-PM contacts as well as major defects in axon regeneration following laser-induced axotomy. Mutations in Atlastin-1 and INPP5K have been linked to various neurological disorders, including hereditary spastic paraplegia and intellectual disability. Thus, the unique distribution of neuronal ER-PM contacts maintained by INPP5K and Atlastin-1 may support neuronal resilience during the onset and progression of these human disorders. *These authors contributed equally to this work.*

**Multiplexed Analysis of Signaling in Diverse Tissue Architectures**

M139

**Non-canonical Wnt signaling promotes directed migration of intestinal stem cells to sites of injury**

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Adult stem cells play important roles in the maintenance and repair of high-turnover tissues, and accurate stem cell positioning is crucial to facilitate localized integration of new cells into damaged epithelia. The extent in which stem cells can migrate in different adult tissue and the mechanisms regulating this behavior, however, is poorly understood. In the adult *Drosophila* intestine, we demonstrate by wholemount *ex vivo* live imaging that intestinal stem cells (ISCs), while largely static under homeostatic conditions, undergo dramatic lamellipodia-dependent cell migration after intestinal damage by bacterial infection or laser ablation. In particular, when localized injury is created by laser ablation, ISC migration is polarized towards the wound. We find that ISC migration is actin-dependent and regulated by non-canonical Wnt signaling, and that these processes are coordinated by nearby enteroendocrine cells (EEs). In response to injury, the metalloprotease, Mmpl1, is activated in EEs to cleave the N-terminal, extracellular domain of the Ptk7 orthologue, Otk. This extracellular release of Otk non-autonomously promotes protrusion formation in ISCs, and is required for ISC migration. We find that ISC migration is closely linked with ISC proliferation, and inhibiting migration not only decreases mitotic activity of ISCs, but also impairs the overall regenerative capability of the *Drosophila* intestine after damage, demonstrating that stem cell migration is required for efficient repair of adult tissue. Altogether, our data deepen our understanding of the mechanisms regulating stem cell migration, and provide insight into how different cell types coordinate to ensure proper tissue regeneration.
Crosstalk Between DBL-1/BMP Signaling and Cuticle Collagen Genes in C. elegans Body Size Regulation

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The regulation of growth and body size is a fundamental property of animal development, and misregulation of these traits is associated with many diseases and disabilities. At the organismal level, growth depends on cell growth, cell division, and the extracellular matrix (ECM), which interact in complex and incompletely understood ways within and between tissues. In the nematode C. elegans, DBL-1, a member of the TGF-β superfamily most closely related to BMP2/4, is a major determinant of body size. In our studies addressing how BMP signaling regulates body size, we identified cuticle collagen genes as both downstream effectors and upstream regulators of the BMP signaling pathway. Cuticle collagens are the most abundant components of the external cuticle, an apical acellular ECM that serves as the outermost layer of the animal’s skin. First, we investigated the transcriptional network through which the DBL-1/BMP pathway regulates body size and identified cuticle collagen genes as effectors of growth control. We demonstrated that DBL-1-regulated cuticle collagens can act as positive regulators (col-41), negative regulators (col-141), or dose-sensitive regulators (rol-6) of body size. Genetic interactions are consistent with these collagen genes acting downstream of DBL-1/BMP for body size regulation. Next, we asked whether depletion of DBL-1-regulated cuticle collagens could modulate DBL-1/BMP signaling in a feedback circuit. We find that depletion of each of these cuticle collagen genes reduces DBL-1/BMP expression in ventral cord motorneurons. We hypothesize that the types of collagens present in cuticle confer specific structural and mechanical properties, and that these properties impinge on body size regulation and feedback regulation of BMP ligand expression. Intriguingly, feedback regulation occurs in spite of the physical separation of the cuticle from the BMP ligand-expressing motor neurons, implying the existence of contact-independent regulation of gene expression. We are therefore testing the hypothesis that biomechanical inputs regulate DBL-1/BMP signaling. We are testing the role of mechanosensitive ion channels and transcription factors expressed in DBL-1-producing motor neurons. We also used Atomic Force Microscopy (AFM) in intact animals to determine how the structure and mechanics of the cuticle differ in genotypes with cuticle collagen mutations. In summary, C. elegans provides a unique in vivo model to study bidirectional interactions between cell signaling and the ECM in the context of the intact organism. We hypothesize that these reciprocal interactions permit robust yet environmentally-responsive control of body size.

Piezo ensures robust tissue size regulation by balancing proliferation, cell size, anisotropy and cell death

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Title: Piezo ensures robust tissue size regulation by balancing proliferation, cell size, anisotropy and cell death
Authors: Nilay Kumar*, Megan Levis*, Mayesha Sahir Mim, Maria Unger, Jeremiah Zartman*Co-first authorsMechanisms ensuring robustness in organ size regulation are critical for proper development. In epithelial tissues, mechanosensitive Piezo channels are critical for maintaining homeostasis through regulations of cell division and apoptosis. Stretch activation of Piezo triggers cell proliferation. It has been hypothesized that calcium spiking from Piezo activates ERK signaling and
induces a G2-to-M transition during cell division. However, the tissue-level mechanistic and functional roles of Piezo during development remain unknown. Here, we have combined pharmacological and genetic approaches to study Piezo’s roles in regulating the development of the *Drosophila* wing imaginal disc, an excellent model organism for epithelial organ development. We investigated the combined outcomes on tissue size, shape and mechanical properties. We found that Piezo overexpression increases the relative concentration levels of cytoskeletal integrin and non-muscle myosin-II leading to increased tissue curvature. Both genetic overexpression and pharmacological activation of Piezo increased proliferation and apoptosis in the tissue. Surprisingly, calcium signaling activity was blocked when Peizo was either overexpressed or knocked down but was increased with acute pharmacological activation. We hypothesize that this loss of calcium activity is due to desensitization of the Piezo channel. Further, knockdown of Piezo increased the numbers of cell neighbors, increased cell area, and decreased cell anisotropy. Piezo overexpression slightly increased the number of cell neighbors and cell area. This highlights the critical role of Piezo in regulating overcrowding within the epithelia during organ growth. In sum, these results support Piezo as a key regulator of epithelial homeostasis through a feedback mechanism that regulates the balance of forces within the epithelium. The outcomes of this feedback loop contribute to the robustness of organ size regulation.

M142  
Identification and functional characterization of rare post-mitotic cell states induced by injury and required for whole-body regeneration in *Schmidtea mediterranea*  
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Regeneration requires coordination of stem cells, their progeny, and distant differentiated tissues. Here, we present a comprehensive atlas of whole-body regeneration in *Schmidtea mediterranea* and identify wound-induced cell states required for tissue repair. Analysis of 299,998 single-cell transcriptomes captured from regeneration-competent and regeneration-incompetent fragments identified transient regeneration-activated cell states (TRACS) in the muscle, epidermis, and intestine. TRACS were stem-cell-division-independent with distinct spatiotemporal distributions and RNAi depletion of TRACS-enriched genes produced regeneration defects. Muscle expression of *notum*, *follistatin*, *evi/wls*, *glypican*-1, and *junctophilin*-1 was required for tissue polarity. Epidermal expression of *agat*-1, *agat*-2, *agat*-3, *cyp3142a1*, *zfhx3*, and *atp1a1* was required for stem cell proliferation. Finally, expression of *spectrin*-B and *atp12a* in intestinal basal cells and *lrrk2*, *cathepsin*-B, *myosin1*e, *polybromo*-1, and *talin*-1 in intestinal enterocytes was required for stem cell proliferation and tissue remodeling, respectively. Together, our results identify cell types and molecules important for regeneration and indicate that regenerative capacity can emerge from coordinated transcriptional plasticity across all three germ layers.
M143
Airgel: a tissue-engineered airway for the investigation of respiratory infections
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Mucociliary clearance is an essential defense mechanism of the airway epithelium. It comes from the coordinated beating of ciliated cells that transports mucus upward in the respiratory tract, thereby eliminating pathogens or harmful particles trapped in the mucus layer. However, a detailed understanding of how clearance arises and how defects in this process favor respiratory infections is still lacking. Current model systems to study mucociliary clearance are limited: conventional cell culture systems lack the 3D architecture and extracellular matrix of the airway epithelium; animal models do not allow for studies with high spatiotemporal resolution; organoids provide an alternative, but because of their closed geometry, they do not permit studies of clearance at the air-liquid interface. To solve this issue, we engineered a novel organoid-based airway model called AirGel. It is composed of a custom hydrogel scaffold patterned into a cylindrical cavity, all embedded in a microfluidic chip. To grow a tube-shaped airway, we line the surface of this cavity with primary human bronchial epithelial cells. We can then establish an air-liquid interface in the lumen, thereby stimulating differentiation. After a few weeks, AirGels contain major airway cell types, recapitulating the respiratory tract’s histological signature. We estimated goblet and ciliated cells to each account for about 15% of all cell types in our model, while in vivo they constitute about 10% and 50% of the epithelium, respectively. Additional optimization of culture conditions is ongoing to increase the proportion of ciliated cells. Moreover, to validate these tissue-engineered organoids as an airway model, we performed a series of biophysical characterization experiments with a focus on clearance. We found that cilia beat at a physiologically relevant frequency and are able to transport secreted mucus unidirectionally along the epithelial surface in AirGels. Owing to their transparent scaffold, AirGels permit live microscopy at high spatiotemporal resolution. We fully leveraged this to investigate the mechanisms of infection by the opportunistic pathogen Pseudomonas aeruginosa. By combining lectin-based staining of secreted mucus and timelapse microscopy, we visualized the clearance and motility of bacteria trapped in mucus. At longer timescales, we also investigated biofilm formation in cystic fibrosis airways: over a few hours, bacterial aggregates formed on stagnant mucus. Overall, these results highlight the potential of AirGels to model acute and chronic infection in the airway. We foresee that AirGel will provide the community with a tool enabling a deeper understanding of the respiratory tract, from global health, developmental biology and cell biology perspectives.

M144
Intestinal organoids for automated screening assays. High content imaging and analysis of organoid morphology.
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3D cell models representing various tissues were successfully used for studying complex biological effects, tissue architecture, and functionality. However, complexity of 3D models remains a hurdle for the wider adoption in research and drug screening. We describe the automated integrated cell culture and high-content imaging system that allows automated monitoring, maintenance, and characterization of organoids. The integrated system included IXM-C HT.ai confocal imaging system, automated CO2 incubator, automated liquid handler (Biomek i7), as well as Collaborative Robot. We
developed methods for automation of cell seeding, media exchange, as well as monitoring development of intestinal organoids. In addition, method allows automation of compound testing and evaluation of toxicity effects. 3D intestinal organoids were developed from primary mouse intestinal cells cultured in Matrigel. Cells were cultured in a media that promotes the formation of 3D structures recapitulating morphological and functional characteristics of intestine. Organoids self-organized and developed into the complex morphology resembling intestinal crypt formation. Using liquid handling system allowed automated seeding cell in Matrigel droplets into 24 well plates, followed by an automated media addition and media exchanges. Organoids were monitored using imaging in transmitted light. Then machine learning-based image analysis allowed detection of organoids and characterization of their size, diameter, and density. For endpoint measurements organoids were then stained with fluorescently labeled antibodies or viability dyes and imaged using automated confocal imaging system. Advanced image analysis allowed 3D reconstitution and complex phenotypic evaluation of organoid structures, including characterization of organoid size and complexity, cell morphology and viability, as well as determining presence and expression levels for differentiation markers. We characterized multiple quantitative descriptors that could be used for studying disease phenotypes and compound effects. 3D image analysis provided quantitation of the organoids number, size distribution, complexity, cell content, viability, volumes, as well as quantitation of cell proliferation and expression of specific markers. We demonstrated concentration-dependent effects of several compounds known to cause toxicity (doxorubicin, cisplatin, taxol). Organoids were also evaluated for responses to TNFa and other inflammatory cytokines. Described methods demonstrate the tools for increase of throughput and automation of organoid assays and compound screening, and also propose analysis approaches that allow to gain information about complex systems, disease phenotypes and compound effects.

Quality Control and Stress Signaling

**M145**

**Unraveling Fundamental Mechanisms of Uromodulin Quality Control and Their Role in Uromodulin-associated Chronic Kidney Disease**

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UMOD is an abundant glycosylphosphatidylinositol (GPI)-anchored protein exclusively expressed in the kidney. When properly folded, UMOD transits from the ER to the apical membrane via the Golgi apparatus. **Little is known about the specific trafficking binding partners of UMOD and the quality control mechanisms operative in the early secretory pathway.** Mutations in UMOD cause UMOD-related autosomal-dominant tubulointerstitial kidney disease (ADTKD-UMOD), characterized by slowly progressive Chronic Kidney Disease (CKD). Mutations disrupt protein folding and promote ER retention, triggering ER-stress pathways and eventually cell death. The detailed mechanisms responsible for
aberrant UMOD protein quality control, are not known. It was recently demonstrated that mutation in another kidney protein, MUC1, leads to entrapment in TMED9-containing vesicles reversible by treatment with the compound BRD4780. **We hypothesize that a similar pathogenic quality control mechanism may be active in ADTKD-UMOD.** Localization and interactomes of UMOD and TMED cargo-receptors was assessed in HEK-293 cells co-transfected with wild type (wt) or mutant (C126R) human UMOD. We also conducted *in vivo* studies in heterozygous (UMOD^{+/-}C125R) and homozygous (UMOD^{C125R/C125R}) mice (homologous to human C126R). The interactomes of wt and mutant UMOD were defined by quantitative mass spectrometry-based affinity proteomics. Several interactors, including members of the TMED family, were enriched in the mutant UMOD interactome. Protein complex immunoprecipitation (Co-IP) in lysates of HEK293 cells co-transfected with wt or mutant UMOD and interacting protein candidates confirmed the results from proteomics studies. Interestingly, when pulling down TMEDs, we found an abundant immature non-glycosylated form of the mutant protein, suggesting that immature UMOD is entrapped in early secretory compartments. *In vivo*, treatment with BRD4780 lead to the apparent disrupted interactions between mutant UMOD and interacting partners which led to the release of UMOD from the early secretory pathway as assessed by Western Blot of kidney lysates and immunofluorescence microscopy of tissue from wild-type, UMOD^{+/-}C125R and UMOD^{C125R/C125R} mice. In summary, **our results suggest that UMOD interacts with the TMED family of cargo-receptors and other proteins that may mediate the pathogenic quality control mechanisms responsible for toxic ER-retention and accumulation.** Shedding light on these new molecular mechanisms may unmask new therapeutic strategies for the treatment of ADTKD-UMOD.

M146
**A combined mechanism for maintenance of skeletal muscle integrity and bioenergetics**

**K. M. Voos,** J. Tzeng, H. Choi, T. Pharr, D. Lorenzo; Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Ankyrin-B (AnkB) is a ubiquitously expressed cytoskeletal-associated protein that positions and stabilizes cell adhesion molecules, membrane transporters, and ion channels at specialized membrane microdomains. Human AnkB variants have been associated with an increased risk for obesity and type-2 diabetes. When expressed in knock-in mice, these variants lead to age- and diet-dependent metabolic dysfunction and AnkB deficiency, of which skeletal muscle is a primary target. However, the extent to which AnkB deficits in skeletal muscle impacts skeletal muscle energy capacity and metabolic homeostasis is unknown. Here, we uncovered novel dual roles of AnkB in skeletal muscle maintenance and bioenergetics. Using a conditional knockout mouse, we determined that skeletal muscle-specific loss of AnkB leads to decreased mouse activity, as assessed by indirect calorimetry, and to transcriptional changes in critical energy-sensing pathways. These mice also fatigued more quickly than littermate controls when subjected to non-voluntary endurance exercise. Our proteomics and biochemical analysis of the AnkB interactome in skeletal muscle revealed that AnkB forms complexes with several mitochondrial proteins and with components of the translation machinery, including elongation initiation factors and ribosomal proteins. Consistent with these findings, skeletal muscle fibers from AnkB knockout mice showed substantial disorganization of the translational machinery, whereas transmission electron microscopy of AnkB-deficient skeletal muscle uncovered an imbalance in mitochondrial fusion/fission events. Together, our data highlights a dual role of AnkB in skeletal muscle metabolism and maintenance that may contribute to energy deficits and reduced health of AnkB-deficient skeletal muscle, and impact systemic metabolism. We will discuss insights into the processes
through which AnkB contributes to the formation of specialized membrane domains that modulate critical homeostatic processes in skeletal muscle.

M147

**Mitochondrial ROS signaling enables repair of injured cells and its disruption contributes to disease onset in LGMD2B**

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Mutations leading to the loss of the C2 domain containing protein dysferlin cause limb girdle muscular dystrophy type 2B (LGMD2B). The loss of dysferlin persistently impairs cellular calcium handling and lysosome fusion, and leads to poor repair of skeletal muscle cells. However, despite the existence of these cellular pathologies, LGMD2B patients do not manifest clinical symptoms until late adolescence. The mechanism of delayed pathogenesis in LGMD2B remains enigmatic, which has hampered therapeutic development. To decipher the delayed onset in LGMD2B, we compared the repair ability of myofibers from young (asymptomatic) and old (symptomatic) dysferlin-deficient mice, and found that myofiber repair is increasingly compromised with disease progression. To investigate the cellular mechanisms for the progressive membrane repair deficit and delayed onset of clinical symptoms in LGMD2B muscles we assessed proteomic changes. For this study, we performed whole muscle proteomics for longitudinal (same muscle over time) and cross-sectional (differentially affected muscles at the same age) differences in in healthy and LGMD2B mouse muscles. These analyses identified upregulation of proteins involved in vesicle trafficking and membrane repair and simultaneous downregulation of mitochondrial electron transport chain (ETC) subunits, without a loss of cellular mitochondrial content. Mitochondrial ROS signaling is activated by injury and is required for sarcolemmal repair, but reduced mitochondrial ETC capacity in LGMD2B muscles reduced injury-triggered mitochondrial ROS production, creating a second hit that exacerbates the repair deficit in dysferlin-deficient myofibers. This deficit can be phenocopied pharmacologically by cytosolic accumulation of mitochondria-derived ROS, providing a comprehensive model for LGMD2B disease onset. According to this model, progressive mitochondrial deficit is the second hit (in addition to the primary dysferlin deficiency), which causes an insurmountable loss of myofiber repair capacity leading to onset of symptoms in LGMD2B. These insights into the onset of LGMD2B identify rebalancing of mitochondrial ROS production as a new therapeutic target to delay or prevent disease onset in LGMD2B.

M148

**Persistent Autophagy of p62 Clients in Quiescent Cells Limits Their Future Proliferative Potential**

A. Murley, K. Wickham, A. Dillin; Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

Reversible cell cycle arrest, known as cellular quiescence, is a feature of numerous cells, including many types of adult stem cells, hepatocytes, lymphocytes, and oocytes, and restricts their proliferation until appropriate cues, such as tissue injury, induce their re-entry into the cell cycle. Quiescent cells employ active mechanisms that limit the accumulation of cellular damage, such as reactive oxygen species, protein aggregates, and dysfunctional organelles, that can negatively influence their survival or reactivation. With organismal aging, however, regulation of cellular quiescence breaks down, often
resulting in the inability of quiescent cells to respond to proliferative cues and re-enter the cell cycle. Using starvation-induced developmental arrest of *C. elegans* as a model for quiescence, we found that mutations in genes functioning in the unfolded protein response of the endoplasmic reticulum (UPR\textsuperscript{ER}) lead to the inability of quiescent cells to re-enter the cell cycle after prolonged periods of arrest, indicating that the UPR\textsuperscript{ER} is one of the processes that protects quiescent cells and ensures their future proliferative potential. Surprisingly, we found in a genome-wide screen that null mutations in core autophagy genes *atg*-4.1 and *atg*-9, as well as mutations in other autophagy genes, strongly suppressed the progressive, terminal cell cycle arrest phenotype of UPR\textsuperscript{ER} mutants. Furthermore, a null mutation in *sqst*-1 (p62/SQSTM1), which functions as a receptor for the autophagic degradation of ubiquitinated proteins and organelles, also suppressed the progressive, terminal cell cycle arrest phenotype of UPR\textsuperscript{ER} mutants. We have expanded our studies to *in vitro* models of quiescence using human cells to dissect the mechanisms through which autophagy inhibition may, in certain contexts, protect quiescent cells and improve their future proliferative potential. These findings point to a complex interplay between the endoplasmic reticulum and lysosomal degradation pathways, including autophagy, in quiescent cells to ensure their future proliferative potential, paving the way towards interventions that may improve the regulation of quiescent cells throughout life.

**M149**

**Ubiquitination and deubiquitination of membrane proteins**

*M. Mariappan, J. A. Culver; Yale University, West Haven, CT.*

Numerous proteins that have hydrophobic transmembrane domains (TMDs) traverse the cytosol and posttranslationally insert into cellular membranes. It is unclear how these hydrophobic membrane proteins evade recognition by the cytosolic protein quality control (PQC), which typically recognizes exposed hydrophobicity in misfolded proteins and marks them for proteasomal degradation by adding ubiquitin chains. Here, we find that tail-anchored (TA) proteins, a vital class of membrane proteins, are recognized by cytosolic PQC and are ubiquitinated as soon as they are synthesized in cells. Surprisingly, the ubiquitinated TA proteins are not routed for proteasomal degradation but instead are handed over to the targeting factor, TRC40, and delivered to the ER for insertion. The ER-associated deubiquitinases, USP20 and USP33, remove ubiquitin chains from TA proteins after their insertion into the ER. We have recently found that mitochondria-targeted membrane proteins are also ubiquitinated in the cytosol and deubiquitinated at the outer membrane of mitochondria. Thus, our data suggest that all posttranslationally targeted membrane proteins are ubiquitinated in the cytosol and are deubiquitinated by membrane-associated deubiquitinases before insertion or translocation across membranes.

**M150**

**Human Signal Peptidase Complex Acts as a Quality Control Enzyme for Multipass Membrane Proteins**

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Ubiquitination and deubiquitination of membrane proteins

Numerous proteins that have hydrophobic transmembrane domains (TMDs) traverse the cytosol and posttranslationally insert into cellular membranes. It is unclear how these hydrophobic membrane proteins evade recognition by the cytosolic protein quality control (PQC), which typically recognizes exposed hydrophobicity in misfolded proteins and marks them for proteasomal degradation by adding ubiquitin chains. Here, we find that tail-anchored (TA) proteins, a vital class of membrane proteins, are recognized by cytosolic PQC and are ubiquitinated as soon as they are synthesized in cells. Surprisingly, the ubiquitinated TA proteins are not routed for proteasomal degradation but instead are handed over to the targeting factor, TRC40, and delivered to the ER for insertion. The ER-associated deubiquitinases, USP20 and USP33, remove ubiquitin chains from TA proteins after their insertion into the ER. We have recently found that mitochondria-targeted membrane proteins are also ubiquitinated in the cytosol and deubiquitinated at the outer membrane of mitochondria. Thus, our data suggest that all posttranslationally targeted membrane proteins are ubiquitinated in the cytosol and are deubiquitinated by membrane-associated deubiquitinases before insertion or translocation across membranes.
While a flurry of luminal and cytoplasmic quality control factors is known to target membrane proteins for degradation, not much is known on how the folding state of multipass membrane proteins is controlled. The ER Signal Peptidase Complex (SPC) is well-known for its function in cleaving off signal sequences from ER-targeted nascent chains of secretory and membrane proteins. In mammalian cells, it is found in two paralogous complexes, each formed by one of the catalytic subunits (SEC11A or SEC11C) and three accessory subunits (SPCS1, SPCS2 and SPCS3), whose function is still ill-defined. By means of an \textit{in silico} approach, we identified several multipass membrane proteins containing a cryptic SPC cleavage site at the N-terminus, despite not having a signal sequence, and even after internal transmembrane domains. Focusing on disease-linked mutations of these non-canonical SPC substrates, we observed that a large fraction is cleaved post-translocationally triggering degradation via an Hrd1-dependent ER-associated degradation (ERAD) pathway. Surprisingly, lack of the accessory subunit SPCS1 prevents this non-canonical cleavage, without affecting signal peptide cleavage of secretory proteins. Moreover, by means of compensatory stabilizing mutations and by redox stress, we demonstrate that protein misfolding and failed complex assembly is the cause of cryptic cleavage site exposure and subsequent SPC-mediated cleavage. We additionally reveal that SPCS1 is upregulated during ER-stress and helps cells coping with it. Finally, our comprehensive SPC substrate search reveals that SPC controls abundance of multiprotein complexes with key functions in secretome dynamics. All in all, our study brought to light a previously unanticipated role of the SPC in targeting proteins into ERAD, with a central role in substrate selection played by the accessory subunit SPCS1.

\textbf{Regulation of Mitosis}

\textbf{M151}

\textit{The Ndc80 complex coordinates with dynein for the initial kinetochore-microtubule capture during early mitosis}

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Kinetochore are initially captured by dynamically unstable microtubules via a ‘search-and-capture’ mechanism in mitotic prometaphase. The microtubule minus-end-directed motor, dynein, is critical for this process as it transports laterally attached chromosomes towards the spindle pole. After chromosome alignment in metaphase, the kinetochore-bound microtubule-binding complex, Ndc80, has been demonstrated to play a central role in stabilizing kinetochore-microtubule (k-MT) attachments. Ndc80 is recruited to kinetochores during early mitosis but it is not clear if this complex contributes to initial k-MT capture. By combining CRISPR/Cas9-mediated knockout approach and RNAi technology, we show that mitotic cells lacking Ndc80 exhibit severe defects in k-MT capture during prometaphase. Mitotic cells rescued with Ndc80 mutants that are deficient in microtubule-binding are unable to execute proper k-MT capture. We find that prometaphase cells inhibited of the dynein-dynactin complexes on the other hand, while they are able to make initial k-MT attachments are unable to execute poleward movement of the chromosomes, which remain tethered to the plus-ends of microtubules. Our studies, for the first time, identify two distinct stages of processive kinetochore capture in prometaphase: an initial dynein-independent stage, where Ndc80 activity is instrumental in initial k-MT attachments and the ensuing dynein-dependent stage, where poleward transport of kinetochores is initiated. Further, we find an interaction between dynein and Ndc80 in prometaphase suggesting a possible kinetochore ‘hand-over’ mechanism between these complexes, essential for
chromosome capture. In summary, our findings suggest a synergy between dynein and the Ndc80 complexes during initial kinetochore capture in prometaphase, which in turn is required for proper chromosome alignment and segregation.

M152

**Coordinated poleward flux of sister kinetochore fibers drives chromosome alignment**

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Chromosome alignment at the spindle equator promotes proper chromosome segregation and depends on pulling forces exerted at kinetochore fiber tips together with polar ejection forces. However, this is not a complete picture because kinetochore fibers are also subjected to forces that drive their poleward flux. Here we introduce a flux-driven centering model that relies on flux that is generated by forces within the overlaps of bridging and kinetochore fibers. This centering mechanism works so that when kinetochores are displaced, the longer kinetochore fiber fluxes faster than the shorter one, moving the kinetochores towards the center. We developed a speckle microscopy assay in human spindles and confirmed the key prediction that kinetochore fiber flux is length-dependent. Experiments together with theory indicate that kinetochores are better centered when the overlaps are shorter and the kinetochore fiber flux markedly slower than the bridging fiber flux. We identify Kif18A and Kif4A as overlap and flux regulators and NuMA as a coupler between the fibers. Thus, length-dependent sliding forces that the bridging fiber exerts onto kinetochore fibers promote chromosome alignment.

M153

**Nak associated protein 1 (NAP1) activates Tank binding kinase 1 (TBK1) to regulate mitosis.**

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Mitosis is a precisely regulated cellular mechanism which governs the fundamental processes like embryogenesis, development, and tissue regeneration. Prenatal mitotic errors can cause birth defects like neurodevelopmental disorders, congenital heart disease, and miscarriage, whereas postnatal abnormal cell division leads to early aging and cancer. Aberrant expression of the serine/threonine kinase, Tank Binding Kinase 1 (TBK1) is associated with several types of cancer such as glioblastomas, breast, and lung cancers. However, its role in the development of cancer or normal cell division has been understudied. Our lab and others identified that loss of TBK1 leads to a reduction in mitosis, cell proliferation, and the accumulation of multinucleated cells. Recruitment of activated TBK1 on the centrosomes of dividing cells is critical for proper mitotic progression. TBK1 activation depends on its ability to bind to an adaptor protein which causes its dimerization and trans-autophosphorylation of the kinase domain. As a multifunctional kinase involved in innate immune signaling, selective forms of autophagy, and mitosis, the subcellular location of the TBK1-adaptor complex dictates its function. Therefore, we sought to identify which adaptor protein was required for TBK1 activation during its role in cell division. Using a combination of shRNA mediated knockdown cell lines, editing of several TBK1 associated genes via CRISPR, and co-immunoprecipitation experiments, we identified the adaptor protein NAK Associated Protein1 (NAP1) to be responsible for activating TBK1 during mitosis. NAP1 KO
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cells displayed similar mitotic and growth defects phenocopying the loss of TBK1. Furthermore, we found that NAP1 is a cell cycle regulated protein mediated by proteasomal degradation. We also found that this NAP1-TBK1 interaction is important across multiple cell types including neural stem cell division. In conclusion, our study uncovers a distinct function of NAP1-TBK1 complex during cell cycle which otherwise has only been linked to the innate immune signaling.

M154
A mitotic stopwatch controlling G1 progression is frequently inactivated in p53-wildtype cancers

The tumor suppressor p53 is a guardian of the genome, protecting against the emergence of aneuploid, genomically unstable cells. However, nearly half of all cancers are p53-wildtype, and a significant proportion of these cancers nonetheless exhibit genomic aberrations. Here we suggest that this may be because although p53 is intact, these cancers have specifically inactivated a p53-dependent mechanism that monitors the amount of time that cells spend in mitosis. We show that prolonged mitosis triggers the formation of a complex between the p53-binding protein 53BP1 and the deubiquitinase USP28. Complex formation is controlled by the mitotic kinase PLK1 and acts as a stopwatch that measures time in mitosis; when mitotic time is greater than a specific threshold, the resulting daughter cells are fated to permanent G1 arrest or death. The mitotic stopwatch acts by stabilizing p53 to control expression of the cyclin-dependent kinase inhibitor p21 and exhibits multi-generational memory, with sub-threshold extensions of mitosis summing across sequential cell divisions. Surveying 20 different p53-wildtype cancer cell types to assess the status of the mitotic stopwatch revealed that it was partially or completely compromised in about 60-70% of the cell lines due to mutation of 53BP1 or USP28, partial suppression of the p53 pathway, or as-yet-unknown mechanisms, consistent with the idea that the mitotic stopwatch acts as a tumor suppressor that translates difficulty of mitotic progression into an alarm that triggers growth arrest. The status of the mitotic stopwatch also correlated with sensitivity to the mitotic inhibitor centrinone, which prolongs mitosis via depletion of centrioles. Cancer cell lines, such as neuroblastoma, that retain an intact stopwatch were highly sensitive to centrinone, suggesting that stopwatch status may have prognostic value in cancer therapy.

M155
The chromosomal passenger complex establishes chromosome biorientation via two parallel localization pathways
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In order to maintain a stable genome, cells have to segregate their chromosomes faithfully. During mitosis, sister chromatids align in the center of the cell and subsequently become attached to the microtubule-based spindle. It is crucial that sister chromatids are attached to microtubules from opposite spindle poles, a process called chromosome biorientation. The attachments between chromosomes and microtubules are mediated by a multi protein complex called the kinetochore. Microtubule-kinetochore attachments are established by a “trial and error” process until the two sister chromatids of each chromosome are bioriented. Due to the stochastic nature of chromosome biorientation, initially many erroneous kinetochore-microtubule attachments are formed, where both
sister chromatids are attached to microtubules emanating from the same pole. Such erroneous microtubule-kinetochore attachments are corrected by the four-member chromosomal passenger complex (CPC). The CPC phosphorylates outer kinetochore targets which leads to a destabilization of the erroneous attachment. During chromosome alignment, the CPC localizes to the inner centromere, the inner kinetochore and spindle microtubules. Here we show that a small region of the CPC subunit INCENP/Sli15 is required to target the complex to all three of these locations in budding yeast. This region, the SAH, is essential for phosphorylation of outer kinetochore substrates, chromosome segregation, and viability. By restoring the CPC to each of these three locations individually, we found that inner centromere localization alone is sufficient for cell viability. In addition, a second pathway consisting of a combination of inner kinetochore and microtubule binding is also sufficient to promote accurate chromosome segregation. Furthermore, we find that the two pathways target the CPC to different kinetochore attachment states, as the inner centromere targeting pathway is primarily responsible for bringing the complex to unattached kinetochores. We have therefore discovered two parallel localization pathways that are both sufficient for CPC activity in chromosome biorientation, both of which depend on the SAH region of INCENP/Sli15.

M156
Plekha5 Regulates Mitotic Progression by Promoting APC/C Localization to Microtubules
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Progression through major cell cycle checkpoints involves spatiotemporal coordination of cytoskeletal dynamics with changes in protein activities, chromosomal dynamics, and signaling events. A major role of the ubiquitin-proteasome system is to orchestrate cell cycle progression through temporally controlled protein degradation programs. The anaphase-promoting complex/cyclosome (APC/C) is the primary E3 ligase controlling all aspects of mitosis, including G2/M transition, early mitosis, the metaphase-anaphase transition, and mitotic exit. The temporal control of APC/C-mediated ubiquitination in these processes is well established, including identities of ubiquitination substrates and accessory factors that modulate E3 ligase activity. Much less is known, however, about the spatial organization of APC/C function, including its potential interplay with the microtubule cytoskeleton, which serves as a signaling platform and whose dynamic behavior is central to advancement through mitosis. Here, we investigate and describe pleckstrin homology domain-containing family A, member 5 (PLEKHA5) as a new regulator of APC/C function in mitosis. We found that PLEKHA5 localizes to the microtubule network and interacts with APC/C. PLEKHA5 knockdown antagonizes mitotic entry and progression, causing a buildup of APC/C substrates implicated in both the G2/M and metaphase-anaphase transitions, in a manner dependent upon the PLEKHA5 interaction with APC/C. In vitro ubiquitination assays showed that APC/C isolated from mitotic cells lacking PLEKHA5 had lower catalytic activity and a decreased association with its key mitotic co-activator CDC20. We investigated the dynamic localizations of the APC/C and the potential role of PLEKHA5 in its regulation by developing a TurboID proximity biotinylation tool to assess microtubule localization of endogenous proteins. Microtubule-targeted TurboIDs revealed that PLEKHA5, APC/C subunits, and the co-activator CDC20 all localized to microtubules both in interphase and in M phase. Importantly, upon PLEKHA5 knockdown, a pool of APC/C lost its microtubule localization, which could explain the decreased association with CDC20. We propose that PLEKHA5 functions as a novel APC/C adaptor to promote its subcellular localization to microtubules and enable APC/C to search for its co-activator CDC20 more efficiently in
one dimension along the microtubule network. In this model PLEKHA5 acts to coordinate the spatial regulation of APC/C<sup>CDC20</sup> and facilitate its efficient polyubiquitination of key mitotic effector proteins to ensure proper progression of mitosis.

**Signaling Hubs: Cilia, Cytonemes, and Second Messengers**

**M157**

**Aurora kinase A proximity map reveals centriolar satellites as regulators of its ciliary function**

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Aurora kinase A (AURKA) is a conserved kinase that plays crucial roles in numerous cellular processes. Although AURKA overexpression is frequent in human cancers, its pleiotropic functions and multifaceted regulation present challenges in its therapeutic targeting. The key to overcoming these challenges is to identify and characterize the full range of AURKA interactors, which are often weak and transient. Previous proteomic studies were limited in monitoring dynamic and non-mitotic AURKA interactions. Here, we generated the proximity interactome of AURKA in asynchronous cells, which consisted of 440 proteins involving multiple biological processes and cellular compartments. Importantly, AURKA had extensive proximate and physical interactions with centriolar satellites, key regulators of the primary cilium. Loss-of-function experiments identified satellites as negative regulators of AURKA activity, abundance, and localization in quiescent cells. Notably, loss of satellites activated AURKA at the basal body, decreased centrosomal IFT88 levels and caused ciliogenesis defects. Collectively, our results provide a resource for dissecting spatiotemporal regulation of AURKA and uncover its proteostatic regulation by satellites as a new mechanism for its ciliary functions.

**M158**

**Searching for Cytonemes: visualizing specialized filopodia in the developing mouse embryo**

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Spread of morphogens to form concentration-dependent signaling gradients is essential for cell fate differentiation during tissue patterning and for stem-cell niche maintenance driving tissue homeostasis. Perturbation of gradient function can result in developmental disorders and cancers. The mechanisms governing morphogen dispersion and gradient establishment in mammals are still debated, due in part to the inability to visualize morphogen gradients in situ. The cytoneme morphogen transport model posits that specialized filopodia extend between morphogen-sending and responding cells to ensure that appropriate signaling thresholds are achieved. How morphogens are transported along and deployed from cytonemes, and whether these processes are conserved across phyla is still unclear. Using the morphogen Sonic Hedgehog (SHH), we recently demonstrated that mammalian cells producing SHH increase cytoneme formation. Dispersion of SHH requires the actin motor protein Myosin 10 (Myo10), which transports SHH containing vesicles to cytoneme tips for release to receiving cells. Loss of Myo10 results in collapse of the SHH gradient in mouse neural tubes, leading to disrupted neuronal fate specification. Ongoing efforts are focused on visualizing cytonemes in the developing mouse neural tube using live multi-photon microscopy (MPM) and Focused Ion Beam Scanning Electron
Microscopy (FIB-SEM). We continue to develop methods to preserve the delicate specialized filopodia in fixed mammalian tissue with the goal of visualizing a working morphogen gradient in a developing embryo. These studies were supported by: NIGMS R35GM122546 (SKO) and by SJCRH. The content is solely the responsibility of the authors and may not represent the official views of the funding agencies. The authors declare no conflicts of interest.

M159

**Arachidonic acid is a direct allosteric activator of Smoothened ciliary trafficking**

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The Hedgehog (HH) signaling pathway is an evolutionarily conserved driver of tissue patterning during development and is essential for tissue homeostasis post-development. HH ligands relieve Patched1 (PTCH1) repression of the G protein-coupled receptor Smoothened (SMO), allowing SMO to accumulate in primary cilia for activation of GLI transcription factors. The molecular mechanisms driving ciliary entry of active SMO are not yet clear. Our lab previously demonstrated that SHH pathway induction leads to SMO-dependent activation of phospholipase cPLA2 at the base of the primary cilium for production of arachidonic acid. Arachidonic acid generated at the ciliary base initiates a feed-forward loop by acting through the transmembrane domain of SMO to promote its ciliary entry and high-level signaling to GLI.

In the present study we demonstrate that arachidonic acid effects on ciliary trafficking are specific to SMO, and that the fatty acid does not impact trafficking of additional primary cilium-localized GPCRs. Molecular docking experiments were used to identify a putative arachidonic acid binding site in the transmembrane domain of SMO. Arachidonic acid shares extensive binding site overlap with the inverse SMO agonist cyclopamine, but anchors through a different residue. We tested the predicted arachidonic anchoring residue by individually mutating arachidonic acid and cyclopamine anchoring residues in murine SMO and interrogated small molecule binding alteration. The arachidonic acid anchoring mutant shows reduced fatty acid association but maintains the ability to bind cyclopamine and the SMO agonist SAG. Functional assays reveal that the arachidonic binding mutant does not accumulate in primary cilia or efficiently signal to GLI following SHH stimulation, suggesting a crucial role for the fatty acid in propagation of the SHH signal response. As such, we propose that arachidonic acid functions as a bona fide allosteric activator of SMO that amplifies the SHH signal response by specifically promoting SMO ciliary entry. These studies were supported by: NIGMS R35GM122546 (SKO) and by SJCRH. The content is solely the responsibility of the authors and may not represent the official views of the funding agencies. The authors declare no conflicts of interest.

M160

**Accelerating imaging-based reverse genetics with spatial optical barcodes and deep learning**

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Advances in DNA synthesis and genome editing have made it easier to establish causal relationships between genes and the cellular behavior they govern. Through pooled CRISPR screens, it is now possible to simultaneously apply thousands of genetic perturbations to a single collection of cells and measure
the impact of these perturbations to reveal causal connections. Additionally, given the recent advances made in imaging technologies and machine learning, imaging these perturbed populations would provide a powerful approach for the discovery of new gene functions by revealing a phenotypic space orthogonal to traditional sequencing-based phenotypic measurements. While this amalgamation of approaches promises to significantly increase the power and utility of CRISPR libraries, it also comes with a set of unique challenges due to the need to link cells to their perturbation; this connection is impossible to know a priori for pooled perturbations. A proposed solution to this problem has been the integration of optical barcoding technologies, which use nucleic acid sequences that can be interpreted with imaging. However, while existing approaches to optical barcodes have made these imaging-based experiments possible, their difficulty and low throughput has placed them outside the reach of most labs. To overcome these previous limitations, I present a new approach to spatial optical barcodes, genomic sequences that are targeted to create multi-color spatial patterns inside of cells. In this approach, we leverage CRISPR-Display to target a set of gRNAs, each of which is assigned a unique integrated RNA tag, to repetitive regions in the genome, thereby creating spatial patterns inside cell nuclei that can be visualized with RNA FISH. I present experiments in which we have identified a set of 10 barcode gRNAs that produce visually distinct patterns - enough to produce a barcoded library with 5,000 perturbations that can be interpreted with a single round of imaging. Our lab's suite of deep learning models for single cell analysis can then identify barcodes and extract phenotypic features. By leveraging deep learning as a framework for barcode identification and single cell analysis, we present a new methodology capable of creating a broadly accessible platform for interrogating dynamic, live-cell phenotypes in high throughput perturbation screens.

M161
**Furin promotes Sonic Hedgehog Signaling by Cleaving the Ligand Deployment Protein Dispatched**

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The 12-pass transmembrane protein Dispatched (DISP) is required for deployment of Hedgehog (HH) family ligands to long-range targets. We recently demonstrated that DISP must be cleaved in its first extracellular loop by Furin proprotein convertase to efficiently release HH from ligand-producing cells. Investigation of processing deficient DISP in mouse cells reveals that cleavage deficient DISP retains the ability to bind vertebrate Sonic Hedgehog (SHH) but demonstrates altered membrane localization in ligand-expressing cells. To test the requirement for DISP cleavage in vivo, we used CRISPR targeting of the endogenous gene to generate a mouse model of defective DISP processing (DISP<sup>CS</sup>). DISP<sup>CS</sup> mice have reduced viability, with surviving animals showing altered neural tube patterning consistent with attenuated SHH signaling activity. Combined, our results support that Furin is an essential modulator of SHH signaling that promotes ligand release through cleavage-mediated activation of the deployment protein DISP. These studies were supported by: NIGMS R35GM122546 (SKO) and by SJCRH. The content is solely the responsibility of the authors and may not represent the official views of the funding agencies. The authors declare no conflicts of interest.
Lipidated FGF induces bidirectional FGF-FGFR signaling through cytonemes
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During morphogenesis, spatiotemporal communication of secreted signals is critical for the coordination of cells. Cells transmit signals target-specifically through cytoneme contacts. We investigated how cytoneme contacts form and why signals are released only though the cytoneme contact sites. In Drosophila, FGF produced in wing imaginal disc cells regulates the development of the disc-associated air sac primordium (ASP). ASP and wing disc FGF source cells extend cytonemes containing FGFR and FGF, respectively on their surfaces. These FGF sending and receiving cytonemes reciprocally guide each other to establish contacts and exchange FGF directly at the contact sites. Further investigation revealed that the contact-dependent reciprocal guidance and signal exchange are controlled by a lipid-modification of the FGF. FGF is GPI-anchored to the source cell surface. This lipid-modification inhibits random FGF secretion, but facilitates contact-dependent target-specific FGF release. We show that wing disc FGF source cells and ASP cells dynamically extend cytonemes and recognize each other by Cell-Adhesion-Molecule/CAM-like FGF-FGFR interactions at the cytoneme contacts. Contact-mediated FGF-FGFR binding induces reciprocal responses in ASP and source cells which polarize their cytonemes toward each other and mutually stabilize the signaling contact sites. FGFR-bound FGF is then released from the source GPI-anchor that enables the FGFR-bound FGF to move to the ASP cell body via ASP cytonemes. Thus, lipid-modification enables the FGF to orchestrate bidirectional FGF-FGFR signaling, which, in turn, controls the target-specific origin of cytoneme contacts and contact-dependent self-regulated FGF release.

The Physical Aspects of the Cell Cycle

Building a synthetic cell: Cell cycle robustness and hysteresis to cytoplasmic density variation
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Cells control their sizes and cytoplasm properties to ensure proper functioning. The density of cytoplasm, where most cellular reactions occur, is highly variable across various physiological and pathological states of cells undergoing growth, division, differentiation, apoptosis, senescence, etc. The challenge of understanding how cytoplasm affects cellular functions is in modulating its density in live cells. Here, by combining microfluidic experiments and modeling to perturb a frog egg cytoplasm in vitro, we found that cell cycles, a ubiquitous cellular process, maintain stable functioning across an incredible range from 0.2X RCD (relative cytoplasmic density) up to 1.46X RCD. Cell cycles arrested in a concentrated cytoplasm (>=1.46X RCD) can recover by diluting it but cannot until it is way below the natural density (0.79X RCD), suggesting the system remembers its history. This phenomenon, called hysteresis, is also common in physics, chemistry, and engineering. We developed a mathematical model to reproduce most experimental observations by assuming that cyclin synthesis & degradation rates and all molecule concentrations depend on cytoplasmic density. Interestingly, Neurohr et al. (Cell 2019) find that in oversized cells (density reduction from ~1.10 to ~1.07), transcription and translation machinery become limiting and do not scale with cell size, suggesting that our assumption of decreased synthesis rate with cytoplasmic density might be appropriate. Our model suggested a subcritical Hopf bifurcation causes differential thresholds to switch between the oscillating/arrested states, producing the observed
hysteresis. The model also predicted that the Cdk1/Wee1/Cdc25 positive feedback does not contribute to the robustness, confirmed by experiments applying inhibitors. Studies also connected cytoplasmic density with cell homeostasis. Fission yeast with higher cytoplasmic density tends to undergo supergrowth at a higher rate to achieve proteome homeostasis (Knapp, Cell Syst 2019). In human cells, cytoplasmic dilution of the cell cycle inhibitor Rb through the G1 growth phase triggers cell division, providing a mechanism to promote cell size homeostasis (Zatulovskiy, Science 2020). Interestingly, while we could tune the cell-cycle period to cyclin variations, we found it robust to density changes of the whole cytoplasm that contains cyclin. We hypothesized that instead of the absolute concentrations of each component, ratios between components matter for the oscillator robustness. This divergent response to changes in the overall concentration (where cell cycle is resilient) versus specific signaling activity (where cell cycle is sensitive) may help decouple possible cell homeostasis control mechanisms from cell cycle tuning capability.

M164

A mechanical G2 checkpoint controls epithelial cell division through E-cadherin-mediated regulation of Wee1-Cdk1

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Epithelial cell divisions must be tightly coordinated with cell loss to preserve epithelial integrity. However, it is not well understood how the rate of epithelial cell division adapts to changes in cell number, for instance during homeostatic turnover or upon wounding of epithelia. Here, we show epithelial cells sense local cell density through mechanosensitive E-cadherin adhesions to control G2/M cell cycle progression. We demonstrate that tensile forces on E-cadherin adhesions are reduced as local cell density increases, which prompts the accumulation of the G2 checkpoint kinase Wee1. This elevated abundance of Wee1 results in inhibitory phosphorylation of Cdk1, and thereby establishes a pool of cells that is temporarily halted in G2-phase. Importantly, these cells are readily triggered to divide upon epithelial wounding, due to the consequent increase in intercellular forces and resulting degradation of Wee1. Our data thus demonstrate that epithelial cell division is controlled by a mechanical G2 checkpoint, which is regulated by cell density-dependent intercellular forces sensed and transduced by E-cadherin adhesions.

M165

Mechanical regulation of mitotic entry

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Within a tissue, cells are continuously subjected to forces exerted by their neighbours. As these cells prepare to enter mitosis, they must ensure that enough space is available to assemble the mitotic machinery without perturbing tissue homeostasis. To do so, cells undergo a series of biochemical reactions regulated by Cyclin B1-CDK1 that allow a reorganization of the acto-myosin cytoskeleton and ensure the coordination of cytoplasmic and nuclear events. Along with the biochemical signals that control mitotic entry, the link between mechanical forces and cell cycle progression has long been
subject of interest. However, the exact nature of the physical forces exerted on mitotic cells and the impact they have on mitotic progression is still poorly understood. Here, using state-of-the-art microscopy, micropatterning, micro-fabrication and cell confinement techniques, we identify a mechanical signal that regulates mitotic entry and early spindle assembly. Our results indicate that mechanically confining cells shortly before nuclear envelope breakdown is sufficient to promote Cyclin B1 nuclear translocation, triggering premature mitotic entry. This mechanical signal requires cPLA2-mediated acto-myosin contractility and nucleus-cytoskeleton coupling through the LINC complex. Importantly, exogenous mechanical stimulation can overcome the lack of cPLA2 or Myosin II activity to promote mitotic entry. Moreover, our results suggest that premature Cyclin B1 nuclear translocation is likely due to a change in nuclear pore complex (NPC) conformation, which facilitates cyclin B1 movement into the nucleus. Finally, we show that this mechanical signal during mitotic entry is essential to promote efficient spindle assembly and ensure mitotic fidelity.

M166
Mitotic spindle architecture is formed by crosslinker-driven coarsening of a homogeneous microtubule mesh into discrete bundles
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The mitotic spindle has a highly organized architecture consisting of evenly distributed bundles of microtubules, which provide spindle integrity and generate forces that move and ultimately segregate chromosomes. These bundles are thought to arise by interaction between antiparallel microtubules and subsequent growth of new microtubules along the old ones. However, this idea has not been tested so far. Here we show, in contrast to the prevailing view, that microtubules self-organize into bundles through a coarsening process driven by crosslinkers. By using STED super-resolution microscopy of spindle cross-sections, we reveal that microtubules are initially uniformly distributed over the region of the future spindle, becoming rearranged into discrete bundles over time. This is reflected in the distribution of the microtubule crosslinker PRC1, which changes from a homogeneous mist-like dispersion to droplet-like structures. To understand the physics of bundle formation, we develop a mean field theory in which we describe microtubules and crosslinkers by density fields, including attraction between microtubules mediated by crosslinkers, repulsion between individual microtubules and entropic interaction among crosslinkers in the Landau-Ginzberg free energy, as well as a non-equilibrium process describing microtubule dynamics. Our theory predicts a phase transition from a homogeneous density state to a state with multiple clusters of crosslinked microtubules, above a microtubule density threshold. This minimum cut off density acquires higher values as the microtubule nucleation rate decreases. The model predicts diverging time scale of bundle formation when the microtubule density approaches the threshold. Our experiments in which we depleted augmin, a complex required for nucleation of new microtubules along pre-existing ones, also showed a longer time scale of bundle formation. Thus, crosslinkers and microtubule dynamics drive a coarsening process in which the initially uniform microtubule network is remodeled into a highly ordered cytoskeletal arrangement of the mitotic spindle.
M167

Mechanical regulation of cell division orientation: investigating the role of NuMA

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Cells within an organism constantly experience a variety of mechanical forces from their surrounding tissue environment. Reading and responding to these forces is crucial to shape and maintain tissues, with errors in this process contributing to failures in embryogenesis and diseases such as cancer. One of the cellular functions regulated by mechanical force is cell division orientation, which is determined by the position of the mitotic spindle. For example, stretching a tissue leads the majority of divisions to align along the axis of stretch. For external forces to regulate division orientation, they must be sensed and relayed to the mitotic spindle, and spindle-associated cortical proteins are likely key to this process. One such candidate, nuclear mitotic apparatus protein (NuMA) has been implicated in orienting the spindle according to force although the mechanistic details remain uncharacterised, especially in a tissue context. Therefore, we utilised the Xenopus laevis embryonic animal cap tissue to which reproducible tensile forces can be applied externally, to understand the role of NuMA in mechanosensitive spindle orientation. Using GFP-tagged NuMA, we show that cortical localisation of GFP-NuMA is dynamic and sensitive to mechanical stretch, with recruitment to the polar cortex earlier during mitosis in stretched tissues. Furthermore, we use mathematical modelling of spindle movements to show that amplifying microtubule-pulling at sites of experimentally observed cortical NuMA, as opposed to other pulling regimes, moves the spindle to an orientation that most closely matches experimental data. Using morpholino-targeted knockdown of endogenous Xenopus NuMA in early embryos, we also show that a reduction in NuMA levels disrupts the ability of cells to orient divisions along the axis of mechanical stretch and cell shape. Interestingly, our data suggest that mechanosensitive spindle orientation through NuMA is an effect of direct force sensing rather than sensing changes in cell shape. Overall, with a combination of live tissue imaging and mathematical modelling, our results indicate that NuMA is vital to orient a mitotic spindle according to external force.

M168

The Astrin-SKAP Complex Lubricates the Kinetochore-Microtubule Interface

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The kinetochore links chromosomes to spindle microtubules to drive chromosome segregation at cell division. To do so, its attachments to microtubules must be both dynamic and strong. While we know nearly all mammalian kinetochore proteins, how these give rise to dynamic yet strong microtubule attachments remains poorly understood. Here, we focus on the Astrin-SKAP complex, which localizes to bi-oriented kinetochores and is essential to chromosome segregation, but whose mechanical role is unclear. Live imaging revealed that SKAP depletion dampens metaphase kinetochore movements, reduces sister kinetochore coordination and increases the tension between sisters. Using laser ablation techniques, we isolated kinetochores associated to polymerizing vs depolymerizing microtubules in cells. Laser ablating kinetochore-fibers, we showed that without SKAP kinetochores move slower on polymerizing microtubules. In turn, ablating kinetochores we showed that without SKAP kinetochore-microtubules depolymerize slower. Additionally, both ablation experiments revealed that without SKAP more force is needed to rescue kinetochore-microtubules to polymerize. Thus, in contrast to previously
described kinetochore proteins that increase the grip on microtubules under force, Astrin-SKAP reduces grip, lowering friction and effectively “lubricating” the interface. Together, our findings suggest a model where Astrin-SKAP lubricates correct attachments to help preserve them.
Evolution of the Cytoskeleton and Cell Division Machinery

SG1

The archaeal origins of eukaryotic cell division
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Eukaryotic cells are complex. This is a consequence of their likely having arisen, over a billion years ago, from a partnership between an archaeal cell and a bacterium. While such complexity is fascinating, it can sometimes mask the core machinery and principles underpinning important cell biological processes. Given this, one might ask whether we can get at fundamental features of a process like eukaryotic cell division by studying similar processes in our closest archaeal relatives? This is the question I aim to explore in this presentation. In doing so, I aim to discuss the challenges of working with our archaeal relatives, the potential pitfalls of comparing "homologues" separated by large evolutionary distances, and the difficulties of inferring phenotype from genotype.

SG2

Activation of cytokinesis in Caulobacter crescentus
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In bacteria, cytokinesis is orchestrated by the tubulin-like GTPase FtsZ, which assembles into a dynamic Z-ring at the division site. FtsZ recruits dozens of proteins to the division site that collectively remodel the cell envelope and split the cell into two. Constriction of the cell envelope is driven by synthesis of peptidoglycan (PG) cell wall by two division proteins, the PG synthases FtsW and FtsI. Work in several bacterial species implicates the dynamic polymerization and depolymerization of FtsZ in the regulation of FtsWI movement and activity. However, the mechanisms linking FtsZ to the regulation of FtsWI are unclear. Recent work in our laboratory has demonstrated that the FtsZ-binding protein FzlA is a critical mediator of signaling between FtsZ and FtsWI in Caulobacter crescentus. Using genetics and advanced imaging approaches, we found that FzlA - which is normally essential for cytokinesis - is dispensable when FtsW is hyperactivated by mutation. Moreover, the average velocity of single molecules of FtsW increases when fzlA is deleted and decreases when fzlA is overexpressed. Conversely, the rate of constriction decreases when fzlA is deleted and increases when fzlA is overexpressed. These data are consistent with a model wherein slow-moving FtsW molecules are actively synthesizing PG, as suggested from work in E. coli. We conclude that FzlA is a limiting factor coupling FtsZ to the activation of FtsWI and, consequently, cytokinesis, in Caulobacter.
Cellular and molecular mechanisms of cell division and chromosome segregation in the thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius*

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Archaea represent one of two domains of life on Earth, from within which eukaryotes emerged, but we know far less about the cell biology of archaeal organisms than we know about bacteria and eukaryotes. For example, we do not understand how most archaea control their shape, organize their intracellular spaces, segregate their DNA, or divide. One reason for this lack of information is that many tools and techniques commonly used to study the cell biology of model bacteria and eukaryotes do not work properly under the more extreme growth conditions required by some model archaea. This is particularly true for high-resolution, live-cell light microscopy, which is still seldom applied to thermophilic prokaryotes mainly because of the difficulty of sufficiently heating samples and high-NA objectives and a lack of fluorescent proteins that fold correctly and fluoresce at high temperatures. *Sulfolobus acidocaldarius* grows optimally at 75-80°C and at pH 2-3. It belongs to the TACK superphylum, sister group to the Asgard archaea from within which eukaryotes have emerged. During cell division, the segregation of chromosomal DNA relies on a system that resembles the bacterial ParAB system called SegABs. It consists of a homolog of the bacterial ParA (SegA), and a DNA binding protein (SegB) that is unique to the archaeal kingdom. While *S. acidocaldarius* lacks homologs of actin or tubulin, it possesses the typical crenarchaeal homologs of the eukaryotic ESCRT-III complex proteins, which they utilize for membrane constriction and abscission during cytokinesis. Basic information about the molecular mechanisms and dynamics of chromosome segregation is still missing. The crosstalk between cell shape, polarity, motility, and DNA segregation in *Sulfolobus* cells is also poorly understood. Here, we describe a simple, commercially available system capable of high-resolution imaging of live cells at 75°C. We performed quantitative time-lapse microscopy of *S. acidocaldarius* cells as they undergo surface motility, form cell-cell contacts, change their shape, and undergo cell division. We also identified the centromere-like sequence segS and solved the crystal structure of the DNA binding protein SegB, shedding light on the molecular mechanisms that underlie chromosome segregation in *S. acidocaldarius*.

SG4

Evolution and function of divergent septin proteins

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*Co-presenters

Septins are a family of GTPases that form a component of the cytoskeleton. Originally identified in budding yeast as a series of mutants defective in cytokinesis, septins have since been implicated in a wide variety of cell biological processes. In opisthokonts (fungi, animals, and related species), multiple septin subunits form hetero-oligomeric complexes, which in turn polymerize into long filaments. These septins can be phylogenetically grouped into 7 clades. In humans and yeast, septin complexes are made of subunits from 1A, 1B, and 2B, and 1A, 2A, 3, and 4, respectively. Some of the groups are represented by multiple septin genes, allowing for modular assembly of different complexes. Group 5 septins are found in the fungal branch of opisthokonts, although it appears to have been lost in some lineages (e.g., *Saccharomyces*). Interestingly, a Group 5 septin in *Aspergillus nidulans*, AspE, is not incorporated into the septin hetero-oligomeric complex, yet its proper localization in cortical regions during development...
requires the other septins. Thus, the monomers or homo-oligomers of Group 5 septins may physically and functionally interact with the oligomeric 1A/2A/3/4 complex to provide an additional layer of diversity to septin structures and function. Outside of opisthokonts, septins are found in some (but not all) eukaryotic lineages, such as green algae, brown algae, ciliates, dinoflagellates, and diatoms. Interestingly, some of these septins are encoded as a single gene in a genome, suggesting that they function as monomers or homo-oligomers. One such single septin, Chlamydomonas reinhardtii SEP1, has been reported to possess an unusually high GTPase activity and homo-dimerize in vitro, which depends on an arginine finger located adjacent to the G4 motif (Pinto et al., 2017). Endogenously tagged SEP1-mNeonGreen localizes on the chloroplast envelope as clumps and also forms a ring at the division plane of this organelle, both of which are dependent on the arginine finger. Analysis of available sequences suggests that arginine fingers are widespread in non-opisthokont septins, with single septins invariably possessing this residue, while septin duplication and diversification within a genome frequently accompany its loss. R-fingers are found in other GTPases that are evolutionarily related to septins (TOCs and GIMAPs) and have been implicated in homo- and hetero-dimerization. These results highlight how Group 5 and non-opisthokont septins provide opportunities to delve into the early evolution of septin-family proteins, especially their evolution from a single gene to multiple genes forming heterooligomeric complexes.

SG5
A gene duplication of a septin provides a developmentally-regulated filament length control mechanism

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Septins are a family of conserved filament-forming proteins that function in a variety of processes including cell cycle progression, cell morphogenesis and autophagy. Despite their conservation from yeast to humans, the number of septin genes within an organism varies and higher eukaryotes express many septin isoforms due to alternative splicing. It is unclear how variability in septin complex composition influences the biophysical properties of septin polymers. Here we report that a complex duplication event within the CDC11 locus in the fungus, Ashbya gossypii, gave rise to two similar, but distinct Cdc11 proteins, Cdc11a and Cdc1b. CDC11b transcription is developmentally regulated producing different ratios of Cdc11a and b complexes during Ashbya’s lifecycle. Moreover, deletion of either CDC11a or CDC11b results in distinct cell polarity defects. Remarkably, despite substantial identity in amino acid sequence, Cdc11a and Cdc11b complexes have distinct biophysical properties with clear filament length and membrane-binding ability differences. Thus, septin subunit composition has functional consequences for filament properties and such functional plasticity can be exploited for distinct biophysical properties and cell functions.
Variation in Septin GTPase Activity as an Evolutionary Driver of Variation in Septin Filament Composition
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The septin family of cytoskeletal proteins evolved from an ancient homodimeric GTPase. The septin GTP-binding pocket is encompassed within an oligomerization interface, but the role of GTP hydrolysis in septin oligomerization remained enigmatic, as did the existence of GTPase-dead septins, of which several evolved independently. The number of septin genes in extant organisms varies widely, from none in most plants to >10 in humans and other vertebrates. In most cases, the building blocks of septin filaments are rod-shaped hetero-oligomers with individual subunits organized in a linear, symmetrical arrangement. In humans, hetero-hexamers with 2:2:2 organization of subunits co-exist with 2:2:2:2 hetero-octamers that incorporate an extra central homodimer. It is not fully understood how cells assemble distinct hetero-oligomers within the same cytoplasm and, when more septin proteins are produced than there are subunit positions to occupy, select from among a pool of multiple candidates. In Saccharomyces cerevisiae, where septin genes were first discovered, septin filaments are made only of hetero-octamers. We discovered that octamers are favored over hexamers because the GTPase-dead septin in S. cerevisiae prefers to recruit, rather than bypass, the central homodimer. Thus loss of GTPase activity restricted the diversity of septin assemblies. We also found evidence that slow GTPase activity by another yeast septin controls the selection between alternative subunits at the ends of octamers and allows cells to tailor septin complex composition to the cytosolic GTP:GDP ratio. Finally, a fission yeast septin appears to have evolved to bind non-guanosine nucleotides, which may reflect altered nucleotide availability during the developmental stage in which it functions. Together, these findings illustrate how evolutionary variation in GTPase activity sculpted the diversity of septin hetero-oligomers.

Characterization of Dictyostelium Cell-Substrate Adhesion Structures During Migration
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Cell-substrate adhesion is pivotal for a variety of cellular processes. In Metazoans, cells use cell-substrate adhesions called focal adhesions (FAs)-nanostructures composed of a multilayered protein complex physically linking the extracellular matrix (ECM) to the intracellular actin cytoskeleton - for efficient migration. Though this machinery was originally believed to be Metazoan specific, genomic sequencing of organisms across the eukaryotic kingdom suggests organisms as evolutionarily distant as Amoebozoa possess putative homologues of core FA components. It is unclear, however, if cell-substrate adhesions in organisms evolutionarily distant from Metazoans are evolutionarily conserved in function and composition to Metazoan FAs. An ideal organism for studying cell-substrate adhesions across evolutionary time is the Amoebozoan, Dictyostelium discoideum. While Dictyostelium cells have been shown to form cell-substrate adhesions for motility, our evolutionary analysis of the presence and absence of Dictyostelium homologues of core FA components suggest Dictyostelium lacks putative homologues of multiple components hypothesized to be critical for FA formation and function based on Metazoan data. These results suggest Dictyostelium are capable of forming cell-substrate adhesions despite lacking clear homologues of known core FA components. Preliminary time-lapse fluorescent microscopy of paxillinB (PaxB), the Dictyostelium homologue of the core FA component Paxillin, and actin shows the two components co-localize at punctate structures at the cell ventral surface, suggestive
of potential cell-substrate adhesions. PaxB and actin punctae are highly dynamic, assembling and disassembling during random cell migration. We are currently using transmission electron microscopy to analyze these punctae at the ultrastructural level. Furthermore, we are further investigating the localization of other known Dictyostelium homologues of core FA components to ventral surface structures during directed and random migration. Investigating the nature and composition of Dictyostelium cell-substrate adhesions will help us elucidate how cell-substrate adhesions have evolved across evolutionary time. This will subsequently aid further research into the evolution of adhesion-dependent mechanisms such as migration, cytokinesis, and pathogenesis.

SG8

Multiple Roles of the Actin Cytoskeleton in Giardia Attachment
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Attachment to the intestinal epithelium is critical to the lifestyle of the protozoan parasite Giardia lamblia. The microtubule cytoskeleton plays a well-characterized role in attachment via the ventral adhesive disc, whereas the role of Giardia’s unconventional actin cytoskeleton is controversial. We identified several novel actin-associated proteins that localize to the microtubule-based ventral adhesive disc. We also found interactors which are enriched in the ventrolateral flange (VLF), a sheet-like membrane protrusion around the cell that forms a gasket-like interface with attached surfaces. Depletion of GlActin by translation-blocking antisense morpholinos resulted in impaired attachment as well as defects in both the VLF and the ventral disc. Proteomics studies have identified over 100 disc-associated proteins (DAPs), but no force-generating protein has been identified among them. Disc and Actin Associated Protein 1 (DAAP1), is highly enriched in two regions of the disc previously observed to gate fluid flow that controls the level of suction under the disc. Knockdown of DAAP1 with CRISPRi impairs gate regulation without causing gross disc morphology changes. This suggests that DAAP1 and GlActin could have a role in regulating the conformational dynamics of the ventral disc. The VLF is known to engage with attached surfaces; our identification of the VLF localized and actin-associated protein Flangin, enabled us to test the role of the VLF in attachment. Intriguingly, we find that the VLF grows in width during mitosis. The VLF’s contribution to attachment may be particularly important during mitosis when the ventral disc is disassembled to prepare for cytokinesis and is not expected to be functional. We found that Flangin-depleted parasites have diminished VLFs that when challenged with fluid shear force in flow chambers had a reduced ability to remain attached, supporting a role for the VLF in attachment. Together this work highlights the central role of GlActin in at least two of Giardia’s mechanisms for maintaining host-parasite interactions.

SG9

A more complex Basal Complex: Mapping novel components of the Toxoplasma gondii cytokinesis machinery portrays an expanded hierarchy of its assembly
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Toxoplasma gondii is an obligate intracellular apicomplexan parasite, currently infecting one-third of the world’s population. In immunocompetent individuals the parasite quickly switches to a dormant stage that causes lifelong but mostly symptomless infection. In immunocompromised patients or unborn children however, the parasite can cause severe and lethal disease, which is directly linked to its lytic cell
division cycle. The lytic cycle consists of host cell invasion, subsequent replication and egress from the host cell, which in subsequent rounds can destroy large proportions of a patient’s tissue. *Toxoplasma* replicates by an internal budding mechanism, hereby forming two new daughter cells in the mother’s cytoplasm (endodyogeny). Budding concludes with the actions of the contractile basal complex (BC), the functional equivalent of the cytokinesis-related ring. The BC’s molecular composition, its order of assembly and its mode of action are incompletely understood but differ substantially from cytokinesis events observed in well-studied systems. To understand this unusual cytokinesis apparatus, we dissected its proteomic composition by proximity-dependent biotin identification (BioID). We identified 11 novel basal complex components (BCCs), portraying the BC as a structure comprising only ~ 30 proteins. Interestingly, the majority of novel BCCs is narrowly conserved in cyst-forming Coccidia, highlighting a specialized cytokinesis machinery tailored to the needs of apicomplexan parasites that initiate budding in the cytoplasm. To understand the architecture of the BC in greater detail, we applied expansion microscopy and thereby assigned novel BCCs to either the complex initiation, its expansion or its mature phase. Intriguingly, the BC functions as a scaffold early in the division process highlighted by a 5-fold symmetry observed in the forming daughter bud, from where the buds extend apically and basally, with different kinetics. Interestingly, we only identify a single essential gene among the novel BCCs, BCC4, arguing that the *T. gondii* basal complex relies on components that exhibit redundant functions. Rapid depletion of BCC4 from the BC however, results in daughter cells with significant wider basal ends and eventually leads to conjoined parasites that are unable to conclude division. BCC4 therefore acts early in the BC’s expansion phase, safeguarding the integrity of the nascent daughter cells, hereby allowing coordinated division to unfold. Since BCC4 harbors no recognizable protein domains, we are currently applying proximity labeling strategies to identify potential interacting partners. In conclusion, our work highlights an unusual cytokinesis apparatus for which we refine a multilayered architecture and hierarchy of assembly.

SG10

**Evolutionary Morphogenesis and the rise of The Fungi**

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Morphogenesis comprises the cellular processes that generate organismal shape. Despite being sister lineages, the morphogenetic programs of multicellular Fungi and Animals are remarkably divergent in their *materia prima* (e.g. cell wall vs. epithelia) and its mechanisms. How morphogenetic programs evolved and diversified in these lineages remains a key question in evolutionary cell biology. Chytrids are members of the deepest lineages of Fungi, and their life cycle alternates between an animal-like ciliated zoospore cell that swims and crawls and a sporangial cell with fungal traits like a cell wall and hyphae. The growth of the sporangial cell results in a multinucleated compartment that is more than a hundred times larger than the initial uninucleated zoospore. During late development, the sporangia switches developmental programs and undergoes cellularization to produce numerous zoospores that are discharged through a pore in the cell wall. During zoospore formation, chytrid sporangia are transiently multicellular and undergo morphogenetic transformations akin to those of animal embryonic development. These transformations include a cytokinetic event that produces a multicellular sphere of tightly packed uninucleated cells. This morula-like sphere undergoes ciliogenesis and synchronous abscission to form motile zoospores. This combination of fungal traits, such as cell wall and hyphae, and an animal-like developmental program makes chytrids uniquely suited to trace the evolution of animal and fungal morphogenesis. We recently developed the chytrid *Spizellomyces punctatus* as a genetically
tractable model system for evolutionary cell biology. We combine live-cell imaging of F-actin, microtubules, myosin-II and membrane dynamics with pharmacological perturbations to characterize the developmental program and morphogenetic transformations underlying chytrid cellularization. Further, we show that Chytrids retain key potential regulators of cellularization that are shared with animals but absent from Dikaryotic fungi. These results provide new insights into the ancestral morphogenetic toolkit present in the common ancestor of animals and fungi as well as innovations unique to the Fungal morphogenetic program.

**SG11**

**The Hippo Pathway Regulates Cytoskeletal Dynamics in a Close Unicellular Relative of Animals**

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Understanding the ancestral functions of animal signal transduction pathways can inform our understanding of how these pathways emerged in evolutionary history and highlight unknown or underappreciated functions of these pathways. The Hippo pathway is a conserved signal transduction pathway that coordinates tissue size, cell proliferation, cell differentiation, cytoskeletal architecture, and cell survival in animals. The core elements of the pathway are the kinase Hippo/MST1, which phosphorylates the kinase Warts/LATS, which phosphorylates the proliferation-promoting transcriptional coactivator Yorkie/YAP1, causing inactivation of Yorkie through cytoplasmic sequestration. To understand the origins of the Hippo pathway, we developed genetic tools in *Capsaspora owczarzaki*, a close unicellular relative of animals and the most basal known organism encoding a complete core Hippo pathway. *Capsaspora* is a filopodiated amoeboid organism that can form multicellular structures through cell aggregation and continued cell-cell adhesion. We generated a homozygous knockout of *Capsaspora* Yorkie (*coYki*) and, in contrast to what might be expected based on studies in animal cells, we detected no difference in cell proliferation between WT and *coYki* -/- cells. However, multicellular aggregates of *coYki* -/- cells showed a clear phenotype, with mutant aggregates being asymmetrical, less circular, and less thick perpendicular to the substrate as compared to WT cells. When examined individually, *coYki* -/- cells showed much greater cortical activity than WT cells, with frequent cortex-disrupting protrusions visible in mutant but not WT cells. We show that these protrusions are actin-depleted blebs, and inhibiting these blebs suppressed the phenotype of *coYki* -/- aggregates, indicating a causal link between cytoskeletal dynamics in individual cells and aggregate morphology. Loss of function of the *Capsaspora* orthologs of the Hippo or Warts kinases results in nuclear accumulation of *coYki*, suggesting that regulation of the subcellular localization of Yorkie by the Hippo pathway is evolutionarily ancient. Together, our results suggest an ancient role of the Hippo pathway in the regulation of cytoskeletal dynamics, suggest that proliferation regulation by the Hippo pathway evolved after the last common ancestor of *Capsaspora* and animals, and establish *Capsaspora* as a tractable system for studying gene function in a close unicellular relative of animals.
Plant cells divide using targeted secretion to build a new cell plate between the two daughter nuclei. The developing cell plate expands often over a large distance from the middle of the cell ultimately reaching a pre-defined area at the cell cortex. At that site, the membrane surrounding the new cell plate fuses with the mother cell membrane resulting in the physical separation of the two daughter cells. Many types of plant cells have a preprophase band (PPB), which is a band of cortical microtubules that forms during prophase predicting where the developing cell plate ultimately fuses. However, some cells, such as the filamentous tissues of mosses, do not have PPBs. It is unclear if and how these cells mark the cell cortex to ensure accurate placement of the cell plate during cytokinesis, particularly in branching cells that exhibit a highly asymmetric form of division. To investigate this, we localized TON1 in the filamentous tissues of moss. TON1 is a highly conserved protein known to localize to the PPB and required for PPB formation in A. thaliana. We found that TON1 localizes to the cross wall adjacent to an emerging branch and to the cortex at the branch emergence site. Interestingly, TON1 co-localizes at these sites with Myo8A, which we have shown previously influences cell division placement. Furthermore, we discovered that Myo8A at the cross wall adjacent to the emerging branch depends on TON1. By deleting TON1, we were able to selectively remove myosin VIII from this site. In comparison to wild type, which has myosin VIII at the adjacent cross wall, we found that ∆ton1 cells rarely generated division planes that intersected the cross wall adjacent to the branching cell. Our data demonstrate that TON1 acts upstream of myosin VIII and together with myosin VIII defines cell division placement in branching cells. Our results suggest that TON1 and myosin VIII comprise a PPB-like structure lacking microtubules, raising questions concerning the composition of the PPB.

Genetic Changes: Physical Causes & Consequences

Multinucleation is observed in at least thirty percent of solid tumors and is associated with an increased tolerance for mutation, resistance to chemotherapy, and invasive potential. Genomic integrity depends on proper execution of the cell cycle, which can be altered through mechanotransduction pathways as the tumor microenvironment stiffens during tumor progression. We recently found that inducers of epithelial-mesenchymal transition (EMT), including transforming growth factor-beta (TGFβ) and matrix metalloproteinases (MMPs), also promote multinucleation in stiff microenvironments through Snail-dependent expression of the filament-forming protein septin-6 and other midbody proteins, resulting in midbody persistence, abscission failure, and multinucleation. Consistently, we observed elevated expression of Snail and septin-6 as well as multinucleation in a human patient sample of metaplastic carcinoma of the breast, a rare classification characterized by deposition of collagen fibers and active EMT. In contrast, a soft microenvironment protects mammary epithelial cells from becoming multinucleated, even in the presence of abnormally high levels of midbody proteins. I will describe data
suggesting that mechanical signaling downstream of tissue stiffening synergizes with oncogenic signaling to promote genomic abnormalities that drive cancer progression.

SG14
The mitotic actin cytoskeleton and endoplasmic reticulum morphology orchestrate patterns of nuclear envelope assembly
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Nuclear envelope assembly is a fundamental cellular process that metazoan cells undergo each cell cycle. Recent work has linked nuclear envelope assembly defects to dysfunctional nuclear envelopes that lack nuclear pore complexes (NPCs) and other ‘non-core’ proteins, resulting in spontaneous loss of nuclear envelope integrity. Disruption of the nuclear envelope exposes chromosomes to the cytoplasm, which generates chromosome fragmentation and initiates innate immune proinflammatory signaling. Despite the importance of nuclear envelope assembly, how the nuclear envelope forms with a complete set of ‘core’ and ‘non-core’ proteins is poorly understood. The nuclear envelope assembles from the mitotic endoplasmic reticulum, which adopts tubular or sheet-like morphology in different human cell lines. Here we show a novel role of the mitotic actin cytoskeleton in organizing the mitotic endoplasmic reticulum that acts in concert with endoplasmic reticulum-shaping proteins CLIMP-63 and RTNs. This mechanism also plays a key role in establishing the pattern of nuclear envelope assembly in different cell types. We show that cells can undergo two distinct patterns of nuclear envelope assembly: tubule filtration and sheet lateral expansion. The extent to which one or the other pathway is used in different cells determines the extent of NPC assembly in the core region. Using a “fenestration tracker”, we show that lagging chromosomes do not assemble NPCs because endoplasmic reticulum sheets that form the fenestrations required for NPC assembly poorly penetrate the spindle region. Experimental perturbation of endoplasmic reticulum morphology independent of the actin cytoskeleton results in defective nuclear envelope assembly and a fragile nuclear envelope that is prone to disruption. These findings identify an equipoise between ER morphology and the mitotic cytoskeleton that generates different functional patterns of NE assembly in different human cells.

SG15
The relationship between constricted migration and 3D genome organization
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A variety of human cell types, from fibroblasts to metastatic cancer cells, sometimes must squeeze through tight constrictions in physiological contexts, dramatically deforming their nuclei. Chromosome structure contributes to nucleus stiffness, and changes to chromosome folding can affect gene regulation, DNA replication, and DNA repair, but still little is known about how the nucleus squeezing of confined migration depends on or is affected by 3D genome structure. In melanoma cells, breast cancer cells, and healthy fibroblasts, we observe rearrangements of heterochromatin and Lamin A in nuclei that have passed through constrictions smaller than their nucleus. Employing genome wide chromosome
conformation capture (Hi-C), we observe striking differences in chromosome spatial compartmentalization in melanoma cells that have passed repeatedly through tight constrictions. These constricted migration 3D genome signatures are partly explained by a selection process that favors the unique structural features of a small subset of cells within the initial population that are already highly migratory. However, there are other changes in 3D genome structure that newly appear after cells have passed through the constriction, suggesting that some chromosome structure changes may be induced by the process of constricted migration. Different clones derived from an initial population of melanoma cells each exhibit somewhat different genome structure changes after constricted migration, but they share common patterns of change across a shared set of genomic regions. In particular, we find that a loss of interaction frequency within heterochromatic (B compartment) regions is a shared feature across clones and between melanoma and breast cancer cells. Some 3D genome alterations we observe are associated with altered expression of genes with roles in migration and metastatic progression. But, we also observe an overrepresentation of regions with no genes among those that change compartments, suggesting that some changes may relate to nucleus physical properties rather than gene regulation. These observations begin to clarify the relationship between the 3D organization of the genome and ability of cells to migrate through constricted spaces.

SG16

Cellular Responses to Chronic Osmotic Stress

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Cells respond to sudden fluctuations in environmental osmolarity by acutely modulating the transport of solutes and water in order to maintain cell size homeostasis and minimize deleterious effects on cellular functions. While most studies on cellular osmotic responses have been primarily performed on plants and yeasts, little is known about how mammalian cells adapt to chronic osmotic stress on a longer time scale. Much of what we know thus far about mammalian osmoadaptive responses come from studies involving the osmoregulatory transcription factor Nuclear Factor of Activated T-cells 5 (NFAT5), which demonstrated that NFAT5 is activated upon hypertonic stress to enhance transcription of osmoprotective genes such as enzymes and transporters that accumulate uncharged small organic osmolytes intracellularly. There is however a scarcity of research that look into the long-term effects of hypotonic stress on cellular behaviors, which possess significant physiological relevance in conditions such as inflammation-associated edema and wound healing. Thus, we set forth to systematically characterize and investigate the long-term consequences of chronic osmotic stress of cell health and functions. We found that while both hyper- and hypotonic stress decreases the rate of cell growth and influence cell cycle progression, they are brought about in distinct manners. Furthermore, we discovered that osmotic stresses also exert profound effects on mitosis, leading to emergence of mitotic errors and potential chromosomal instabilities. Overall, these defects in cell cycle progression and genome instability induced by chronic osmotic stress could significantly alter cellular signaling and functions. Currently, we are in the process of elucidating the physicomechanical and biochemical mechanisms in which mammalian cells adapt to chronic osmotic stress. Looking forward, we hope that these finding will extend our fundamental understanding on how cells respond and adapt to osmotic stresses, and shed some light on how chronic osmotic stress may potentially contribute to pathogenic processes such as aging and cancer progression.
ORAL PRESENTATIONS

SG17

Oncogenic Ras alters cell shape and mechanics in mitosis to promote loss of tissue integrity and the proliferation of individual cells

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To divide within a tissue, cells require physical space in which to assemble a mitotic spindle and accurately segregate chromosomes. Cells generate this space by changing shape as they enter mitosis to become spherical, a process driven by rearrangement of acto-myosin filaments. Mitotic rounding is accompanied by cell stiffening, allowing cells to push outwards against their environment. Limiting mitotic rounding by confining cells under stiff hydrogels leads to defects in spindle formation and chromosome segregation, resulting in whole chromosome losses and unviable karyotypes. We investigated the effect of oncogene activation on cell shape and mechanics during cell division in normal epithelial cells. We found that induction of oncogenic RasV12, for as little as five hours, activates downstream MEK/ERK signaling to enhance mitotic rounding and stiffening in single cells. These Ras-dependent changes allow single cells to round up and divide faithfully when confined underneath stiff hydrogels, limiting the chromosome segregation errors observed in non-transformed cells. Ras/ERK-dependent changes to mitotic actin organisation led to additional changes when cells are cultured as an epithelium. Mitotic spindle orientation is disrupted meaning that cells frequently divide out of the epithelial plane. In addition, changes to post-mitotic actin repolarisation prevent daughter cells from taking up the space previously occupied by their mothers, thus disrupting tissue integrity. In epithelial monolayers, this results in bilayering and loss of tissue polarity, while in 3D spheroids, these changes lead to loss of spherical morphology and local invasion. These findings demonstrate how oncogene activation can affect the physical process of cell division to promote loss of normal tissue architecture but also then facilitate accurate chromosome segregation in single cells outside the normal tissue context. This reveals the direct contribution of alterations to mitotic mechanics to oncogenic phenotypes.

SG18

3d-stiffness maximizes heritable dna changes as revealed with live cell creporters

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Whether a cell’s DNA sequence is affected by the mechanics of its microenvironment is unclear, despite abundant evidence that gene expression, viability, and other basic processes are highly mechanosensitive. Here, live-cell monitoring of changes to chromosome copy numbers is developed and used in diverse perturbations, particularly compressed mitosis as imaged \textit{in vivo}. Such squeezing kills some dividing cells, but rare stem cells and cancer cells show heritable loss (~1%) of mono-allelic GFP/RFP-tagged constitutive genes as Chromosome-reporters (Creporters). Flattening spreads the mitotic spindle and limits microtubule-chromosome attachment, favoring mis-segregation and Creporter loss; however, inhibiting the high level of Topoisomerase in such cells suppresses chromosome compaction and loss. Tumor suppressor Myosin-IIA, in contrast, stabilizes compressed chromatin against fragmentation and knockdown increases Creporter loss. Solid human tumors and teratomas show similar Creporter trends in mice, and single-cell-sequencing identifies rare chromosome changes
exclusively in normal, 3D-infiltrative cells while also relating specific chromosome losses to altered
growth and cytoskeleton-driven motility.

SG19
**Kras and mechanical compression drive evolution toward a pancreatic cancer genotype**
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The tumor microenvironment has altered mechanical properties compared to normal tissue; pancreatic
tumors develop particularly high compressive stress. We developed a simplified model to study the
effects of compression on non-transformed pancreas duct cells, with or without the activation of
oncogenic KRAS. We found that compression synergizes with KRAS activation, leading to nuclear
mechanical instability and a high rate of mitotic errors. These mitotic errors led to stereotyped
aneuploidies. TP53 status and KRAS activation strongly affected the fitness landscape and pattern of
aneuploidies that evolved under compression. Tetraploid cells became far more aneuploid than diploid
cells, with a bias towards cancer-associated arm gains. Finally, after one month of evolution, cells
became resistant to mechanical compression. Thus, mechanical compressive stress may contribute to
the evolution of pancreatic cancer genotypes.

SG20
**Mechanisms of Inherently Low Fidelity of Chromosome Segregation in Human Pluripotent Stem Cells**
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Fidelity of chromosome segregation is crucial in cell division and chromosomes must be accurately
segregated to produce euploid daughter cells with an equal distribution of chromosomes. Errors in
chromosome segregation result in the gain or loss of whole chromosomes, producing aneuploid cells
with abnormal numbers of chromosomes. In normal human somatic cells, chromosome segregation is
regulated so that aneuploidy is rare. In contrast, during human development, chromosome segregation
errors are surprisingly common in human pluripotent embryonic cells, resulting in aneuploidy being the
leading cause of miscarriages and birth defects. Yet, the underlying mechanisms remain poorly
understood, especially for mitotic errors. Here, we directly compare mitosis in human pluripotent stem
cells (hPSCs), including human embryonic stem cells (hESCs) and human induced pluripotent stem cells
(hiPSCs), and somatic cells to investigate the causes of chromosome segregation errors in hPSCs. Using
quantitative live-cell imaging, immunofluorescence microscopy and chemical approaches, we show that
mitotic error rates are significantly elevated in hPSCs compared to somatic counterparts, with lagging
chromosomes being the most common error. Moreover, mitotic error rates are significantly elevated in
hiPSCs compared to isogenic somatic fibroblasts demonstrating that different genetic backgrounds do
not account for the mitotic error rates observed. We further demonstrate that improper kinetochores-
microtubule (k-MT) attachments cause lagging chromosomes in hPSCs. In addition, we use chemical
compounds to show that decreasing K-MT attachment stability or prolonging mitotic duration
significantly decreases the frequency of mitotic errors, including lagging chromosomes, in hPSCs. Thus,
our results demonstrate that k-MT attachment error correction is inefficient in hPSCs accounting for the
high mitotic error rates, but we can improve error correction efficiency by decreasing K-MT attachment
stability or by prolonging mitosis. Importantly, our data suggests that the fidelity of chromosome
segregation is inherently linked to the developmental stages and our results provide new strategies for how to improve the genome stability of hPSCs growing in culture which is critical for the success of regenerative and reproductive medicine.

SG21
Delivering insights into organ homeostasis and regeneration through in vivo genome-wide screens
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Our ability to understand and modulate mammalian physiology and disease requires knowing how all genes contribute to any given phenotype in the organism. Genome-wide screening using CRISPR-Cas9 has emerged as a powerful method for the genetic dissection of cellular processes, but the need to stably deliver single guide RNAs to millions of cells has largely restricted its implementation to ex vivo systems. These ex vivo systems cannot reproduce all of the cellular phenotypes observed in vivo nor can they recapitulate all of the factors that influence these phenotypes. There thus remains a pressing need for high-throughput functional genomics in a living organism. Here, we establish accessible genome-wide screening in the mouse liver and use this approach to uncover the complete regulation of cellular fitness in a living organism. We discover novel sex-specific and cell non-autonomous regulation of cell growth and viability. In particular, we find that the class I major histocompatibility complex is essential for preventing immune-mediated clearance of hepatocytes. Our approach provides a comprehensive picture of cell fitness in a living organism and highlights the importance of investigating cellular phenomena in their native context. Our screening method is robust, scalable, and easily adapted to examine diverse cellular processes using any CRISPR application. We have hereby established a foundation for accessible high-throughput functional genomics in a living mammal, enabling unprecedented insight into mammalian physiology and disease.

SG22
Members of the MRE11-NBS1-RAD50 complex promote proper kinetochore-microtubule attachments and faithful chromosome segregation in a DNA damage-independent manner
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Faithful genome maintenance and inheritance are essential for proper cell function and require the coordinated efforts of many intricate pathways. One of the major mechanisms by which cells maintain genome stability is the DNA damage response (DDR) pathway. Although once thought to be interphase-specific, we have previously identified a novel, DNA damage-independent, and mitosis-specific role for the DDR kinase ATR. Despite their important role in maintaining genome stability, we currently understand little about the functions of DDR proteins in mitosis. We hypothesized that other members of the DDR pathway have novel DNA damage-independent mitotic functions to promote proper chromosome segregation and protect genome stability. The MRE11-RAD50-NBS1 (MRN) complex promotes DNA double-stranded break repair by processing DNA ends and promoting DDR kinase signaling. To determine whether the MRN complex promotes proper chromosome segregation independent of its known DDR functions, we used immunofluorescence to score mitotic defects in cells lacking NBS1 and RAD50. We identified that NBS1- and RAD50-deficient cells have increased chromosome segregation errors, including increased rates of lagging chromosomes, which are due to
mitotic defects rather than unresolved interphase DNA damage. Furthermore, we have identified that NBS1 and RAD50 localize to prometaphase kinetochores in a DNA damage-independent manner, suggesting that members of the MRN complex may function in a novel mitotic pathway to promote proper chromosome segregation. We hypothesized that increased lagging chromosomes in NBS1- and RAD50-deficient cells are caused by defects in kinetochore-microtubule (k-MT) attachments. In support of this hypothesis, we found that cells lacking NBS1 and RAD50 have hypostable prometaphase k-MTs. Moreover, prometaphase RAD50 interacts with MCAK/Kif2C, a microtubule depolymerase that destabilizes k-MTs as part of the mitotic error correction machinery. These data suggest that MRN complex members have a novel and DNA damage-independent mitotic function to stabilize kinetochore-microtubules and promote proper chromosome segregation. Therefore, the MRN complex promotes genome stability throughout the cell cycle as part of multiple discrete mechanisms.

SG23

**Force generation of KIF1C is impaired by pathogenic mutations**

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Intracellular transport is essential for neuronal function and survival. The fastest neuronal transporter is the kinesin-3 KIF1C. Mutations in KIF1C cause hereditary spastic paraplegia and cerebellar dysfunction in human patients. However, neither the force generation of the KIF1C motor protein, nor the biophysical and functional properties of pathogenic mutant proteins have been studied thus far. Here we show that full length KIF1C is a processive motor that can generate forces up to 5.7 pN. We find that KIF1C single molecule processivity relies on its ability to slip and re-engage under load and that its slightly reduced stall force compared to kinesin-1 relates to its enhanced probability to backslip. Two pathogenic mutations P176L and R169W that cause hereditary spastic paraplegia in humans maintain fast, processive single molecule motility in vitro, but with decreased run length and slightly increased unloaded velocity compared to the wildtype motor. Under load in an optical trap, force generation by these mutants is severely reduced. In cells, the same mutants are impaired in producing sufficient force to efficiently relocate organelles. Our results establish a baseline for the single molecule mechanics of Kif1C and explain how pathogenic mutations at the microtubule-binding interface of KIF1C impair the cellular function of these long-distance transporters and result in neuronal disease.

SG24

**Synergistic Killing of Ovarian Cancer Cells by SYK Inhibitor and Paclitaxel via Disruption of Nuclear Envelope Integrity**

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Ovarian cancer is the most dangerous cancer of the female reproductive system. The dismal survival rate is often associated with resistance to standard chemotherapy drug paclitaxel (PTX) in recurrent ovarian cancer. Our group previously discovered that chemoresistance in ovarian cancer is linked to
hyperactivity of the spleen tyrosine kinase (SYK) signaling pathway. SYK inhibition could restore the sensitivity of ovarian cancer cells to PTX in vitro as well as in vivo and SYK inhibitor Fostamatinib is under clinical trial for ovarian cancer treatment. In this work, we discovered that the combined treatment of R406, pre-drug of Fostamatinib, and PTX, can synergistically and effectively kill non-dividing ovarian cancer cells. We observed this synergetic killing is accomplished through a mechanism distinct from mitotic arrest in dividing cancer cells resulting from PTX-only treatment. Immunofluorescence revealed that the expression of lamin A/C, a key protein in maintaining nuclear envelope integrity, is reduced 82% by R406 treatment. Applying microrheology methods, we examined the viscoelastic properties of the compromised nuclear envelope and observed that the nuclei of R406-treated ovarian cancer cells are significantly softer, with 90% reduction in elastic modulus. The softened nucleus is highly susceptible to nuclear deformation and chromatin leakage. Indeed, when combined with PTX, the co-treatment induced a fragmented nuclei morphology with leaked genomic DNA detected in cytoplasm in 75% of the co-treated cells. Furthermore, the co-treated cells exhibited 10-fold higher apoptosis rate compared to the control. Western blot results of cGAS-STING detection in the co-treated cells lead us to hypothesize that when PTX is used in combination with R406, the compromised nuclear envelope with deformed nuclei results in DNA leakage into the cytoplasm, triggering apoptosis through the cGAS-STING pathway. Our result indicated that by breaching nuclear envelope integrity via SYK inhibition, ovarian cancer chemoresistance can be bypassed. Furthermore, this novel killing mechanism may be applied to treat a wide array of cancers in addition to ovarian cancer.

SG25
Single-cell Analysis Reveals Predictable Quantitative Patterns of DNA Damage Checkpoint Adaptation
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Why biological quality-control systems fail is often mysterious. Checkpoints in yeast and animals are overridden (adapt) after prolonged arrests allowing self-replication to proceed despite the continued presence of errors. Although critical for biological systems, checkpoint adaptation is not understood quantitatively or at the system level by experiment or theory. To uncover potential patterns in checkpoint adaptation, we derived the mathematically optimal checkpoint strategy, balancing the trade-off between risk and opportunities for growth. The theory predicts the optimal override time based on two inputs, the statistics i) of error correction and ii) of survival. We applied the theory experimentally to the DNA damage checkpoint in budding yeast whose override is not understood quantitatively, functionally, or at the system level. Using a novel fluorescent construct which allowed cells with DNA breaks to be isolated by flow cytometry, we quantified i) the probability distribution of repair for a double-strand DNA break (DSB), including for rare events deep in the tail of the distribution, as well as ii) the survival probability after override. Based on these two measurements, the optimal checkpoint theory predicted remarkably accurately the DNA damage checkpoint override times as a function of DSB numbers, which we also measured for the first time precisely. Thus, a first-principles calculation uncovered hitherto hidden patterns underlying the highly noisy checkpoint override process. Our multi-DSB results revise well-known bulk culture measurements and show that override is a more general phenomenon than previously thought. Further, we showed that override is an advantageous strategy in cells with wild-type DNA repair genes. The universal nature of the balance between risk and self-replication opportunity makes our analysis relevant to a plethora of other biological systems as well.
SG26
Improved transcript discovery in single cell RNA-Seq with CRISPRclean
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Four different cell types including cardiomyocytes, neuronal nuclei, muscle biopsies and blood cells from AML samples were analyzed to determine a 100 gene panel of overexpressed, non-differentiated genes as candidates for CRISPR based depletion. The gene list consists of mostly ribosomal and mitochondrial derived protein coding transcripts with a co-efficient of variation of 1.1 or less when comparing cases and controls. These candidate transcripts take up approximately 40% of sequencer reads on average from a 10x Genomics prepared scRNA seq library. CRISPR guides were designed for even spacing of approximately 50bp across the target transcripts. 10x Genomics libraries previously prepared and sequenced to approximately 20,000 reads per cell were used as templates for the 100 gene panel depletion using CRISPRclean (Jumpcode Genomics). In this presentation we will describe the increase in unique transcript identification after depletion when normalized to the untreated control samples.

How Cells Build on the Micron Scale

SG27
Combinatorial codes of mitochondrial nucleoid composition govern the regulation of mtDNA replication
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Mitochondria form dynamic reticular networks essential to cellular energy generation, amino acid synthesis, lipid modification and heme production. Central to mitochondrial function is the mitochondrial genome (mtDNA); a small multi-copy DNA molecule maintained within the innermost mitochondrial matrix compartment by machineries distinct from those of the nuclear genome. MtDNAs are packaged into protein-nucleic acid complexes termed mitochondrial nucleoids which are distributed throughout the organellar network. Local gene expression from a subset of nucleoid complexes is thought to enable an efficient systemwide supply of mtDNA-encoded respiratory chain subunit proteins. However, the mechanisms by which mtDNA nucleoid abundance, distribution, and gene expression may be regulated to enact information flow throughout mitochondrial networks necessary to support function has remained unclear. To molecularly define the sub-population of replicating nucleoid complexes we localized known mtDNA nucleoid proteins within cultured cancer cells via super-resolution microscopy in preliminary immunofluorescence studies, finding that nucleoid populations are apparently highly heterogeneous in composition within single cells. We next leveraged publicly available CRISPR KO screening data to identify genetic co-dependencies between the dedicated mitochondrial DNA polymerase POLG and other mitochondrial genes. Hierarchical clustering by pairwise growth effect correlations assigned 240 genes to a single cluster; importantly, this cluster contained all known components of the mitochondrial DNA replisome, as well as mitoribosomal proteins, mitochondrial tRNA biosynthetic machinery, components of respiratory complexes I and V and multiple uncharacterized proteins. These findings raised the possibility that mtDNA synthesis may occur within a module of a macroassembly associated with the inner mitochondrial membrane in which replication, transcription and translation may be spatiotemporally coincident. We next explored this idea by
systematically visualizing candidate proteins from our cluster of interest relative to nascent mtDNA synthesis via metabolic labeling and super-resolution microscopy, identifying subsets that co-localize with, or adjacent to, actively replicating mtDNA within mitochondria. Ultimately, we aim to elucidate the combinatorial codes of nucleoid composition that govern the regulation of mtDNA replication and/or transcription, working toward a systems-level understanding of the governance of mtDNA copynumber and distribution in human cells.

SG28
Building axonal branches: Molecular insights from cryo-electron tomography
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Neurons display highly complex and polarized morphologies that form an intricate network to facilitate information processing and brain homeostasis. These complex morphologies are built by an extraordinary orchestration of various cellular processes including the dynamic reorganization of cytoskeleton and relocalization of cellular organelles. Axonal branching is a key event during neuronal development that allows the formation of new connections and thus increasing the complexity of neural circuits. However, our understanding of branch formation is sparse due to technical limitations. Using in situ cellular cryo-electron tomography on primary mouse neurons, we directly visualized the remodeling of organelles and cytoskeleton structures at axon branches. Based on our observations, we present how different cellular processes including organelle dynamics and transport, local protein synthesis and cytoskeletal rearrangements take place at different stages of building the axon branch. Together, we observe that axon branching points are a hotspot for several cellular activities that coordinate closely to facilitate the neuronal development.

SG29
E-cadherin adhesion assembly and signaling on synthetic membrane substrates
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E-cadherin plays an essential role in the formation and maintenance of the epithelial tissue, which, in turn, is fundamental to our normal development. This involves the extracellular domain-mediated transcellular homotypic interaction outside the cell and the intracellular domain-mediated formation of a mechanosensory E-cadherin-beta-catenin-alpha-catenin complex inside the cell. To further understand the mechanism underlying adhesion assembly and signaling, we reconstituted E-cadherin adhesion on a hybrid live cell-supported lipid bilayer platform. Our initial results using E-cadherin-functionalized bilayers with different viscosities revealed a requirement for reduced E-cadherin diffusion for efficient clustering which was coincident with retraction of filopodia extended by the cells, suggesting the involvement of a step of kinetic nucleation in the process. Post assembly, E-cadherin clusters showed a dynamic interaction with the actin cytoskeleton. Further, micron-scale clustering of E-cadherin was associated with the conformational activation of alpha-catenin, which persisted in the absence of actomyosin tension suggestive of a posttranslational modification of the protein. Additionally, single-molecule experiments appear to suggest a force-dependent binding of alpha-catenin with clusters of E-cadherin-beta-catenin complex. Together, these results indicate that the cellular actomyosin cytoskeleton plays a crucial role in the assembly and maintenance of E-cadherin adhesion.
ORAL PRESENTATIONS

SG30

A cellular mechanochemical wave of contraction during tissue morphogenesis
T. Lecuit; IBDM, Marseille, FRANCE.

Morphogenesis seeks to understand how tissue shape changes emerge from molecular interactions. The prevailing framework in developmental biology explains how genetic and biochemical information controls cellular mechanics, in particular contractility mediated by actomyosin networks, and thus cell and tissue shape changes. As such, development is viewed as a program following deterministic rules. However, newly reported contractile dynamics, namely pulses, flows and waves, cannot be explained in this framework: they are self-organized in that they depend on local mechano-chemical interactions, stochastic processes and feedbacks that cannot be accounted for by upstream genetic control. We explore the contributions of genetic control and self-organization in Drosophila embryos. Our current work leads us to consider that morphogenetic information emerges from the dynamic interplay between genetics, mechanics and geometry.

SG31

Actin Organization and Dynamics in Motile Toxoplasma gondii Parasites

Apicomplexan parasites such as Plasmodium and Toxoplasma, which cause malaria and toxoplasmosis, respectively, use a motility mechanism called gliding to migrate over host cells and through extracellular matrix. Unlike the well-studied eukaryotic motility mechanisms of swimming and crawling, gliding does not involve cilia or cell shape change. Gliding is powered by a thin layer of filamentous (F)-actin and a specialized myosin (MyoA) at the cell surface, confined between the plasma membrane and a membranous scaffold termed the inner membrane complex. In this study, we used single-molecule imaging and active matter theory to examine the basis of this unique mode of motility. Tracking single actin and myosin molecules in living Toxoplasma gondii revealed that actin can undergo rapid directional movement, while myosin is largely immobile, consistent with anchorage to the inner membrane complex. However, F-actin transport direction was strikingly heterogeneous, suggestive of a dynamic or self-organized system, rather than uniformly following the fixed polarity of underlying microtubules as previously believed. To understand how actin polarity and velocity patterns could arise from actomyosin interactions alone, we developed a continuum model for filament self-organization constrained by the geometry of the parasite outer membrane. In the absence of filament turnover, this model predicted the emergence of actin patches that recirculate up and down the cell, a “cyclosis” that we observed experimentally for drug-stabilized actin bundles in live parasites. The addition of actin turnover, along with F-actin polymerization at the parasite apical end, enabled the emergence of a second steady-state mode, in which actin polarity and transport is largely rearward. These results suggest that polarized actin dynamics govern the transition between experimentally observed bidirectional (patch or pendulum) and unidirectional (helical, circular, twirling) gliding modes. Notably, the two self-organized actin polarity patterns predicted can explain previously puzzling observations about Toxoplasma and Plasmodium gliding mechanics and the effect of actin regulatory mutants on gliding behavior. In summary, this work combines experiment and theory to present a framework for Toxoplasma actomyosin self-organization during gliding, improving our physical understanding of this understudied class of eukaryotic cell motility.
SG32

**Polar chromosomes in human cells congress by microtubule pivoting**

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During mitosis, the cell forms a spindle that equally segregates chromosomes into two daughter cells. Soon after nuclear envelope breakdown (NEB), kinetochores on chromosomes are captured by microtubules nucleated at the spindle pole. Some chromosomes immediately find themselves in the area between two spindle poles, yet 7 out of 46 chromosomes, on average, are positioned behind the pole at NEB in human cells. Once they approach the pole, the question remains how these unfavorably positioned polar chromosomes make their way across the centrosome as a physical barrier and reach the spindle body, from where they can continue their congression towards the equator. Here we show that astral microtubules attached to kinetochores of polar chromosomes pivot around the spindle pole and thereby transport polar chromosomes towards the spindle body. Stimulated emission depletion (STED) imaging of fixed samples showed that, during pivoting, both kinetochores of a polar chromosome typically attach to a single microtubule laterally, with other more complex attachments observed less frequently. This was consistent with the presence of the checkpoint protein Mad2 on both kinetochores, as well as with the analysis of end-binding protein 3 (EB3) comets which predominantly passed close to both kinetochores. The angle that the kinetochores of these chromosomes form with the spindle axis changed during the period of rapid spindle elongation, indicating the role of centrosome separation in the pivoting of microtubules attached to kinetochores of polar chromosomes. By using different kinesin-5 (Eg5) inhibitors to perturb the sliding of antiparallel microtubules and either stop or reverse spindle elongation, we confirmed that pivoting occurs due to a hydrodynamic drag force created by centrosome movement. Confocal and STED live-cell microscopy revealed that just before the end of pivoting, spindle body microtubules capture the distal kinetochore of a polar chromosome, leading to an increase in interkinetochore distance and landing of the kinetochore pair on the spindle surface. Pivoting as a mechanism of chromosome congression ensured timely mitosis, as polar chromosomes that failed to reach the spindle body by the end of spindle elongation significantly delayed anaphase onset.

Altogether, we propose a model in which pivoting of microtubules around the spindle pole, driven by spindle elongation, promotes the movement of peripheral chromosomes towards the spindle body and consequently their proper congression to the spindle equator.

SG33

**Mammalian Kinetochore-Fibers Regulate Their Length and Dynamics Individually, Independent of Spindle Poles**

**M. Richter, S. Dumont; UCSF, San Francisco, CA.**

At each cell division, the spindle builds itself from tubulin building blocks, with nanometer-scale components giving rise to a micron-scale structure. While many proteins modulating mammalian spindle length are known, how they work together to set spindle length remains poorly understood. To probe the role of spindle architecture in setting the length of spindle substructures, and vice versa, we inhibit dynein to generate spindles whose kinetochore-fibers (k-fibers) no longer focus and connect at poles. We find that these k-fibers have an unchanged mean length, albeit a broader length distribution, and that their microtubules “flux” outward normally. Furthermore, these k-fibers grow back after being laser
ablated, doing so by suppressing apparent minus-end depolymerization as they grow, all without a pole. This is in contrast to previous models where the pole and pole-associated forces regulate microtubule minus-end dynamics. However, pole-inhibited spindles fail to correctly segregate chromosomes, suggesting that k-fibers require global cues provided by the pole for proper coordination during anaphase. These findings support a model where spindle length, but not its function, is an emergent property of individual k-fibers, rather than of the whole spindle through the pole.

SG34
Mechanism of kinetochore fiber maturation by Augmin
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Accurate chromosome segregation during mitosis is required to prevent chromosomal instability, a hallmark of human cancers implicated in tumor evolution, metastasis and drug resistance. In mammals, chromosome segregation relies on the formation and maturation of a thick bundle of microtubules that attach at the kinetochore region of each chromosome to form kinetochore fibers (k-fibers). How k-fibers mature from initial kinetochore-microtubule attachments remains a key fundamental question. Here we used the low chromosome number (N=3) and distinctively large kinetochores (up to 2 μm in length) of Indian muntjac cells, to directly investigate the molecular mechanism underlying k-fiber maturation. By combining functional analyses of 65 conserved mitotic proteins, with fixed- and live-cell super-resolution CH-CH STED nanoscopy, we identified Augmin as the main driver of k-fiber maturation. Augmin is an octameric Y-shaped complex that recruits γ-tubulin to pre-existing microtubules, triggering microtubule nucleation and contributing to rapid microtubule amplification in the spindle. Surprisingly, we found that Augmin promoted kinetochore microtubule turnover by sustaining centrosome-independent microtubule growth from kinetochores and poleward flux. Tracking of microtubule growth events within k-fibers revealed an angular dispersion of ~40°, consistent with Augmin-mediated branched microtubule nucleation. Indeed, Augmin depletion reduced the frequency of microtubule growth events within k-fibers and prevented normal repair after acute k-fiber injury by laser microsurgery. Altogether, our work directly elucidates how Augmin mediates k-fiber maturation in mammals.

SG35
A gelation transition enables the self-organization of bipolar metaphase spindles
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The mitotic spindle is a highly dynamic bipolar structure that emerges from the self-organization of microtubules, molecular motors, and other proteins. Sustained motor-driven poleward flows of short dynamic microtubules play a key role in the bipolar organization of spindles. However, it is not understood how the local activity of motor proteins generates these large-scale coherent poleward flows. Here, we combine experiments and simulations to show that a gelation transition enables long-ranged microtubule transport causing spindles to self-organize into two oppositely polarized microtubule gels. Laser ablation experiments reveal that local active stresses generated at the spindle midplane propagate through the structure thereby driving global coherent microtubule flows.
Simulations show that microtubule gels undergoing rapid turnover can exhibit long stress relaxation times, in agreement with the long-ranged flows observed in experiments. Finally, our model predicts that, in the presence of branching microtubule nucleation, either disrupting such flows or decreasing the network connectivity can lead to a microtubule polarity reversal in spindles. We experimentally confirm this inversion of polarity by abolishing microtubule transport in spindles. Overall, we uncover an unexpected connection between spindle rheology and architecture in spindle self-organization.

SG36

**Real-time Chromatin Assembly on Naked DNA in Xenopus Egg Extract**

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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.

SG37

**Nucleosomes as liquid-like organisers of chromatin**

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Liquid-liquid phase separation (LLPS) is an important mechanism that helps explain the membraneless compartmentalization of the Cell nucleus. Because chromatin phase separation is a collective phenomena (i.e., emerging from dynamic formation of thousands of molecular interactions), linking biophysical features of individual nucleosomes to LLPS modulation remains an open challenge. In this talk, I will discuss our new multiscale chromatin approach—integrating atomistic representations of DNA and proteins, a chemically-specific coarse-grained model of oligonucleosomes, and a minimal model of chromatin—that can resolve individual nucleosomes within sub-Mb chromatin domains and phase-separated systems (Farr et al. Nat Communs 2021). I will also discuss how using this model, we find that
nucleosome thermal fluctuations, which become significant at physiological salt concentrations, destabilize the 30-nm fiber. In its place, nucleosome breathing favours stochastic folding of chromatin into a liquid-like ensemble. I will also discuss why nucleosome breathing also promotes the intrinsic LLPS of chromatin by simultaneously boosting the transient nature and heterogeneity of nucleosome-nucleosome contacts and the effective nucleosome valency. Our work highlights how the plasticity of nucleosomes is a key element in the liquid-like behaviour of nucleosomes within chromatin, and the regulation of chromatin LLPS.

Membrane Biology of Virus Entry and Assembly

SG38
Lipid-dependent budding and spread of emerging pathogenic viruses
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Lipid enveloped viruses replicate and bud from the host cell where they acquire their lipid coat. Lipid-enveloped viruses include dangerous pathogens such as coronaviruses (SARS-CoV-2, etc.), filoviruses (Ebola virus and Marburg virus) and paramyxoviruses (Nipah virus, Hendra virus, etc.). Despite understanding some of the basics of how these viruses cause disease and enter host cells, not much is known on how these dangerous pathogens interact with host cell lipids to achieve new virion formation. The viral matrix or membrane protein regulates assembly and budding from the host cell membrane, connecting the viral lipid envelope to the viral nucleocapsid. Depending on the virus family, this assembly and budding may occur at the plasma membrane or the ER-Golgi intermediate compartment. This presentation will detail the biophysical and biochemical basis of how these emerging pathogens hijack host lipid membrane and metabolic networks to form new virus particles that undergo release from the host cell.

SG39
Understanding Influenza A virus assembly using quantitative fluorescence microscopy
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Influenza A virus (IAV) is a respiratory pathogen that causes seasonal epidemics with significant mortality. One of the most abundant proteins in IAV particles is the matrix protein 1 (M1), which is essential for the virus structural stability. M1 organizes virion assembly and budding at the plasma membrane (PM), where it interacts with other viral components. The recruitment of M1 to the PM as well as its interaction with the other viral envelope proteins (hemagglutinin, neuraminidase, matrix protein 2) is controversially discussed in previous studies. A quantitative characterization of protein-protein interactions in living (infected) cells is needed in order to understand virus assembly from a molecular point of view. To this aim, we use cutting-edge microscopy techniques that can provide quantitative information also in complex biological samples. Such approaches are based on fluorescence microscopy and, more specifically, on the mathematical analysis of equilibrium fluctuations (e.g., scanning fluorescence cross-correlation spectroscopy and number & brightness). Our recent results deepen our understanding of how viral proteins interact at the plasma membrane of infected cells and demonstrate that our experimental approaches can be successfully applied to investigate complex networks of protein-protein and protein-lipid interactions in biological systems.
SG40

**Nanophysiology of Influenza Virus Infection**

**C. Sieben;** Helmholtz Centre for Infection Research, Braunschweig, GERMANY.

For many enveloped viruses, the plasma membrane represents the first obstacle to overcome during cell entry and the site of progeny virus assembly. Since viruses are nanoscale entities, these processes require a finely tuned spatio-temporal regulation at the nanoscale. Influenza A viruses (IAV) bind cells using the viral protein hemagglutinin recognizing sialylated plasma membrane glycans, IAVs primary attachment factors (AF). Since AFs cannot fulfill a signaling function, the virus needs to activate downstream factors in order to trigger endocytosis. The epidermal growth factor receptor (EGFR) was shown to be activated and transmit IAV entry signals but how IAV engages and activates EGFR remains unclear. We used quantitative super-resolution microscopy to study the lateral organization of IAV attachment factors as well as its functional receptor at the scale of the virus-cell interface (<100 nm). We show that SA and EGFR are organized in partially overlapping nanoclusters in the apical plasma membrane of permissive A549 cells. Within AF clusters, that are distinct of microvilli, the local AF concentration, a parameter that directly influences virus-cell binding, strongly increases towards the cluster center, thereby representing a multivalent virus-binding platform. Our quantitative analysis allowed us to simulate virus-membrane interaction suggesting that IAVs perform an explorative movement dominated by the local SA concentration, which could be confirmed by live-cell single-virus tracking. For EGFR, we find clusters of rather low molecule abundance. Virus binding activates EGFR but interestingly this process occurs without a major lateral EGFR redistribution, suggesting the activation of preformed long-lived clusters. Our results provide a first step towards understanding the nanophysiology of influenza virus infection. We are able to relate the structural organization of the cell surface with its functional role during virus-cell binding and receptor activation.

SG41

**A BECLIN1-DERIVED PEPTIDE PROMOTES VIRUS ENTRY IN EARLY ENDOSONES BY ACTIVATING THE BECLIN1-VPS34-ATG14L COMPLEX**

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Tat-Beclin1 (TB1) is a therapeutic peptide consisting of the cell-penetrating polybasic domain of HIV-1 Tat fused with a portion of Beclin1. TB1 is under clinical evaluation as a putative autophagy inducer with potential therapeutic value. Here, we report that TB1 promotes the early stages of virus infection in human cells. TB1 enhanced infection by VSV and HIV-1 in a cell-type dependent manner but had no effect on infection by Influenza A virus. While virus attachment to cells is not affected by TB1, the peptide facilitates entry at or before the membrane fusion step, when viral components gain access to host cytosol. TB1 was previously shown to influence Beclin1 activity in cells and in vitro by supporting its attachment to membranes, and this was dependent on a single amino acid residue, F270. Addition of TB1, but not the F270S mutant peptide, to cells enhanced virus infection, and this effect was associated with elevated levels of PI3P in early endosomes. These results suggest that TB1 activates the enzymatic activity of PI3K class III (Vps34), the major producer of cellular PI3P. Therefore, we silenced Beclin1 or other members of the PI3K class III complexes to which it belongs (Beclin1-Vps34-ATG14L (complex 1) and Beclin1-Vps34-UVRAG (complex 2)). In Beclin1-deficient cells, TB1 lost the ability to promote VSV infection. Furthermore, knockdown of ATG14L, but not UVRAG, abrogated the effect of TB1 on infection.
Together, these findings indicate that TB1 promotes virus entry by activating PI3K class III complex 1, which is known to be involved in autophagosome formation and endocytic trafficking. The impact of TB1 on infection was maintained in cells lacking ATG9b, suggesting that activation of canonical macroautophagy is not the mechanism by which TB1 promotes virus entry. Instead, our data suggest that TB1 promotes fusion between incoming virus particles and early endosomes by promoting PI3P deposition by Beclin1-Vps34-ATG14L. This is consistent with the observation that entry of Influenza A virus, which undergoes fusion in late endosomes, is unaffected by TB1. These results reveal virus co-opting of a PI3K class III complex for efficient entry into cells and offer new targets for the design of pan-viral entry inhibitors.

SG42

S-acylation controls SARS-CoV-2 membrane lipid organization and enhances infectivity

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SARS-CoV-2 virions are enveloped by a lipid bilayer that assembles at intracellular membranes, through the action of various structural membrane proteins. Here, we studied how S-acylation regulates SARS-CoV-2 structural proteins, in particular, the Spike glycoprotein which is responsible for binding and fusion to target-cells. We show that during SARS-CoV-2 infection, the predominant activity of the S-acyltransferases ZDHHC20 but also ZDHHC9 modifies Spike and other viral membrane proteins. Spike is modified shortly after translation on its unusual 10 cytosolic cysteines and within the ER and Golgi, where CoV-envelopes normally assemble. Using a multidisciplinary approach, we established that S-acylation controls biogenesis and degradation of Spike, and drives the formation of localized ordered cholesterol and sphingolipid rich lipid nanodomains, within viral budding sites and SARS-CoV-2 virions. Finally, we show that S-acylation of Spike allows the formation of viruses with enhanced fusion capacity, highlighting the potential of S-acylating enzymes and lipid biosynthesis enzymes as novel therapeutic anti-viral targets.

SG43

Sneaking out with the trash: How Coronaviruses get out of cells.

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Βετα-Coronaviruses are a family of positive-strand enveloped RNA viruses that include the severe acute respiratory syndrome-CoV2 (SARS-CoV2). Much is known regarding their cellular entry and replication pathways, but their mode of egress remains uncertain. Using imaging methodologies and virus-specific reporters, we demonstrate that Βετα-Coronaviruses utilize lysosomal trafficking for egress, rather than the biosynthetic secretory pathway more commonly used by other enveloped viruses. This unconventional egress is regulated by the Arf-like small GTPase Arl8b and can be blocked by the Rab7 GTPase competitive inhibitor CID1067700. Such non-lytic release of Βετα-Coronavirus results in lysosome deacidification, inactivation of lysosomal degradation enzymes and disruption of antigen presentation pathways. This coronavirus-induced exploitation of lysosomal organelles for egress.
provides insights into the cellular and immunological abnormalities observed in patients and suggests new therapeutic modalities.

SG44
The Roles Played by the Plasma Membrane Phospholipids in HIV-1 Particle Assembly and Host Protein Incorporation
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The HIV-1 structural polyprotein Gag drives the virus particle assembly at the plasma membrane (PM). Gag localization to the PM is regulated by binding of its N-terminal highly basic region (HBR) to tRNAs and an acidic phospholipid, PI(4,5)P2. At the PM, the nascent virus particles are known to incorporate a subset of host membrane proteins in addition to viral glycoprotein Env and viral genomic RNA. However, for most of these membrane proteins, the functional significance of their incorporation into virions and mechanisms promoting the incorporation process remain to be determined. In polarized CD4+ T cells, HIV-1 Gag localizes to the rear-end protrusion known as uropod. We found that even when cells are not polarized, Gag colocalizes with a subset of uropod-associated host transmembrane proteins, i.e., PSGL-1, CD43, and CD44, at the PM. In contrast, other uropod-directed transmembrane proteins are excluded from HIV-1 Gag clusters at the cell surface. As for functional significance of uropod-directed proteins in HIV-1 replication cycle, we and others have shown that PSGL-1 and CD43 recruited into nascent virions prevent virus attachment to the target CD4+ T cells. By contrast, we found that virion-incorporated CD44 facilitates virus particle binding to lymph node stromal cells known as fibroblastic reticular cells, which in turn mediate trans-infection of CD4+ T cells that contact with these stromal cells. Thus, incorporation of these uropod-associated transmembrane proteins into nascent virus particles modulate virus spread either negatively or positively. Using super-resolution localization microscopy, we showed that polybasic sequences in the cytoplasmic juxtamembrane regions of CD43, CD44, and PSGL-1 are essential for coclustering with Gag multimers at the PM. We also observed that the positive charge of the Gag HBR is necessary for the recruitment of PSGL-1 to Gag clusters, suggesting the presence of a negatively charged entity that can bind both Gag HBR and juxtamembrane polybasic sequences of the transmembrane proteins. Notably, we observed that depletion of the cellular PI(4,5)P2 reduces incorporation of CD43, CD44, and PSGL-1 into virus particles. Altogether, these results suggest the possibility that an acidic phospholipid cluster induced by Gag multimerization at virus assembly sites interacts with the juxtamembrane polybasic sequences of CD43, CD44, and PSGL-1 and thereby recruits these transmembrane proteins into assembling virions.

SG45
How physics assists in assembling HIV
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In order to assemble their envelope, viruses such as HIV have to locally recruit the host membrane components required for their function and at the same time, bend the host plasma membrane to form the viral bud. In HIV, Gag self-assembly is essential for deforming the membrane but a significant fraction of Gag clusters do not successfully grow into a mature viral particle, suggesting that other cellular components are needed for a successful completion of the process. With cell biology and in vitro
experiments, we will show that the I-BAR protein IRSp53 assists in viral budding (1) i) by its own ability to spontaneously cluster on the cell plasma membrane due to its intrinsic curvature (2) and to locally recruit Gag (1) ii) by contributing to membrane curvature (3) and lowering the free energy barrier involved in the progression of the budding process. References (1) K. Inamdar et al. \textit{eLife} 10, e67321 (2021) (2) F.C. Tsai et al, \textit{in preparation} (3) C. Prévost et al, \textit{Nat. Commun.} 6, 8529 (2015)

\textbf{Not Just a Cellular Railroad: Microtubules as Cargoes and Signaling Centers}

SG46

\textbf{Microtubule Sliding in the Axon: an Old Topic Meets the Future}


The decades-long controversy of whether microtubules slide in the axonal projections of neurons has been resolved in recent years. There is now clarity that microtubules slide in axons, which is consistent with microtubule behavior in a variety of cell types. Even so, the most important questions and challenges face new generations of cell biologists and neuroscientists. How can the sliding of microtubules be visualized in a cell type whose geometry and packing density of microtubules make it very difficult? What functions are fulfilled by the sliding? What mechanisms cause the sliding and what mechanisms regulate the sliding? What happens when the sliding is dysregulated? We have proposed that microtubule sliding is directly relevant to the plus-end-out polarity orientation of microtubules in axons. This is based on \textit{in vitro} studies showing that their sliding by motor proteins is a powerful means by which microtubules can be organized into arrays of uniform orientation. Studies from various labs in recent years have shown that microtubule polarity flaws arise in the axon when various microtubule-related proteins are disrupted. Here, we argue that polarity sorting of microtubules by molecular motors is a fundamental contributor to regulating the orientation of axonal microtubules but that this mechanism does so against a backdrop of other factors that vary significantly depending on the type of neuron and species of animal. Some of these factors assist in preserving the plus-end-out pattern of microtubules while other factors present significant risks to the fidelity of the plus-end-out pattern but exist to carry out crucial functions for the axon. An asset of a motor-driven process for polarity sorting microtubules in the axon is that it is an ongoing dynamic process capable of clearing mal-oriented microtubules from the axon throughout the life of the neuron. We posit that dysregulation of these various factors leads to microtubule polarity flaws that are a significant contributor to axonal degeneration in a variety of neurological diseases.

SG47

\textbf{The Role of Kinesin-1 Mediated Microtubule Sliding in Regulation of Insulin Secretion}

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Pancreatic beta cells have to secrete only a sub-population of available insulin granules to reduce blood sugar to normal levels but yet avoid hypoglycemia. This requires tight coordination between intracellular insulin storage and secretion. We have previously shown that cytoskeletal polymers microtubules (MTs) critically govern this coordination. Molecular motors use MT tracks to transport and park insulin granules at specific cellular locations. In beta cells, MT network is complex, with interlocked, non-radial network in the cell interior, where secretory insulin granules are trapped by looped transport, and the sub-
membrane MT bundle, which promotes insulin granule withdrawal from the secretion sites. Due to these features, MT network limits insulin secretion in healthy beta cells. Given a spike in glucose concentration the microtubule network is remodeled to allow for robust secretion. Here, we report that motor-dependent repositioning of existing MTs is an essential feature of glucose-triggered MT remodeling. Using real-time imaging of microtubules labeled with single-molecule fiducial marks, we demonstrate that high levels of glucose rapidly induce MT movements. Kinesin-1, a molecular motor that is highly expressed in beta cells and activated downstream of glucose signaling, is capable of transporting MTs along MT tracks, a process known as MT sliding. We found that glucose-triggered MT movements are abolished by inactivation of kinesin-1 or introducing mutations preventing attachment of MTs as a cargo. We show that microtubule sliding is important for cellular distribution of MT minus ends and is influencing the overall MT directionality and architectural features. This means that kinesin-1 likely has a dual role in insulin secretion regulation: directly, via insulin granule transport, and indirectly, via remodeling MT network. Interestingly, our computational 3D data analysis indicates that kinesin-1 inhibition causes an aberrant insulin granule clustering, which is consistent with their facilitated trapping at aberrant accumulations of microtubule minus ends. Ongoing work will utilize kinesin-1 mutant incapable of microtubule sliding but capable of insulin granule transport, combined with computational simulations, to reveal the specific contributions of MT sliding into regulation of insulin granule positioning and secretion. Overall, here we report a novel mechanism whereby glucose stimulation induces MT network remodeling, and, subsequently, MT-dependent control of insulin granule transport for secretion.

SG48
Map7 anchors kinesin-1 to slide parallel and antiparallel microtubules
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Microtubules are polarized protein polymers that are arranged in same directions (parallel) or opposite directions (antiparallel) in cells. Such arrangements allow microtubules to use motor proteins to slide over each other and contribute to diverse cellular processes, including mitosis, migration, axonal growth, cell differentiation, and nuclear migration. Many motor proteins are known to slide antiparallel microtubules, either via multiple motor domains walking on opposing microtubules (e.g. kinesin-5 and cytoplasmic dynein) or by motor domains linked to non-motor microtubule binding sites (e.g., kinesin-1 and kinesin-14). However, neither mechanism supports efficient parallel microtubule sliding, because motors walking simultaneously on both parallel microtubules generate opposing forces that usually cancel out. Here, we report an anchorage mechanism that facilitates the plus-end directed kinesin-1 motor to slide both parallel and antiparallel microtubules. This mechanism involves MAP7, a lattice-bound microtubule associated protein that also interacts with kinesin-1. Using an in vitro microtubule-sliding assay, we demonstrate that 1) MAP7 anchors a truncated but active kinesin-1 motor to slide adjacent microtubules in a dose-dependent manner; 2) the resulting sliding velocity is influenced by MAP7 bound to the motor side of microtubules as well as microtubule orientations; 3) microtubule sliding observed is in either parallel or antiparallel orientation; 4) preferential motor anchorage on one side of the microtubules by MAP7 encourages parallel microtubule sliding; and 5) parallel microtubule sliding is slower than anti-parallel sliding. Taken together, our study reveals a novel and versatile two-component molecular system that can regulate microtubule sliding and subsequent microtubule reorganization in diverse cellular processes.
Deciphering the mechanics of crosslinked microtubule networks in mitosis
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Cells organize cytoskeletal networks to perform complex physical tasks such as segregating chromosomes during mitosis. For example, the dynamic mitotic spindle network is built from microtubules that are organized, bundled, and transported by motor and non-motor proteins that produce ‘active’ pushing and ‘passive’ frictional forces. How these mesoscale forces are regulated at the micron-scale by ensembles of nanometer-sized proteins in order to properly move chromosomes has been unclear. We are addressing this knowledge gap by characterizing crosslinked microtubule bundles under load using optical tweezers and single molecule fluorescence microscopy. We have found 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, operate in two distinct modes to control microtubule sliding. In the first mode, PRC1 forms high-density clustered aggregates at microtubule tips to significantly impede kinesin-driven microtubule sliding activity. In the second mode, PRC1 ensembles behave as 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 ensembles can adopt multiple states that differentially regulate microtubule motions to establish stable rates of both chromosome and pole separation during cell division. 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.

Kinesin-6 Klp9 orchestrates spindle elongation by regulating microtubule sliding and growth
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Mitotic spindle function depends on the precise regulation of microtubule dynamics and microtubule sliding. Throughout mitosis, both processes have to be orchestrated to establish and maintain spindle stability. We show that during anaphase B spindle elongation in Schizosaccharomyces pombe, the sliding motor Klp9 (kinesin-6) also promotes microtubule growth in vivo. In vitro, Klp9 can enhance and dampen microtubule growth, depending on the tubulin concentration. This indicates that the motor is able to promote and block tubulin subunit incorporation into the microtubule lattice in order to set a well-defined microtubule growth velocity. Moreover, Klp9 recruitment to spindle microtubules is dependent on its dephosphorylation mediated by XMAP215/Dis1, a microtubule polymerase, creating a link between the regulation of spindle length and spindle elongation velocity. Collectively, we unravel the mechanism of anaphase B, from Klp9 recruitment to the motors dual-function in regulating microtubule sliding and microtubule growth, allowing an inherent coordination of both processes.
SG51

Motor guidance by long-range communication through the microtubule highway

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Coupling of motor proteins within arrays drives muscle contraction, flagellar beating, chromosome segregation, and other biological processes. Current models of motor coupling invoke either direct mechanical linkage or protein crowding, which rely on short-range motor-motor interactions. In contrast, coupling mechanisms that act at longer length scales remain largely unexplored. Here we report that microtubules can physically couple motor movement in the absence of short-range interactions. The human kinesin-4 Kif4A changes the run-length and velocity of other motors on the same microtubule in the dilute binding limit, when 10-nm-sized motors are separated by microns. This effect does not depend on specific motor-motor interactions because similar changes in Kif4A motility are induced by kinesin-1 motors. A micron-scale attractive interaction potential between motors is sufficient to recreate the experimental results in a computational model. Unexpectedly, our theory suggests that long-range microtubule-mediated coupling not only affects binding kinetics but also motor mechanochemistry. Therefore, motors can sense and respond to motors bound several microns away on a microtubule. These results suggest a paradigm in which the microtubule lattice, rather than being merely a passive track, is a dynamic medium responsive to binding proteins to enable new forms of collective motor behavior. This talk will also highlight new simulation methods for modeling microtubule crosslinking and sliding.

SG52

Microtubule Dynamics During Plant Cell Division Plane Orientation

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Cell divisions are accurately positioned to generate cells with the correct size and shape. In plant cells, the new cell wall is built in the middle of the cell by vesicles trafficked along an antiparallel microtubule and microfilament array called the phragmoplast. The phragmoplast expands towards a specific location at the cell cortex called the division site, but how it accurately reaches the division site is unknown. We observed microtubule arrays that accumulate at the cell cortex during the anaphase to telophase transition in maize epidermal cells. Before the phragmoplast reaches the cell cortex, these cortical-telophase microtubules transiently interact with proteins at the division site, including the microtubule-binding protein TANGLED1. Microtubule plus-end pausing at the division site aligns the cortical-telophase microtubules perpendicular to the division site. The cortical-telophase microtubules are incorporated into the phragmoplast as it reaches the cell cortex primarily by parallel bundling. Microtubule addition into the phragmoplast positions it accurately to reach the division site. This suggests that division site localized proteins are critical for organizing cortical-telophase microtubules to mediate phragmoplast positioning as it reaches the cell cortex.
SG53

Cytoplasmic dynein-1 cargo diversity is mediated by the combinatorial assembly of FTS-Hook-FHIP complexes

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In eukaryotic cells intracellular components are organized in space and time by the microtubule cytoskeleton and defects in this process are linked to neurodevelopmental and neurodegenerative diseases. Cargos that move on microtubules are connected to cytoplasmic dynein-1 (dynein) and kinesins via adaptor proteins. While ~40 kinesins transport cargos toward the plus end of microtubules, a single dynein is responsible for cargo transport in the opposite direction. How a single dynein transports a wide variety of different cargos is unknown. A cargo adaptor complex known as the FTS-Hook-FHIP (“FHF”) complex was shown to play a role in dynein-cargo interactions in mammalian cells and fungi. Here, we identify distinct FHF complexes composed of FTS, Hook (Hook1, 2 or 3) and FHIP (FHIP1A, 1B, 2A or 2B) proteins. Using proximity-dependent biotinylation and mass spectrometry we determine the protein interactomes of each FHIP protein. Using live-cell imaging and in vitro reconstitution we show that different FHF complexes associate with distinct motile cargos. Complexes composed of FTS, FHIP1B, and either Hook1 or Hook3 co-localize with Rab5-tagged early endosomes. We reconstitute these interactions in vitro, showing that FHIP1B directly interacts with GTP-bound Rab5. In contrast, complexes composed of FTS, FHIP2A and Hook2 co-localize with Rab1A-marked ER-to-Golgi cargos and FHIP2A is important for the formation and motility of Rab1A-marked membrane tubules. Our findings suggest that the combinatorial assembly of different FTS-Hook-FHIP complexes is one mechanism dynein uses to achieve cargo specificity.

SG54

Cytoskeletal regulation of a transcription factor by DNA mimicry

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A long-established strategy for transcription regulation is the tethering of transcription factors to cellular membranes. In contrast, the principal effectors of Hedgehog signaling, the Gli transcription factors, are regulated by microtubules in the primary cilium and the cytoplasm. How Gli is tethered to microtubules remains unclear. We uncover DNA mimicry by the ciliary kinesin Kif7 as a mechanism for the recruitment of Gli to microtubules, revealing a new mode of tethering a DNA-binding protein to the cytoskeleton. Gli increases the Kif7-microtubule affinity and consequently modulates the localization of both proteins to microtubules and the cilium tip. Thus, the kinesin-microtubule system is not a passive Gli tether but a regulatable platform tuned by the kinesin-transcription factor interaction. We re-tooled the unique DNA-mimicry-based Gli-Kif7 interaction for inhibiting the nuclear and cilium localization of Gli. This strategy can be potentially exploited for downregulating erroneously activated Gli in human cancers.
Cytoplasmic dynein is essential in motoneurons for retrograde cargo transport that sustains neuronal connectivity. Little, however, is known about dynein’s function on the postsynaptic side of the circuit. Using genetic, immunolocalization, and electrophysiology studies, we have identified distinct postsynaptic roles for dynein at neuromuscular junctions (NMJs). We have found that dynein punctae accumulate specifically on the postsynaptic side of glutamatergic synaptic terminals. Postsynaptic dynein is required for the localization of PI(4,5)P₂, a phospholipid membrane component, and a number of membrane-associated proteins including components of the spectrin cytoskeleton. Skittles, a phosphatidylinositol 4-phosphate 5-kinase that produces PI(4,5)P₂ to organize the spectrin cytoskeleton, also localizes specifically to glutamatergic synaptic terminals and this localization is dynein dependent. Further, depletion of postsynaptic dynein results in enlarged ionotropic glutamate receptor (iGluR) clusters and an increased amplitude and frequency of mEJPs. PI(4,5)P₂ levels do not affect iGluR clustering and dynein does not affect the levels of iGluR subunits at the NMJ, suggesting a unique transport independent function of dynein at the NMJ in clustering iGluRs. As dynein punctae closely associate with iGluR clusters, we propose that dynein physically stabilizes iGluRs at the postsynaptic membrane for proper synaptic transmission.

Regulation of the ciliary proteome by ubiquitin
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Primary cilia organize multiple signaling pathways including phototransduction, olfaction and Hedgehog signaling. A central principle of cilia-based signaling is the dynamic regulation of protein, lipid, and second messenger compositions of the cillum. For instance, G protein coupled receptors (GPCRs) undergo regulated exit from cilia gated by their activations state. In past work, we found that β-arrestin, a sensor of the activation state of GPCRs, directs the ubiquitination of activated GPCRs inside cilia and that ubiquitinated GPCRs are subsequently removed from cilia by the BBSome and the retrograde intraflagellar transport machinery. In other contexts - e.g. the mammalian photoreceptor- ubiquitin marks proteins that accidentally enter cilia and must be removed. The ciliary ubiquitination machinery and the BBSome thus represent a quality control pathway that removes unwanted proteins from cilia either constitutively or on-demand. How ubiquitinated proteins are specifically recognized by the BBSome remains an unsolved question. We know that the ubiquitin chains that are attached to unwanted ciliary proteins are linked at lysine 63 (K63), similarly to proteins destined for lysosomal degradation. However, there are no K63Ub recognizing modules currently known in the ciliary trafficking machinery. We have leveraged proteomics of primary cilia in kidney cells and in photoreceptor to identify candidate UbK63-recognizing proteins and screened these candidates in cell-based assays and in mouse retina. Our studies provide some of the first mechanistic insights into the emerging regulation of the ciliary proteome by ubiquitination.
SG57

Multiple motor effector proteins drive autophagosomal transport in axons


Autophagy is a degradative pathway required for neuronal homeostasis; autophagy defects are observed in neurodegenerative diseases including Parkinson’s and Huntington's disease. In neurons, autophagosomes form constitutively at the axon terminal and mature by fusing with lysosomes during dynein-mediated transport to the soma. However, it is unknown how the dynein-autophagosome interaction is regulated during this maturation. We now identify a series of handoffs between dynein effectors as autophagosomes transit along the axons of primary murine hippocampal neurons. Through both live-cell imaging and proximity ligation assays, we find the scaffold protein JIP1 is significantly enriched on nascent autophagosomes in the distal axon, where it inactivates kinesin to initiate the long-range dynein-driven transport of autophagosomes. The transit of autophagosomes along the axon shaft requires neurodegeneration-associated protein Huntingtin and its interacting partner HAP1. In vitro binding and single molecule motility assays demonstrate that HAP1 enhances microtubule binding of the dynein-dynactin complex and activates dynein-driven motility. HAP1 interacts with components of dynein and dynactin via canonical interaction sites (CC1 box motif and Spindly motif). We also identify a novel dynactin p150\textsuperscript{Glued} binding motif in the HAP/TRAk and HOOK families of dynein effectors. Point mutations in HAP1 that disrupt its dynein-dynactin binding sites have a dominant negative effect on autophagosomal transport. Surprisingly, we find that the related motor scaffolds JIP3 and JIP4, canonically associated with lysosomes, both affect autophagosomal motility in the mid- and proximal axon. However, they perturb autophagosomal motility in opposite ways: JIP3 drives the retrograde motility of autophagosomes while JIP4 appears to activate anterograde motion of autophagosomes, causing increased competition between dynein and kinesin and more frequent pausing. Single molecule motility assays and binding experiments show these two very similar motor effectors function differently, especially in the context of autophagosomes. Finally, while inhibiting autophagosomal transport is known to disrupt their maturation, we now find that inhibiting autophagosomal maturation pharmacologically via Bafilomycin A1 treatment or genetically via a dominant negative syntaxin-17 mutant disrupts the recruitment and activity of dynein effectors. Thus maturation state and cofactor activation are tightly linked. Collectively, these results describe a novel maturation-based motor effector handoff on neuronal autophagosomes key to autophagosomal motility, cargo degradation, and ultimately axonal health. Supported by NIH grant R35 GM126950.

Size, Charge, Force, and Entropy at the Cell-cell Interface

SG58

The molecular basis of cell-cell recognition

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The relationship between the structure and binding properties of various cell-cell adhesion proteins and adhesive specificity will be described. The findings are based on 3D structure determination, biophysical measurements and cell assays, with the design and interpretation of experiments guided by theory and multi-scale simulations. Physical principles underlying specific cell-cell recognition will be discussed with
special emphasis on the molecular basis of neuronal recognition mediated by the clustered protocadherins. A particularly novel finding is that these proteins form ordered structures at the interface between neurons and that these play a crucial role - to be described - in the barcoding of neurons so that each develops a unique identity allowing it to distinguish self from non-self.

SG59

**Structural and functional basis of the transsynaptic teneurin-latrophilin complex**

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Teneurins and latrophilins are both conserved families of cell adhesion proteins that mediate cellular communication and play critical roles in embryonic and neural development. However, their mechanisms of action remain poorly understood. In the past several years, three-dimensional structures of teneurins and latrophilins have been reported at atomic resolutions and revealed distinct protein folds and unique structural features. In this presentation, we will discuss these structures which, together with structure-guided biochemical and functional analyses, provide hints for the mechanisms of trans-cellular communication at the synapse and other cell-cell contact sites.

SG60

**Phase separation in synapse formation and function**

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Emerging evidence indicates that liquid-liquid phase separation, the formation of a condensed molecular assembly within another diluted aqueous solution, is a means for cells to organize highly condensed biological assemblies with broad functions and regulatory properties in different subcellular regions. Molecular machineries dictating synaptic transmissions in both presynaptic boutons and postsynaptic densities of neuronal synapses are such biological condensates. In this talk, I will present our recent work showing how phase separation can build dense synaptic molecular clusters, highlight unique features of such condensed clusters in the context of synapse formation and plasticity, and discuss how aberrant phase-separation-mediated synaptic assembly formation may contribute to dysfunctional signaling in psychiatric disorders.

SG61

**The molecular specificity of JAM-C on cerebellar granule neurons connects adhesive recognition to migration in a mouse model for cerebellar development**

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The laminar organization of neurons in the cerebellum enables cognition and motor function. After birth, cerebellar granule neurons (CGNs) exit their germinal zone in the external granule layer (EGL) via glial-guided migration. How these CGNs decide where and when to start this migration has not been answered because it requires 1) identifying the specific molecular interfaces that initiate this transition and 2) developing the techniques to study these cell-cell adhesions at sufficient resolution in the developing cerebellum. The Solecki lab previously showed that the initiation of EGL exit requires
junctional adhesion molecule (JAM-C) adhesive recognition. JAM-C mediates cell-cell adhesion through lateral (cis) and intercellular (trans) interactions with other JAMs. JAM-C is the only JAM in the CGNs, but its cognate JAM-B and -C receptors are expressed on the Bergmann glia that guide EGL exit. In this work, I replaced the endogenous JAM-C in CGNs with mutant constructs that modulate cis and trans interactions to study how JAM-C adhesion enables CGNs to recognize and migrate along the Bergmann glia. I evaluated the impacts of cis and trans binding on the amount of adhesion by expressing these JAM-C constructs fused to the pH-sensitive fluorescent protein pHluorin. With this tool, I observed that the cis and trans interactions are required for generating larger adhesion plaques, and the loss of these interactions inhibits EGL exit in live cerebellar slice cultures. This supports the hypothesis that JAM-JAM interactions underlie EGL exit. I showed that both cis and trans interactions regulate JAM-C adhesion to JAM-B by imaging complementary adhesion-specific fluorescent probes (JAM-C-pHluorin and JAM-B-SNAPtag labeled with a pH sensitive Janelia Fluor) in a co-culture of CGNs and glia. Comparatively, loss of trans binding is more deleterious. This experiment was used to evaluate adhesion formation but not stability. To measure the relative adhesion stability, I used fluorescence recovery after photobleach (FRAP) to determine that the loss of cis interactions but not trans increased the rate of JAM-C diffusion at CGN adhesions. Together these results suggest a cooperative mechanism by which JAM-C adhesions are initiated in trans and stabilized by cis binding. Expansion Microscopy of the JAM-C adhesions in cerebellar tissue suggest that it orients actin assembly in the CGNs since JAM-C adhesions appeared to exclude clathrin-coated pits. This result shows that JAM-C can also influence the assembly of migration machinery at sites of adhesion. This work is a technologically ambitious approach that examines how the molecular specificity of JAM-C controls where and when CGNs exit the EGL.

SG62
Adherens junction-mediated membrane compartmentalization creates a spatial switch for Notch signaling and function
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Adherens junctions (AJ) juxtapose the surfaces of neighboring cells, coupling them spatially and mechanically and creating two discrete microdomains within each cell membrane. Here, we report that two membrane microdomains organized by AJs serve distinct and necessary mechanistic functions in the sequential molecular processing for Notch activation. Membrane domains outside of AJs are home to Notch ligand-receptor engagement initializing activation. Conversely, membrane domains within AJs serve to concentrate the final cell-surface activation step of Notch-intermembrane proteolysis by localizing γ-secretase activity. AJs induce protein compartmentalization by means of cholesterol-dependent γ-secretase recruitment and size-dependent protein segregation, preventing enzyme-substrate interactions hence suppressing nonspecific activation. Ligand-induced ectodomain shedding of Notch eliminates its size constraint, leading to its translocation into AJs for processing by γ-secretase. This mechanism regulates ventricular zone-neural progenitor cell differentiation in developing mice and modifies the processing of other proteins like APP. Our study suggests an unprecedented role of AJs creating specialized membrane microdomains that choreograph γ-secretase-dependent receptor activation.
**SG63**

**Measuring cell surface molecular heights and barriers to cell-cell contacts**

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The height and flexibility of cell surface proteins has emerged as an important factor in a broad range of cell-cell interactions. Plasma membranes crowded with tall proteins can sterically block binding of short proteins to prevent target recognition by immune cells and can present an energetic barrier that blocks the ability of fusogens to drive cell-cell fusion. This talk will describe a method for measuring cell surface molecular heights on native cell membranes and explore the energetic barriers created by membrane-bound proteins that prevent close contact between membranes. The height measurement method is based on superresolution localization of fluorescent probes at the base and tip of a molecule of interest, and we demonstrate that it can be used to map the surfaces of immune cells and target cells. We also use an in vitro assay to explore how membrane-bound proteins can present a major energetic barrier to fusion beyond that presented by bare membranes. This work demonstrates new quantitative tools to explore the complex role played by cell surface topography at cell-cell contacts.

**SG64**

**The septate junction protein Bark beetle is required for Drosophila intestinal barrier function and homeostasis**

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In the intestine, the epithelial barrier is maintained by tight junctions (TJs) in mammals and septate junctions (SJs) in insects. The intestinal barrier allows paracellular flow of water, ions and nutrients across the epithelium, while maintaining food matter and microbes inside the intestinal lumen. Age-related loss of intestinal barrier function has been found across multiple species, including *Drosophila melanogaster* and humans. The age-related causes of barrier dysfunction remain unknown. The tricellular junction (TCJ) is a specialized region of the SJ where three adjacent cells meet. Previous studies by our lab indicated that mis-localization of the TCJ protein Gliotactin (Gli) is correlated with aging. Depletion of Gli in young flies leads to loss of intestinal homeostasis, including increased intestinal stem cell (ISC) proliferation, a hallmark of aging. In the embryonic epithelium, the TCJ protein Bark beetle (Bark) is required to recruit Gli to the TCJ. We hypothesized that Bark would be required for maintenance of intestinal homeostasis and barrier function, similar to Gli. Indeed, depletion of Bark from the TCJ of enterocytes in a young fly posterior midgut (PMG) increased ISC proliferation, accelerated age-associated intestinal barrier loss, and shortened lifespan, suggesting Bark is required for maintenance of intestinal homeostasis. In accordance with our previous RNAseq data, which indicated that transcription of SJ protein genes is not decreased with age, simple overexpression of Bark does not rescue age-associated loss of barrier function. Notably, unlike in the embryonic epithelium, Gli is required for Bark localization to the TCJ in the PMG. In addition, our data show that Bark becomes mis-localized with age, similar to Gli. Antibody staining for Gli and Bark shows a decrease in intensity at the TCJ in intestines from aged flies, with a modest, but significant, increase in Bark staining at the BCJ and in the cytoplasm. Our findings provide additional evidence that age-related TCJ dysfunction could be due to a decrease in TCJ proteins at the TCJ. In summary, the TCJ protein Bark is required at the TCJ to maintain intestinal homeostasis and barrier integrity in *Drosophila*. Our work on the mechanisms leading
to loss of the intestinal barrier will provide insight into strategies to treat age-related gastrointestinal diseases, such as cancer.

SG65
A positive feedback loop between mesendoderm cell migration and interstitial fluid relocalization is required for embryonic axis formation in zebrafish
K. Huljev, C. Heisenberg; Inst Sci/Technol Austria, Klosterneuburg, AUSTRIA.

Accumulation of interstitial fluid (IF) between embryonic cells is a common phenomenon in vertebrate embryogenesis. IF has long been speculated to play a role in embryo patterning and morphogenesis, but direct evidence supporting such functions is still sparse. Here we show that the relocalization and accumulation of IF ahead of the migrating anterior axial mesendoderm (prechordal plate, ppl) is critical for ppl cell protrusion formation and migration, a key process in embryonic axis formation. We further show that ppl cell migration and IF accumulation are engaged in a positive mechanical feedback loop, where internalized ppl tissue, moving in between the epiblast and yolk cell, compress the overlying epiblast tissue, causing IF to relocate from the epiblast surface to the epiblast-yolk cell interface directly ahead of the advancing ppl. This accumulation of IF, in turn, facilitates ppl cell protrusion formation and migration by opening up the space into which the ppl moves, and thus the ability of the ppl to trigger the relocalization of IF by pushing against the overlying epiblast. Thus, embryonic axis formation relies on a positive mechanical feedback loop between tissue movement and IF relocalization.

SG66
The distinct roles of cell and tissue mechanics in vertebrate body axis elongation
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During embryonic development, cells physically interact with each other to sculpt tissues and organs. While cells control their individual mechanics, it is the emergent mechanics at the supracellular scales that determines tissue flows and guides morphogenesis. In this talk, I will discuss the specific roles of cell- and tissue-scale active stresses in the formation of the zebrafish body axis. I will also present a new numerical framework (Active Foam Model) that allows us to simulate tissue dynamics and study the connection between cell behavior and the emergent tissue mechanics at supracellular scales.

SG67
Physical regulation of intercellular communication by the cellular glycocalyx
M. Paszek; Cornell University, Ithaca, NY.

Generation of membrane curvature is compulsory for some of life’s most sophisticated forms of intercellular communication. For instance, cells bend their membranes into spherical vesicles and thin, finger-like projections to package and deliver complex messages to other cells. We have learned that cells can bend their membranes into such forms through assembly of membrane-anchored biopolymers in the cellular glycocalyx. Like a compressed gas hovering over the membrane, flexible glycocalyx polymers generate a pressure that makes the formation of curved membrane features easier. Since the physical behavior of the glycocalyx ensemble is well described by theories from polymer physics, we can predict how to trigger specific membrane morphologies simply by editing the types and numbers of
polymers in the glycocalyx. To do so, we are beginning to adopt powerful strategies from synthetic biology to create a systematic approach for glycocalyx engineering. We design and genetically encode new biopolymers with the specific intent to manipulate the chemical and physical structure of the glycocalyx. Beyond understanding membrane shape regulation, these tools are providing new insights into how the glycocalyx controls intercellular interactions, including recognition and killing of tumor cells by immune cells.

TUESDAY, DECEMBER 7, 2021

Building the Cell

SG68
Phase behavior and function of mitochondrial transcriptional condensates
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Phase separation is involved in organizing many cellular structures, including genomes. The contents of human mitochondria, primarily their genome (mitochondrial DNA, mtDNA) and their RNA granules, assemble via phase separation in the mitochondrial matrix. We have previously shown that the main packaging protein TFAM mediates the in vitro and in vivo condensation of heterogenous, viscoelastic droplets, constituting mt-nucleoids. However, the functional relevance of concentrating the mitochondrial transcriptional machinery into condensates as opposed to keeping the components dilute in the cell is unclear. We have used mitochondrial transcriptional condensates as a model system to probe the functional properties of multi-component — DNA-RNA-protein — condensates. We found that purified core mitochondrial transcriptional machinery, including template mtDNA, the polymerase POLRMT, and transcription factor TFB2M, partitioned with TFAM to form highly heterogenous, viscoelastic droplets in vitro, which recapitulated the dynamics and behavior of mt-nucleoids in vivo. We showed that transcription occurred within the mitochondrial condensates in vitro, that the kinetics of RNA production were dampened within condensates, and that components had slow diffusivities. A strong structure-function relationship of the condensate is indicated by the fact that de novo RNA production led to significant structural changes, whereby droplets transitioned into vesicle-like structures. Computational simulations and perturbation of mito-transcription in vivo recapitulated the transcription-dependent reorganization observed in vitro. We conclude that the mitochondrial transcriptional machinery thus serves as a model system for the biophysics and kinetics of transcriptional condensates. Our results point to phase separation as an evolutionarily conserved mechanism of genome organization and function.

SG69
Entangled Architecture of Rough Endoplasmic Reticulum (RER) and Vacuoles Enables Topological Brakes in Cytoplasm of an Ultra-fast Giant Cell
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Understanding extremes in biological systems provides novel insights into the fundamental limits of life. To study intracellular adaptations under extreme forces generated by giant cells, we study the ultrafast
contractions (50% body length contraction within 5-10 msec, peak acceleration 15g) in *Spirostomum ambiguum* as a model system. How can a single cell adapt to such large acceleration repeatedly over its lifetime without sub-cellular damage? Utilizing transmitted electron microscopy (TEM) and confocal imaging, we discover a novel fenestrated cubic membrane architecture of rough endoplasmic reticulum (RER) wrapping around vacuolar meshwork forming three-dimensional sheets spanning the entire cell, in both relaxed and contracted states. The RER forms a continuous lumen wrapping around vacuoles with a nearly uniform inter-organelle spacing of 60nm. We explore the mechanical role of the entangled RER-vacuole architecture to understand how giant cells dissipate energy in such a short time scale (5-10 msec). We use a simple model with an overdamped molecular dynamics simulation where the RER-vacuolar meshwork is captured as hard colloid particles entangled with inextensible strings, undergoing large deformation. Our simulations reveal that the topological confinement can induce jamming at a volume fraction significantly below critical value while preserving spatial relationships among vacuoles and reduce the peak kinetic energy in the system. Our findings suggest a new role of RER-vacuolar meshwork in a giant cell, *Spirostomum ambiguum*, which can be considered as a metamaterial that applies topological brakes, dampening the intra-cellular motion and preserve the spatial architecture of organelles under extreme motility events.

SG70

**Mitotic Spindle Chirality Provides a Passive Mechanical Response to Forces and Depends on Microtubule Motors and Crosslinkers**

**M. Trupinic**, B. Kokanovic, I. Ponjavic, I. M. Tolic; Ruder Boskovic Institute, Zagreb, CROATIA.

Mechanical forces produced by motor proteins and microtubule dynamics within the mitotic spindle are crucial for the movement of chromosomes and their segregation into the emerging daughter cells. In addition to linear forces, rotational forces or torques are present in the spindle, reflected in the left-handed twisted shapes of microtubule bundles that make the spindle chiral. However, the biological role and molecular origins of spindle chirality are unknown. By developing methods to measure spindle twist, we show that spindles have highest twist values at the beginning of anaphase. To test whether the spindle reacts to an external force by changing the twist, we compressed the spindles along its long axis, which indeed resulted in stronger left-handed twist. Inhibition or depletion of motor proteins that perform chiral stepping, Eg5/kinesin-5 or Kif18A/kinesin-8, decreased the twist, suggesting that these motors regulate twist by rotating microtubules around one another within the bridging fibers or at the spindle pole. Depletion of the microtubule crosslinker PRC1 and the nucleator augmin decreased the left-handed twist or even caused right-handed twist, which indicates that PRC1 contributes to the twist by constraining free rotation of microtubules within the bridging fibers, and augmin by nucleating bridging microtubules. Overall, round spindles were more twisted than elongated ones, implying a correlation between bending moments and twist. In conclusion, spindle twist is largely dependent on the shape of the spindle and controlled by molecular activities within the bridging fibers and at the spindle poles. We propose a physiological role for spindle chirality in providing a passive mechanical response to forces, decreasing the risk of spindle breakage under high load.
SG71

**Cytoplasmic Trade Winds Push Actin Polymerization to the Leading Edge**

**C. G. Galbraith**, U. Boehm, J. A. Galbraith; 1OHSU, Portland, OR, 2Janelia Research Campus, Ashburn, VA.

Net polymerization at the front of treadmilling actin networks is necessary for cell protrusion and migration. The biochemistry underlying treadmilling -- polymerization of monomer at one end of filaments and depolymerization at the other -- is so well understood that it has been reconstituted in-vitro. However, inside the cell, sustaining this cycle of network treadmilling implies recycling monomers. How depolymerized monomer is moved to sites of new filament growth at the cell front is unknown. Here we report that monomer is transported to the cell front by advection, diffusion enhanced by cytoplasmic fluid flow. Advection is restricted to the lamella, which our 3D single-molecule reconstruction of the actin cytoskeleton reveals is an isolated compartment, separated from the rest of the cytoplasm by a semi-permeable actin-myosin wall. Myosin II contraction at the wall creates a cytoplasmic flow that is non-specific. The flow moves actin binding, adhesion, and even inert proteins forward. Behind the wall, in the cell body, diffusion dominates protein movement. Our results indicate the lamella functions as a novel “membrane-less” cytoplasmic compartment; with a specific cellular function: continuously pushing proteins forward to spatially bias protrusion and forward migration.

SG72

**A pooled single-cell CRISPRi screen for regulators of U2OS cell shape and actin organization**

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Image-based genetic screens are powerful platforms for identifying novel regulators of cell properties and behaviors. While image-based screens are typically arrayed (i.e. perturbations are kept in separate wells), recent technologies have enabled a pooled format that promises to increase scalability and reduce technical variability. We conducted a pooled image-based CRISPRi screen to identify regulators of U2OS cell shape and actin organization in a targeted library of 1118 guide RNAs spanning 366 genes. The large, widely varied shapes of U2OS cells provide an ideal system to study genetic determinants of complex morphological states. First, to read out the guide RNA in each cell, we optimized in situ sequencing technologies to maximize the efficiency and confidence of DNA barcode readout from fixed cells. Second, we captured the phenotype and genotype of 1.5 million cells by imaging the actin cytoskeleton, nucleus, and DNA barcodes, and successfully assigned guide RNAs to 86% of cells. A persistent challenge in image-based screens is how to rigorously describe high-dimensional phenotypes. To address this, we extracted single-cell morphological profiles with CellProfiler and captured the key axes of variation using principal component analysis (PCA). We identified genes producing distinct shifts along these axes upon knockdown and quantitatively represented their phenotypes by computing linear discriminant axes. Visualizing cells that closely traverse these axes enables systematic interpretation of high-dimensional perturbation effects. Finally, we compared this approach with a complementary method for dimensionality reduction that avoids human-selected features: a convolutional neural network autoencoder applied directly to single-cell images. These approaches revealed multiple classes...
of morphological phenotypes, arising from knockdown of expected and new regulators. For example, we identified a large cluster of hits associated with contractility, including non-muscle myosin IIA (MYH9), and another with hits associated with adhesion, like vinculin (VCL). The clustering of genes, such as MYH9 and SET domain protein 3 (SETD3), suggests novel functional interactions. Furthermore, we found phenotypes distinct from these two main clusters, such as the elongation and aberrant actin distribution resulting from alpha II-spectrin (SPTAN1) knockdown. The quantitative dissection of relationships between complex phenotypes reveals how different molecular regulators act along specific axes to control cell morphological variation. Though developed to characterize hits in a large-scale screen, our approaches for systematically identifying and visualizing high-dimensional image phenotypes can be applied to diverse cell biological datasets.

SG73
Measuring the Spatial Organization of Signaling on the 3D Cell Surface
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The cell membrane is a highly dynamic structure, serving as a platform for organizing cell signaling. The spatial scale of signaling organization on the plasma membrane ranges from local nano- and microscale clusters to global bursts of signal that span the entire cell. The convoluted morphology of cells complicates the quantitative analysis of signaling patterning on the 3D cell surface. We recently demonstrated how to map fluorescence signals proximate to the 3D cell surface onto the surface itself, representing the cell as a triangle mesh and correcting for microscopy-induced artifacts. Here we describe how to extract the spatial scales at which these signals are organized, doing so in a way that produces both a readily interpretable measure and a mathematically complete dimensionality reduction of signaling on the 3D cell surface. To evaluate spatial organization on the irregular manifold of the cell surface, we employed the Laplace-Beltrami operator, which is conceptually similar to the Fourier transform. However, unlike the Fourier transform which always uses the same sine-like basis, the Laplace-Beltrami operator generates an orthonormal basis specific to each cell’s geometry. We projected surface signaling into the Laplace-Beltrami basis, and then characterized the signal’s spatial scales via the Dirichlet energy measured across a narrow range of Laplace-Beltrami modes. The Dirichlet energy describes how variable the gradient of a function is over a particular geometry. We applied our algorithm to diverse data sets including synthetic images, high-resolution light-sheet microscopy images, and high-throughput confocal data sets. In particular, we compared the spatial scales of simulated polka dot patterns on synthetic spherical versus elongated cells and real blebby versus lamellipodial cells. We found that our extracted spatial signaling scales are independent of cell morphology, and thus establish a framework that can be used to compare the organization of signaling across diverse cell types and conditions. For example, we measured the spatial scales of PI3-Kinase activity on the 3D surface of MV3 melanoma cells before and after PI3-Kinase inhibition. PI3-Kinase polarizes in a front-to-back fashion, a configuration that we would expect to have a low spatial frequency. As expected, we found that PI3-Kinase activity could largely be recapitulated via low modes, and that inhibiting PI3K substantially reduced polarization. Our computational framework extracts spatial signaling patterns even on highly convoluted 3D morphologies, enabling the investigation of cells in physiologically relevant environments, as well as the analysis of morphology-signaling coupling.
Mapping cell structure across scales by fusing protein images and interactions

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The eukaryotic cell is a multi-scale structure with modular organization across at least four orders of magnitude. Two central approaches for mapping this structure - protein fluorescent imaging and protein biophysical association - each generate extensive datasets but of distinct qualities and resolutions that are typically treated separately. Here, we integrate immunofluorescent images in the Human Protein Atlas with ongoing affinity purification experiments from the BioPlex resource to create a unified hierarchical map of eukaryotic cell architecture. Integration involves configuring each approach to produce a general measure of protein distance, then calibrating the two measures using machine learning. The evolving map, called the Multi-Scale Integrated Cell (MuSIC 1.0), currently resolves 69 subcellular systems of which approximately half are undocumented. Based on these findings we perform 134 additional affinity purifications, validating close subunit associations for the majority of systems. The map elucidates roles for poorly characterized proteins, such as the appearance of FAM120C in chromatin; identifies new protein assemblies in ribosomal biogenesis, RNA splicing, nuclear speckles, and ion transport; and reveals crosstalk between cytoplasmic and mitochondrial ribosomal proteins. By integration across scales, MuSIC substantially increases the mapping resolution obtained from imaging while giving protein interactions a spatial dimension, paving the way to incorporate many molecular data types in proteome-wide maps of cells.

Uncovering Mechanistic Rules that Drive Emergent Cell Shape and Colony Dynamics Through Agent-Based Modeling

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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. One way we address this goal is by developing modular, open-source computational models of cell behavior that can be adapted to study various contexts and broadly utilized by the community. Here, we use agent-based modeling (ABM)—an intuitive, bottom-up modeling framework in which autonomous agents follow rules guiding their actions and interactions within a dynamic environment—to uncover fundamental rules driving hiPS cell dynamics. We integrate an existing ABM framework (https://github.com/bagherilab/ARCADE) with a 3D Cellular Potts-based representation of cell shape to study underlying rule-based mechanisms driving multi-scale, emergent hiPS cell shape and colony dynamics. We perform sensitivity analyses across relevant temporal and spatial resolutions, differential adhesion, and framework-specific parameters on a simple model with basic rules guiding cell proliferation and apoptosis. Using a spherical harmonics expansion, we quantify simulated cell shape and apply dimensionality reduction methods to compare simulated shape modes with experimentally observed values. Using cluster and network analysis, we quantify simulated colony dynamics and compare observed trends to experimental observations.
Similarities between simulated and experimental cell populations identify possible explanatory mechanistic rules while differences suggest the need to modify rules in order to capture observed emergent phenomena. For example, the inability to recreate specific morphologies in simulation (e.g., cell “tilting”) suggests that certain parameters (e.g., adhesion) could introduce cell polarity and correct the disparity between simulation and observation. The tight integration of model development, experimental observation, and expert insight enables hypothesis generation, guides experimental design, and facilitates interdisciplinary collaboration. The modular framework can be easily adapted to interrogate diverse scales, behaviors, and systems, from stem cell differentiation to wound healing.

SG76
Emergence of synchronized multicellular mechanosensing from spatiotemporal integration of heterogeneous single-cell information transfer
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We quantitatively characterize how noisy and heterogeneous behaviors of individual cells are integrated across a population toward multicellular synchronization by studying the calcium dynamics in mechanically stimulated monolayers of endothelial cells. We used information-theory to quantify the asymmetric information-transfer between pairs of cells and define quantitative measures of how single cells receive or transmit information in the multicellular network. We find that cells take different roles in intercellular information-transfer and that this heterogeneity is associated with synchronization. Cells tended to maintain their roles between consecutive cycles of mechanical stimuli and reinforced them over time, suggesting the existence of a cellular “memory” in intercellular information transfer. Interestingly, we identified a subpopulation of cells characterized by higher probability of both receiving and transmitting information. These “communication hub” roles were stable - once a cell switched to a “communication hub” role it was less probable to switch to other roles. This stableness property of the cells led to gradual enrichment of communication hubs that was associated with the establishment of synchronization. Our analysis demonstrated that multicellular synchronization was established by effective information spread from the (local) single cell to the (global) group scale in the multicellular network. Altogether, we suggest that multicellular synchronization is driven by single cell communication properties, including heterogeneity, functional memory and information flow.

Cells in the Wild: Environmental Influences on Cell Morphology and Behavior

SG77
Variation in resistance of Paramecium cells to an endonuclear parasite: geographic and lineage-specific patterns
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Resistance to infection can vary in hosts across both geographical and genetic distance, shaped by local interactions between hosts and parasites and by genetic background. We quantified the amount and distribution of variation of the trait of resistance to Holospora infection in a worldwide collection of strains of its ciliate host, Paramecium caudatum, by using controlled inoculations in experimental mass cultures. Paramecium cells can be resistant to the Holospora parasite at several points in the infection process, over several days. We measured infection prevalence at early (1 week post-inoculation) and late (2-3 week) timepoints, by scoring for presence of the bacterial cells in the nucleus of the host. Experiments were performed in replicates and across two labs. We found substantial variation in resistance among strains, and determined that the trait of resistance was heritable, with upper-bound heritability estimates greater than 0.5. Strain estimates of resistance were repeatable between laboratories and ranged from total resistance to near-complete susceptibility. We found that early measurements provided higher estimates of resistance heritability than did later measurements, possibly due to diverging epidemiological dynamics in replicate cultures of the same strains. Genetic distance (based on a neutral marker) was positively correlated with the difference in resistance phenotype between strains \( (r = 0.45) \), essentially reflecting differences between highly divergent clades within the host species. Clade A strains, mostly European, were less resistant to the parasite overall than non-European clade B strains. At a smaller geographical scale (within Europe), strains that are geographically closer to the parasite origin (Southern Germany) were more susceptible to infection than those from further away. These patterns are consistent with a picture of local parasite adaptation.

**SG78**

**Single-cell transcriptomics and genomics are key methods to understanding the evolutionary diversity of protists**

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Our study and understanding of eukaryotic microbes (protists), and in particular their transcriptomes and genomes, has historically been predicated on our ability to culture these organisms. This is time-consuming and thus expensive, and at worst can be extremely difficult—the majority of protists are thought to be free-living phagotrophs, feeding on other protists or prokaryotes. Protists also are extremely diverse in their morphology, exhibiting for example many different kinds of locomotion. Ultimately only a small fraction of protists have ever been brought into culture to be studied more closely, understating the diversity that is found in many protist groups. In the last decade, single-cell transcriptomics and genomics methods have enabled study of protist groups without the need to culture. Applying single-cell methods on cells isolated from environmental samples enables the collection of molecular data, linking it to microscope imagery. Single-cell transcriptomic data can be used in a variety of ways, but one current focus in the field is understanding the evolutionary diversity of understudied protist taxa and their phylogenetic placement. Euglenids are one such group, with little molecular data outside their heavily-studied phototrophic species available (e.g. *Euglena gracilis*). Their phagotrophic counterparts are phylogenetically more diverse and show large morphological variation, yet very little molecular data has been collected, leaving their evolutionary history in particular convoluted. Single-cell transcriptomes generated from phagotrophic euglenids are complete enough to be used in multigene phylogenies, showing that they are basal to phototrophic euglenids, and fall into several evolutionary diverse subgroups, most of which have only superficial morphological similarities. While single-cell genomics are inherently more difficult due to the structure of eukaryotic genomes, they can still be a valuable tool in investigating protists organelar genome diversity. Single-cell genomics
has been used on several cell-sorted free-living cells, phylogenetically placing them among several
groups of Euglenozoa, including euglenids and kinetoplastids. Interestingly this data also enabled partial
reconstruction of mitochondrial genomes, which helps our understanding of the convoluted nature of
euglenozoan mitochondrial genomes and their evolution.

SG79

Hydrodynamic influence of extracellular structures in heterotrophic flagellates
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Most eukaryotic organisms have motile flagella, also called cilia when appearing in many numbers.
Flagella have a conserved basic structure but serve many different purposes, from propulsion in
unicellular protists and spermatozoa, to transport functions in ciliated epithelia in mammalians and
resource acquisition in flagellates. In heterotrophic flagellates, flagella are crucial for generating efficient
and sufficient feeding currents, a requirement which has potentially given rise to construction of diverse
extracellular structures either around the cell body or on the flagella in response to the associated
hydrodynamic conditions. Some cells such as choanoflagellates are collared, and some
choanoflagellates, in addition, construct a very ornate basket-like structure around the cell, known as
the lorica, which potentially increases the prey capture efficiency. In many unicellular protists, the
flagellum itself is equipped with either a vane, fibers of glycoly, or thick, rigid, and tubular hairs.
Here, we show that hydrodynamic interactions between the collar and vane in choanoflagellates, and
between hairs in hairy flagellates are key to generating sufficient feeding current. Finally, in choanocyte
chambers of sponges with many beating flagella, we show how the presence (and the absence) of a
gasket-like structure, forming a canopy above the collar filters, ensures efficient pumping and filtration
in sponges with different body types. At low Reynolds number, viscosity impedes predator-prey contact,
but the presence of these extracellular structures improves the flagellar flow-driving force and structure
of the feeding current to secure the success and key role of heterotrophic flagellates in the microbial
food webs.

SG80

The Effect of Mucus-like Viscoelastic Stress on Giardia Growth
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Microorganisms’ ability to navigate and survive in mucus, a viscoelastic, non-Newtonian fluid, often
holds the key to infection. Giardia lamblia is a flagellated mucus-interacting intestinal protozoan parasite
that infects both humans and other mammals. However, most in vitro cultures and experiments in
Giardia research are conducted in low viscosity, Newtonian culture media, or buffered salt solutions.
Thus, there is insufficient understanding of how the mechanical and chemical components of mucus act
in concert to affect the behaviors of Giardia. Here, we investigate how Giardia behavior changes in
viscoelastic environments created by the addition of biocompatible polymers to the regular culture
media. The polymer provides tunable viscoelasticty depending on its concentration in solution, allowing
a viscoelastic range consistent with the gastrointestinal mucosal environment. We performed these
experiments in a novel micro-culture chamber system that permitted systematic imaging of the culture surfaces. First, we found no significant change in cell doubling time between the Newtonian and the viscoelastic media. Furthermore, in traditional Newtonian culture media, cells attach predominantly to the floor of culture chambers, while in viscoelastic media, we found a significant fraction of cells attached to the ceiling of the culture chambers. Finally, Giardia grow evenly dispersed in Newtonian media, whereas in viscoelastic media, Giardia grow in aggregates. This finding supports previous in vivo research showing Giardia colonize primarily in high-density foci in the duodenum of mice. In addition, preliminary transcriptome analyses indicate a relatively small number of genes with expression levels that are significantly altered in viscoelastic cultures, with surface proteins as the most prominent gene category. The absence of a growth rate effect, coupled with the aggregate formation, suggests a nuanced effort to conserve energy. Our studies are the first effort in the field to investigate how viscoelastic materials alter giardia growth patterns and transcriptome. Our results provide insights into cell responses to mechanical stresses, especially with respect to mucosal environments.

SG81

**Molecular Swiss Army Knives: Tardigrade CAHS Proteins Mediate Desiccation Tolerance Through Multiple Mechanisms.**

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Tardigrades, also known as water bears, make up a phylum of small, but robust animals renowned for their ability to survive extreme stresses including desiccation (near complete drying). How tardigrades survive desiccation is one of the enduring mysteries of animal physiology. CAHS proteins are a family of tardigrade specific intrinsically disordered proteins. CAHS proteins are necessary for tardigrades to robustly survive desiccation, increase desiccation tolerance when heterologously expressed, and protect sensitive biological material from extreme drying in vitro. We show that CAHS D, an intrinsically disordered protein belonging to a unique family of tardigrades proteins, undergoes a liquid-to-gel phase transition in a concentration dependent manner. We identify a mechanism of gelation for CAHS D, which, distinct from other gelling proteins such as gelatin, relies on intermolecular beta-beta interactions. Gelation of CAHS D promotes the slowing of diffusion and coordination of residual water. Slowed diffusion and increased water coordination correlate with the ability of CAHS D to present protein unfolding during desiccation. Conversely, slowed diffusion and water coordination do not correlate with the prevention of protein aggregation during drying. Our study demonstrates that distinct mechanisms are required for holistic desiccation tolerance, and that protectants, such as CAHS D, can act as ‘molecular swiss army knives’ capable of providing protection through several different mechanisms simultaneously.

SG82

**Cold-responsive progenitors from the vascular smooth muscle lineage are a critical source of thermogenic adipocytes**

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The regulation of body temperature, thermoregulation, is a fundamental homeostatic process in warm-blooded organisms. Brown adipose tissue (BAT) is a specialized type of adipose that is primarily responsible for regulating body temperature through adaptive thermogenesis. Brown adipocytes have an exceptional ability to oxidize biological fuels and generate heat to maintain euthermia in a cold environment. Harnessing the potential of thermogenic adipocytes to enhance energy expenditure offers an attractive strategy against obesity and metabolic disorders. Prolonged cold exposure promotes de novo recruitment of brown adipocytes to maximize thermogenesis. However, the cellular source and pathways involved in the expansion of the thermogenic adipocyte pool have remained elusive. Using single-cell transcriptome analysis of human and murine BAT, we have recently identified a previously unrecognized population of cold-responsive adipocyte progenitors derived from the vascular smooth muscle (VSM) lineage. VSM-derived adipocyte progenitors (VSM-APCs) express the temperature-sensitive cation channel, Trpv1. Lineage tracing studies revealed that the Trpv1pos cells are a distinct population of adipocyte progenitors that contribute to the brown adipocyte pool in vivo. Cold exposure promotes the proliferation and differentiation of Trpv1pos progenitors into highly thermogenic adipocytes. Selective inhibition of de novo adipogenesis from Trpv1pos progenitors impaired the cold-induced development of thermogenic adipocytes, indicating the critical contribution of VSM-APCs to adipose cold adaptation and thermogenesis. Analysis of the ligand-receptor interactions in adipose tissue identified the paracrine signaling network mediating the crosstalk between the VSM and other adipose resident cells. Together, this work established a new paradigm for cold-induced thermogenesis that relies on a reserved pool of thermogenic adipocyte progenitors stimulated specifically in response to cold. This new model for the development of thermogenic adipocytes could be critical in designing strategies to target thermogenic adipose tissue as a therapeutic approach for obesity and its metabolic sequelae.

SG83
Chemotactic responses in infectious life stages of the fungal pathogen B. dendrobatidis alter spore distribution throughout the environment.
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The freshwater fungus Batrachochytrium dendrobatidis (Bd) is a lethal pathogen responsible for an ongoing pandemic devastating frog populations globally. B. dendrobatidis belongs to a group of fungi known as chytrids, which are prolific parasites across all branches of the tree of life. Bd’s infectious life-stage is a highly motile, unicellular spore capable of both flagellar swimming and actin-based crawling. Understanding the infection process of Bd will help untangle the ongoing ecological disaster while allowing us to better respond to future emerging chytrid pathogens like B. salamandrivorans. Host-parasite encounter rate is a critical parameter in disease ecology because even small biases in these rates have incredible consequences on the frequency of reinfection, rate of spread, parasite load, and host mortality. Little is currently known about how Bd locates and recognizes its hosts while navigating through the water column. Here we show that Bd responds to a variety of chemical cues by altering patterns of stereotyped swimming behaviors. Our quantification of Bd motility — both swimming and
crawling — reveals distinct patterns of “searching” motility in response to different classes of chematoattractants. Our results suggest chemotaxis by Bd sores can skew the host-parasite encounter rate, likely contributing to its devastating success as a pathogen worldwide. Understanding the mechanisms underlying Bd chemotaxis will illuminate the adaptations making Bd such a successful pathogen while opening the door for a better understanding of chytrid disease across the tree of life.

SG84

Hydrostatic pressure drives cell separation and fluid uptake during wound healing in zebrafish epidermis

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The skin epithelium acts as the barrier between an organism's internal and external environments, which in freshwater organisms requires withstanding a large osmotic pressure differential. During wound healing this barrier is breached, and it is not clear how epithelia can rapidly adapt to the large environmental disruption caused by the mixing of internal and external fluids of vastly different osmotic composition. The epidermis of the larval zebrafish has a superficial outer layer of cells and a basal layer of cells beneath that rests atop a collagenous extracellular matrix. After wounding, the superficial epidermal cells on the wound margin form a contractile actomyosin cable that constricts and cinches the wound closed, while the basal epidermal cells rapidly migrate toward the wound margin. We have observed that, following acute injury, the basal epidermis undergoes a dramatic process of cell separation that resembles hydraulic fracturing. This separation starts in basal cells nearest the wound and then propagates at a constant rate through the tissue spanning several hundred microns, all while the periderm remains intact. Electron microscopy analysis suggests that basal cells remain connected via thin tethers composed of desmosomes and adherens junctions, while large gulfs (>1 µm) appear between the bulk cytoplasm of neighboring cells. Separation is completely inhibited by incubation in external media isotonic with interstitial fluid, suggesting that osmotic pressure gradients drive separation. Subsequent to the separation event, large macropinosomes ranging from 1 to 5 µm are formed in the basal cells. These macropinosomes engulf fluorescent dextran added to the external medium, confirming that they contribute to clearance of external fluid from the body of the wounded animal. This process partially depends on myosin II activity, as fish treated with blebbistatin exhibit reduced propagation of separation away from the wound, as well as fewer macropinosomes. We conclude that excess external fluid entry through the wound and subsequent closure of the wound through actomyosin purse string contraction in the peridermal cell layer causes fluid pressure buildup in the extracellular space of the fish epidermis. This excess fluid pressure causes cells to separate and eventually the fluid is cleared through macropinocytosis.

SG85

Phenotyping perceptive protists

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All lifeforms are environmentally intelligent. Even single-cells sense and respond readily to environmental changes, especially in the wild. In mobile organisms, behavioural responses can be
tracked with high-speed imaging and computer vision, using movement as a dynamic read-out of behaviour and physiology. Here we devise a novel droplet microfluidics assay to encapsulate protists and track their motility patterns over long timescales of hours. We compare and contrast two species of green algae, *C. reinhardtii* - a freshwater biflagellate, and *P. octopus* - a marine octoflagellate, to reveal their surprisingly stereotyped behaviours and emergence of distinct motility macrostates. We further evolve our channel design to deliver on-demand environmental perturbations, to capture in real-time how single cells respond to sudden changes in their microhabitat. By coupling single-cell entrapment with unprecedented tracking resolution and duration, our approach offers unique and potent opportunities for diagnostics, drug-screening, and for querying the genetic basis of organismal behaviour.

Chromosome Dynamics and Aneuploidy in Development

SG86

**Chromatin compartment formation via chromatin-binding phase-separating proteins**

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Chromatin compartments are areas of the nucleus organized into functional domains that contain specific chromatin regions, like heterochromatin or super-enhancers. It has been postulated that the biophysical process of phase separation organizes these compartments, but the role of chromatin binding in targeting and resultant phase behavior have yet to be explained. Here we investigated the role of chromatin binding on phase separation and compaction of two abundant chromatin binding/phase separating proteins, BRD4 and HP1a. BRD4 is a bromodomain-containing protein of the BET family that binds acetylated histones and promotes transcription by localizing to enhancers and recruiting Mediator, while HP1a is a chromodomain-containing protein essential for constitutive heterochromatin that binds to methylated histones and maintains transcriptional silencing of repetitive loci. Using the Corelet system in human cancer cells, we find that the phase diagrams of full-length chromatin binding proteins BRD4 and HP1a do not have a clear boundary at low valencies, perhaps because their binding to multivalent chromatin loci can compensate. Interestingly, disrupting BRD4 chromatin binding through a small molecule inhibitor, JQ1, or HP1 chromatin binding via a point mutation in the chromodomain results in more canonical phase diagrams that have a clear valence distinction. Additionally, these constructs with disrupted chromatin binding form fewer, larger droplets that are not tethered to chromatin loci, suggesting that interaction with chromatin may regulate size, number and positioning of phase-separated chromatin domains. Next we studied the role of targeting specificity by swapping BRD4’s acetyl-binding bromodomain with HP1’s methyl-binding chromodomain. With these swapped constructs, we find that HP1 droplets targeted to acetylated chromatin are capable of reversibly compacting these regions, and BRD4 droplets targeted to methylated regions can reversibly decompact these regions. We postulate that chromatin domains are created by like-like interactions between phase-separation-prone proteins that are targeted to specific loci through interactions with epigenetic marks or specific DNA sequences, and are functionalized through secondary recruitment of enzymes. Understanding the formation and function of these chromatin domains is important because BRD4 and other chromatin-binding/phase-separating proteins are commonly mutated in cancers. Disrupting chromatin binding of BRD4 in cancer cells via JQ1 has therapeutic effects, suggesting that altering phase separation of chromatin-binding proteins may be a novel therapeutic avenue for cancer treatment.
ORAL PRESENTATIONS

SG87

**Replication timing maintains the global epigenetic state in human cells**  
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The temporal order of DNA replication [replication timing (RT)] is correlated with transcription, chromatin modifications and three-dimensional genome architecture; however, causal links have not been established, largely because of an inability to manipulate the global RT program. We show that loss of RIF1 causes near-complete elimination of the global RT program. Loss of temporal control over replication results in widespread alterations in chromatin modifications and genome compartmentalization that accumulate with successive cycles of altered RT as well as stochastic alterations in transcriptional regulation that increase in severity with time. Despite widespread changes in the epigenome, cells retain their cell identity. These results demonstrate that RT is essential to maintain the global epigenetic state. We are currently investigating a) whether these changes occur at replication forks when chromatin assembles at the wrong time; b) the extent to which the disruption of replication timing results from changes in replication origin usage or alterations in the timing of origin firing and; c) the impact of epigenome disruption on the ability of stem cells to commit to alternative fates.

SG88

**Maternal epigenetic inheritance by Polycomb repressive complexes**  
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Genomic imprinting regulates parental allele-specific gene expression in mammals. DNA methylation had been the only known primary epigenetic mark that is deposited in gametes and governs genomic imprinting. A few years ago, we have discovered that polycomb repressive complex 2 (PRC2)-mediated histone H3 lysine 27 trimethylation (H3K27me3) is transmitted from oocytes to embryos and regulates DNA methylation-independent (non-canonical) imprinting in mice (Inoue et al., 2017 Nature). How H3K27me3 is properly established during oogenesis and inherited by embryos remains largely unknown. In this talk, I will introduce the role of polycomb repressive complex 1 (PRC1), which deposits mono-ubiquitylation at lysine 119 of histone H2A (H2AK119ub1), in the regulation of non-canonical imprinting. We show that reduction of H2AK119ub1 by depletion of Polycomb group ring finger 1 (PCGF1) and PCGF6—essential components of variant PRC1—leads to failure in H3K27me3 establishment at derepressed genes in oocytes. The H3K27me3 deficiency is irreversibly inherited by embryos and causes bi-allelic expression of non-canonical imprinted genes, which results in embryonic sublethality and placental enlargement at term. Thus, our study identifies PCGF1/6-PRC1 as an essential player in maternal epigenetic inheritance.

SG89

**RARE competition: Regulation of Hoxb nascent transcripts by multiple RAREs**  
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During development of an embryo, the anterior-posterior (A-P) body axis has to be properly defined, in order for body segments and organs to form in their correct locations. A family of transcription factors, the *Hox* genes, play an important role in determining this A-P body axis. In mammals, there are four *Hox*
gene cluster (A-P) that are arranged on four different chromosomes. For proper A-P axis formation, the spatial and temporal domains of \textit{Hox} genes within the clusters must be precisely initiated and strictly maintained during development. Therefore, it is critical to understand the regulatory inputs that dictate the expression of \textit{Hox} genes. Interspersed within the \textit{Hox} gene clusters are shared enhancer elements, retinoic acid response elements or RAREs, that enable multiple \textit{Hox} genes to incorporate signals from factors such as Retinoic Acid. We sought to understand how shared enhancers regulate multiple genes; are genes activated simultaneously, in some defined order, or through stochastic interactions? To answer these questions, we optimized the single molecule fluorescent \textit{in situ} hybridization technique (smFISH) to look at newly synthesized or nascent \textit{Hoxb} transcripts in mouse tissue sections. We found that three RAREs - DE, B4U, and ENE while having individual inputs into regulating \textit{Hoxb} genes, also appear to work together to ensure proper levels of nascent transcripts in the neural tube and adjacent somites. Furthermore, we see that these RARE have different inputs along the axial level of the embryo, such that DE plays a greater role anteriorly while B4U plays a greater role posteriorly. In the DE-B4U double mutants, we observe that antagonism or competition by individual DE and B4U RAREs is neutralized, as levels of nascent transcripts in the double mutant appear similar to wildtype. Results from the triple DE-B4U-ENE mutants highlight that these RAREs are critical for nascent transcription of \textit{Hox} genes, and is a step towards better understanding of enhancer-promoter interactions required for regulating genes that ensure proper embryonic development.

SG90

**A cycle of X-chromosome inactivation and reactivation demarcates mouse \textit{in vitro} germ cells with meiotic and oogenic potential**

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The early mammalian germ cell lineage is characterized by extensive epigenetic reprogramming, which is required for the maturation into functional eggs and sperm. In particular, the epigenome needs to be reset before parental marks can be established and then transmitted to the next generation. In the female germ line, reactivation of the inactive X-chromosome is one of the most prominent epigenetic reprogramming events, and despite its scale involving an entire chromosome affecting hundreds of genes, little is known about its kinetics and biological function. Here we investigate X-chromosome inactivation and reactivation dynamics by employing a tailor-made \textit{in vitro} system to visualize the X-status during differentiation of primordial germ cell-like cells (PGCLCs) from female mouse embryonic stem cells (ESCs). We find that the degree of X-inactivation in PGCLCs is moderate when compared to somatic cells and characterized by a large number of genes escaping full inactivation. Nevertheless, PGCLCs that fail to undergo X-inactivation show an abnormal gene expression signature and deficiencies in meiotic entry. Subsequent to X-inactivation we observe gradual step-wise X-reactivation, which is mostly completed by the end of meiotic prophase I. Cells deviating from these progressive kinetics and undergoing X-reactivation too rapidly fail to enter a meiotic trajectory. Our data reveals that a fine-
tuned X-inactivation and -reactivation cycle is a critical feature of female germ cell developmental competence towards meiosis and oogenesis.

SG91
**Mechanisms that promote acentrosomal spindle assembly and stability during oocyte meiosis**
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Although centrosomes serve as microtubule organizing centers that guide the formation of bipolar spindles during mitosis, oocyte meiosis of many species occurs in their absence. It has been shown that the poles of human acentrosomal spindles often split apart after they have formed, and that this instability leads to chromosome segregation errors. However, the mechanisms that promote the stability of acentrosomal spindles are not well understood. To address this question, we are using the auxin-inducible degron system in *C. elegans* to acutely remove candidate factors from pre-formed oocyte spindles, to identify proteins required to maintain spindle integrity. Using this approach, we found that the XMAP215 homolog ZYG-9 is required both to establish spindle bipolarity and also to maintain acentrosomal pole stability throughout meiosis. Depletion of ZYG-9 from stable bipolar spindles caused fragmentation of spindle poles and reversion to a multipolar spindle state, reminiscent of the unstable poles in human oocytes. Additionally, depletion of TAC-1, a protein known to interact with ZYG-9 at centrosomes, prevented proper ZYG-9 localization to the meiotic spindle and caused similar spindle phenotypes. Intriguingly, however, depletion of ZYG-9 from a monopolar oocyte spindle had no effect on pole morphology, demonstrating that ZYG-9 is not always required for microtubule minus ends to form a stable pole structure and suggesting that ZYG-9 may regulate the stability of bipolar spindles more globally. In line with this idea, quantification of GFP::ZYG-9 fluorescence revealed that although ZYG-9 is enriched at oocyte spindle poles, there is also a substantial population of this protein across the entire spindle. Moreover, fluorescence recovery after photobleaching (FRAP) experiments revealed that ZYG-9 is highly dynamic at acentrosomal poles when compared to another known pole protein, displaying similar dynamics to tubulin itself. Together, these data support a global role for ZYG-9 in regulating spindle stability and demonstrate that proper regulation of microtubule dynamics in the bipolar spindle is required to maintain the integrity of acentrosomal poles.

SG92
**Genomic inference of the origins of aneuploidy in human preimplantation embryos**
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Aneuploidy is the leading cause of human pregnancy loss and congenital disorders. While meiotic aneuploidies affect all cells and are deleterious, many forms of mosaic aneuploidy may be compatible with healthy birth. We recently developed a statistical approach to quantify aneuploidy in single-cell RNA-seq data, uncovering prevalent forms of mosaicism that were hidden to biopsy-based studies. Using this approach, we inferred that 74% of embryos possessed mitotic aneuploidies, while 31% of embryos possessed meiotic aneuploidies. We found no enrichment of aneuploid cells in the trophoblast compared to the inner cell mass, although we do detect such enrichment in data from
later postimplantation stages. Finally, we observed that aneuploid cells up-regulate immune response genes and down-regulate genes involved in proliferation and metabolism, consistent with stress responses documented in other systems. Given these observations, the ability to distinguish meiotic and mitotic aneuploidies, as well as abnormalities in genome-wide ploidy may prove valuable for enhancing IVF outcomes. To this end, we developed a new statistical method for distinguishing these forms of aneuploidy based on analysis of low-coverage whole-genome sequencing data—the current standard in the field. Our approach overcomes the sparse nature of the data by leveraging allele frequencies and linkage disequilibrium measured in a population reference panel, offering insight into the origins of aneuploidy.

SG93
Parental genome unification is highly error-prone in mammalian embryos
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Most human embryos are aneuploid. Aneuploidy frequently arises during the early mitotic divisions of the embryo, but its origin remains elusive. Human zygotes that cluster their nucleoli at the pronuclear interface are thought to be more likely to develop into healthy euploid embryos. I will present our data that show how the parental genomes cluster with nucleoli in each pronucleus within human and bovine zygotes. Parental genome clustering is required for the reliable unification of the parental genomes after fertilization. During the migration of intact pronuclei, the parental genomes polarize toward each other in a process driven by centrosomes, dynein, microtubules, and nuclear pore complexes. The maternal and paternal chromosomes eventually cluster at the pronuclear interface, in direct proximity to each other, yet separated. Parental genome clustering ensures the rapid unification of the parental genomes on nuclear envelope breakdown. However, clustering often fails, leading to chromosome segregation errors and micronuclei, incompatible with healthy embryo development. In conclusion, our data reveal why nucleolar clustering correlates with the formation of healthy euploid embryos. In addition, we used automated analysis of nucleolar trajectories and clustering, and found new parameters usable by in vitro fertilization clinics to select healthy embryos.

SG94
Aneuploidy disrupts cellular physiology and metabolism
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An abnormal number of chromosomes or aneuploidy accounts for most spontaneous abortions as missegregation of a single chromosome during development is often lethal. Humans with trisomies for chromosomes 13 or 18, which cause Patau and Edwards syndromes, respectively, are born with severe developmental defects and die soon after birth. Only individuals with trisomy 21, which causes Down syndrome, can live to adulthood but show cognitive disabilities, increased risk for leukemias, autoimmune disorders, and clinical symptoms associated with premature aging. Notably, the incidence
of aneuploidy increases with age in both somatic and germline tissues in apparently healthy individuals. The mechanisms by which aneuploidy affects cellular function to cause Down syndrome or promote aging are not well understood. Our studies revealed that aneuploidy disrupts the integrity and morphology of the nuclear membrane. Because mutations that affect nuclear morphology cause premature aging, we hypothesize that the aneuploidy effects on the nucleus drive phenotypic anomalies associated with premature aging in Down syndrome. In addition, to characterize aneuploidy-driven phenotypes in human cells, we performed global transcriptome, proteome, and phenotypic analyses of primary fibroblasts from individuals with Patau (trisomy 13), Edwards (trisomy 18), or Down syndromes. On average, mRNA and protein levels were increased by 1.5-fold in all trisomies, with a subset of proteins enriched for subunits of macromolecular complexes showing signs of post-transcriptional regulation. Lastly, we show that several aneuploidy-associated phenotypes are present in trisomy 21 cells, including lower viability and increased dependency on serine-driven lipid biosynthesis. Our studies establish a critical role of aneuploidy, independent of triplicated gene identity, in driving cellular defects associated with Down syndrome.

Emerging Novel Regulatory Mechanisms of the Cytoskeleton

SG95

Regulation of cytoplasmic actins at the nucleotide and amino acid level

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β- and γ-cytoplasmic actins are ubiquitously expressed in every cell type and are nearly identical at the amino acid level but play vastly different roles in vivo. The molecular mechanisms underlying this functional distinction are not fully understood. We previously found that their essential roles in embryogenesis and mesenchymal cell migration critically depend on the nucleotide sequences of their genes, rather than the amino acid sequence of their proteins. However, it is unclear which gene elements underlie this effect and whether the slight difference in the amino acid sequences of these two actins plays any physiological role. Here we addressed the specific roles of the coding and amino acid sequences in β- and γ-cytoplasmic actins’ intracellular and organismal functions. First, to address the nucleotide coding sequence role, we used stable polyclonal populations of immortalized mouse embryonic fibroblasts with exogenously expressed actin isoforms and their ‘codon- switched’ variants. When targeted to the cell periphery using β-actin 3’UTR, β- and γ-actin have differential effects on cell migration. These effects directly depend on the coding sequence. Single-molecule measurements of actin isoform translation, combined with fluorescence recovery after photobleaching, demonstrate a pronounced difference in β- and γ-actins’ translation elongation rates in cells, leading to changes in their dynamics at focal adhesions, impairments in actin bundle formation, and reduced cell anchoring to the substrate during migration. Our results demonstrate that coding sequence-mediated differences in actin translation play a key role in cell migration. Second, to address the role of the amino acid sequence-level difference in the β- and γ- actin proteins, we used Actbc-g mice, which completely lack β-actin protein
and produce γ-actin from both of the cytoplasmic actin genes. We discovered that while these mice are viable, they appear to have defects in specialized cytoplasmic actin-based protrusions in epithelial cells - microvilli. These defects lead to structural disorganization of the microvilli in the retina and the small intestine. Mass spectrometry-based studies suggest that these changes potentially arise via altered actin binding of several associated proteins to the specialized filament networks. In the retina of Actbc-g mice, lack of β-actin protein leads to reduced light sensitivity, suggesting that β-actin function is required for the proper light response in amino acid-dependent manner. Together, our results show specific roles of nucleotide and amino acid sequences in actin isoform regulation: from silent substitutions to highly conserved amino acid differences.

SG96

The Actg1 Nucleotide Sequence is Required for Normal Mouse Survival

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Mammalian actin is a family of six unique isoforms including four muscle specific actins and the two cytoplasmic isoforms: β- and γ-actin, expressed from the Actb and Actg1 genes, respectively. The cytoplasmic actins differ by only four amino acids but have non-redundant functions, with mutations in either gene causing significant clinical phenotypes in humans. Actb knockout (KO) mice are embryonic lethal while Actg1 KOs are viable with decreased post-birth survival and progressive hearing loss. Editing Actb to express γ-actin protein (Actb⁻γ) revealed that β-actin protein is not essential as these mice were phenotypically normal with the exception of progressive hearing loss (X. Patrino et al., 2018 Proc. Natl. Acad. Sci. USA). To determine if mice exclusively expressing γ-actin from the Actb gene are viable, we crossed the Actb⁻γ mice with Actg1⁻/⁻ mice to generate an Actb⁻γ/Actg1⁻/⁻ line. We found that Actb⁻γ⁺⁻/Actg1⁻/⁻ mice, hereafter referred to as Actb⁻γ/Actg1⁻/⁻, are viable but show decreased birth rates and increased postnatal mortality. The Actb⁻γ⁺/Actg1⁻/⁻ mice comprised only 7.48% of pups at weaning and 50% died by age 100d. Most surprisingly, quantitative western blot analysis of Actb⁻γ⁺/Actg1⁻/⁻ tissues showed that total actin expression was not different from WT and γ-actin expression remains constant, suggesting that the Actg1 nucleotide sequence is required for survival. Also interesting, Actb⁻γ⁺/Actg1⁻/⁻ skeletal muscle presented with none of the myopathy phenotypes manifested by muscle-specific Actb or Actg1 KO mice suggesting that the Actb-expressed γ-actin is sufficient to compensate for loss of endogenous γ-actin. However, the Actb⁻γ⁺/Actg1⁻/⁻ mice did present with a unique muscle weakness phenotype not observed in muscle-specific Actb KO, Actg1 KO, or Actb⁻γ⁺ mice highlighting a more complicated role for cytoplasmic actin in muscle that will require further investigation. Additionally, preliminary data suggests that Actb⁻γ⁺/Actg1⁻/⁻ mice present with a more severe hearing loss starting at 5 weeks, compared to 16 weeks for either Actb⁻γ⁺ or Actg1⁻/⁻ mice suggesting that compensation does not occur in all tissues. In primary mouse embryonic fibroblasts (MEFs) harvested from Actb⁻γ⁺/Actg1⁻/⁻ mice, there was no measured difference in cell proliferation or migration. Our results demonstrate significantly impaired survival in mice lacking an intact Actg1 allele while maintaining constant levels of γ-actin protein, thus demonstrating the importance of the Actg1 nucleotide sequence. Further studies that aim to characterize the unique function of Actg1 will provide important insights into the differential roles of nucleotide sequences and their protein constituents.
Lateral interactions between beta-tubulins determine the temperature dependence of microtubule dynamics
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Microtubules play essential roles in all eukaryotes; therefore, understanding the exquisite temperature dependence of tubulin activity is important for understanding how organisms adapt to different temperatures and respond to acute temperature change. Previous work from our lab and others shows how low temperatures disrupt microtubule dynamics - catastrophes become more frequent and rates of both polymerization and depolymerization decrease. Because of this temperature dependence, we hypothesize that tubulins from cold-adapted species may alter one or more of these properties to allow for functional microtubule networks at low temperatures. In this study, we tested this hypothesis by comparing the amino acid sequences of 10 β-tubulins from cold-adapted species and identified two unique substitutions on either side of the lateral interface that are individually sufficient to alter the temperature dependence of microtubule dynamics. In addition to altering tubulin activity, one of the substitutions, tub2-S56D, increases the requirement for Bim1/EB regulation of microtubule dynamics and selectively disrupts mitotic spindle function at high temperatures. Our findings provide insight into how the mechanism of microtubule dynamics may be tuned for life at different temperatures and reveal interplay between the lateral interface and the conformational changes that accompany tubulin’s nucleotide cycle.

Tubulin isotypes are optimized for distinct spindle function mechanisms during mitosis in budding yeast
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Dynamic cytoskeletal filaments called microtubules (MTs) are vital for a wide range of cellular processes in eukaryotes. They are made from polymerized tubulin, a heterodimer of α- and β-subunits. Most organisms harbor multiple isotypes of either subunit, and these distinct tubulin variants copolymerize to form cellular MTs. However, the functional relevance of tubulin isotypes has remained largely obscure. We investigated the role of the two α-tubulin isotypes, Tub1 and Tub3, in the budding yeast, Saccharomyces cerevisiae. With gene replacement we created otherwise isogenic cells expressing only single isotypes but at levels comparable to total tubulin in normal cells. Comparative Synthetic Genetic Array (SGA) screens using these strains revealed common interactions between the isotypes and genes involved in various cellular processes, demonstrating that normal MT function is compromised in the absence of either isotype. Moreover, the isotypes also display unique interactions with specific components in these same processes, indicating they each contribute distinct properties to cellular MTs. Cell biological assays and quantitative imaging confirm that mitotic spindle positioning and function are compromised in the absence of either isotype. Throughout mitosis, Tub1 and Tub3 differentially recruit key components of the Dyn1- and Kar9-dependent pathways to astral MTs, and each works to preferentially optimize one of these two major spindle positioning mechanisms. During anaphase the isotypes differentially influence the localization of the opposing motors, Cin8 (kinesin-5) and Kar3 (kinesin-14), and antagonistically regulate spindle elongation and overall length. Together these results
reveal that tubulin isotypes facilitate the specific activities of distinct motors and other MT-associated proteins, and yield insights into how multiple isotypes allow highly conserved microtubules to function in diverse cellular processes.

SG99

**Developing a Systematic Screen of Reader Proteins for Tubulin Tyrosination-Detyrosination Cycle**

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Tubulin is post-translationally altered by a suite of modifications, including acetylation, methylation, phosphorylation, polyglutamylation, and detyrosination. It has been proposed that tubulin post-translational modifications (PTMs) serve as a code that diversifies the functional properties of microtubules (MTs), in both proliferating and terminally differentiated cells. PTMs alter MT function by changing the way that MT-associated proteins (MAPs) interact with MTs. Despite widespread acceptance of this principle, systematic screens to identify “readers” that recognize specific PTMs have not been carried out. Our objective was to develop a systematic screening pipeline to identify readers for tubulin PTMs. We optimized our pipeline by targeting tubulin detyrosination of alpha-tubulin, the oldest known tubulin PTM. In our approach, we program cells with a writer - in this case, the detyrosinase VASH1-SVBP - and screen cell lysates using quantitative mass spectrometry for proteins that preferentially associate with tyrosinated or detyrosinated MTs. As expected, we identified CAP-Gly proteins (e.g., CLIP-170 and p150(glued)) as readers for tyrosinated MTs. We also discovered that Echinoderm MAP-like 2 (EML2), a poorly characterized MT-associated protein, is a novel reader of tyrosinated MTs. We show that recombinant EML2 directly interacts with tyrosinated MTs much better than with detyrosinated MTs. To identify the domain responsible for the recognition of tyrosinated MTs, we visualized the electrostatic surface of closely related protein EML1 and found the presence of a highly positively charged patch on a characteristic beta-propeller structure. Molecular dynamics simulations show that the highly negatively charged tubulin C-terminal tail docks with this region of EML2. The binding of the C-terminal tail of tubulin to EML2 is reinforced by a hydrophobic clamp comprised of 2 aromatic residues that are adjacent to the positively charged patch. Point mutations in the patch and the neighboring aromatic residues drastically weakened the microtubule localization of EML2 in HeLa cells. In summary, we report a new pipeline to identify readers of tubulin PTMs and show that EML2 is a new reader of tyrosinated MTs. In principle, our approach can be modified to identify readers of any PTM (provided that writers are known) establishing a powerful method to aid in deciphering the tubulin code.

SG100

**Setdb1 regulates microtubule dynamics**

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SETDB1 is a methyltransferase responsible for the methylation of histone H3-lysine-9, which is mainly related to heterochromatin formation. SETDB1 is overexpressed in various cancer types and is associated with an aggressive phenotype. In agreement with its activity, it mainly exhibits a nuclear
localization; however, in several cell types a cytoplasmic localization was reported. Here we show that during interphase a substantial cytoplasmic pool of SETDB1 is partially colocalized with microtubules. Co-localization of SETDB1 and microtubules was found also during mitosis of both mouse and human cells. Silencing of SETDB1 enhanced microtubule polymerization rate as measured by recovery of microtubules from nocodazole treatment and tracking of microtubule plus-ends by GFP fused to EB1 in live cells. On the other hand, over-expression of SETDB1 reduced microtubule polymerization rate as measured by recovery of microtubules from nocodazole treatment. Thus, suggesting that SETDB1 is a microtubule destabilizing protein. In agreement with these results, silencing of SETDB1 reduced the cellular proliferation rate by 20%, while increasing unsuccessful mitotic events by 5-fold and attenuating mitotic progression. Significant over-expression of wild-type or catalytic dead SETDB1 attenuated MT polymerization in a similar manner, suggesting that SETDB1 affects MT dynamics by a methylation-independent mechanism. In search for the mechanism by which SETDB1 affects MT dynamics we found interaction between SETDB1 and the tubulin deacetylase HDAC6. Silencing of SETDB1 led to an increase in tubulin acetylation, a post-translational modification that is associated with stabilized microtubules. Overall, our data suggest a model in which SETDB1 may affect microtubule dynamics by interacting with both microtubules and HDAC6 to enhance tubulin deacetylation. Overall, our results suggest a novel cytoplasmic role for SETDB1 in the regulation of microtubule dynamics.

SG101
Direct Talin-Actin Binding is Required for Mammalian Development
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During animal development, cell-extracellular matrix (ECM) adhesion plays an essential role in the assembly and maintenance of complex tissues and organs. The integrin family of transmembrane adhesion receptors are the main links between the ECM and the intracellular actin cytoskeleton, and their functions are carefully regulated to ensure proper tissue development and homeostasis. Talin, which is a cytoplasmic protein that links integrins to the actin, is known to act as a molecular scaffold for the assembly of the integrin adhesion complex and it is extremely important for mammalian development. Talin has three actin-binding sites, and the ABS3 site located at the C-terminal end of talin is thought to be essential for integrin-mediated mechanical signaling. Specifically, it has been proposed that when integrin-bound talin links to actin through ABS3, it allows the mechanical force to be transduced to the cytoplasmic adhesion complex. Such force transduction stretches talin and exposes its cryptic binding sites that recruit additional components like vinculin. This can promote more cytoskeleton interactions and thus further reinforcing the integrin-mediated cell-ECM adhesion. To explore the role of ABS3 in vivo, we generated mice containing the K2443D/V2444D/K2445D (KVK/DDD) mutation in Tln1 that abolishes actin-binding through ABS3. From heterozygote intercrosses, we have observed that the Tln1KVK/DDD mutants showed developmental defects and reduced sizes starting at embryonic day (E)8.5 and became resorbed around E11.5. By analyzing the cellular phenotypes associated with the Tln1KVK/DDD mutation in primary fibroblasts derived from the mouse embryos, we have found that such mutation affected the ability of focal adhesions (FAs) to grow and mature and caused defects in cell spreading and migration. Such mutation also influenced the cell adhesion strength and traction force. In addition, the Tln1KVK/DDD mutant MEFs revealed a distinct chiral swirling actin pattern instead of the typical parallel linear stress fiber. In summary, our data have suggested that talin’s ABS3 site has specific roles in mediating mechanical feedback to reinforce adhesions as cells
spread by organizing the actin cytoskeleton into stress fibers, and direct talin-actin binding plays important roles during early mouse development.

SG102
**Ssna1 stabilizes dynamic microtubules and detects microtubule damage**

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Sjögren’s Syndrome Nuclear Autoantigen 1 (SSNA1/NA14) is a microtubule-associated protein with important functions in cilia, dividing cells and developing neurons. However, the direct effects of SSNA1 on microtubules are not known. We employed *in vitro* reconstitution with purified proteins and TIRF microscopy to investigate the activity of human SSNA1 on dynamic microtubule ends and lattices. We find that SSNA1 modulates all parameters of microtubule dynamic instability - slowing down the rates of growth, shrinkage and catastrophe, and promoting rescue. SSNA1 accumulation on dynamic microtubule ends correlates with the growth rate slow-down. Furthermore, SSNA1 prevents catastrophe when soluble tubulin is removed or sequestered by Op18/Stathmin. Finally, SSNA1 detects spastin-induced damage and inhibits spastin’s severing activity. Therefore, SSNA1 is both a potent microtubule stabilizing protein and a sensor of microtubule damage; activities that likely underlie SSNA1’s cellular functions.

SG103
**Septins are Necessary for Detachment and Protrusion Formation in Border Cell Migration**

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Collective cell migration is crucial for development and the preferred mode of migration by metastatic tumors, but much about this process is unknown. The border cell cluster in the *Drosophila* ovary is an ideal *in vivo* model for collective cell migration, as border cells migrate on and between nurse cells. The cytoskeleton is a critical regulator of cell migration. Septins, now considered the fourth element of the cytoskeleton, remain unexplored in collective systems. The objective of this study is to understand the functions and necessity of septins in border cell migration. Using RNAi, mutants, and over-expression, we investigated the impact of septins on border cell migration and cluster morphology through fixed and live imaging. We used high-resolution Airyscan imaging paired with Tissue Cartography to generate 3D models of the surface of the cluster. We found that knocking down any of the five *Drosophila* septins significantly impacted border cell migration and cluster morphology through detachment failure, failure to form stable forward-directed protrusions, and a loose blebbby morphology. Overexpressing septins also dramatically impacted migration, with an inverse effect on cluster morphology. As septin subunits function by forming higher order structures with each other such as filaments and rings, we investigated if septin monomers interacted in our model. Clonal knockdown of Septin 1 (Sep1) or Septin 2 (Sep2) led to a significant loss of Peanut (Pnut, considered *Drosophila* Sep3) in the follicle cells and border cells. Similarly, knockdown of Sep1 or Pnut also caused a loss of Sep2. These findings suggest that Sep1, Sep2, and Pnut form structures with each other in the follicle cells and border cells independently of Sep4 and Sep5, which have no impact on Pnut or Sep2 protein expression. To uncover the mechanistic role of septins in border cell migration, we tested potential candidates that may interact with septins. We observed co-localization between septins and nonmuscle myosin II in fixed imaging, and then explored
further through live imaging. Amazingly, dynamic myosin flashes completely co-localized with dynamic septin expression. This suggests an interaction between septins and myosin during border cell migration. For example, septins may act as a scaffold for myosin, recruiting it and stabilizing protrusions. In conclusion, we found that septins are necessary for the detachment of the border cell cluster and for protrusion formation, while too much septin induces an excess of curvature and prevents migration. Sep1, Sep2, and Pnut interact to form structures and may interact with myosin.

SG104

Integrated cell and in situ structural biology of Arp2/3 Complex Isoform-regulated Cell Motility and Lamellipodium Architecture

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The heptameric Arp2/3 complex is essential for forming branched actin networks and is thus involved in intracellular trafficking, cell shape control, and cellular motility. The mechanisms underlying Arp2/3-dependent actin branch junction formation, including structural changes of the complex and binding to pre-existing actin filaments, remain incompletely understood. This is partly due to the inaccessibility of the branch junction for in vitro structural approaches. We recently employed cryo-electron tomography (cryo-ET) and subtomogram averaging (STA) to obtain a 9Å structure of the branch junction from lamellipodia of migrating cells, providing the first precise description of the active Arp2/3 complex in its native environment. In order to further understand how the Arp2/3 complex regulates lamellipodia architecture, we generated knockout cell lines deficient in either one or both isoforms of the ArpC5 subunit. We analyzed these lines employing an integrated approach combining cell biology and structural biology. Employing cryo-ET and STA on lamellipodia, we obtained ~8 Å reconstructions of ArpC5 isoform-specific branch junctions, revealing striking differences in the stability of the other subunits. Further, our ultrastructural and cell biological assays show different geometrical arrangements of actin filament networks and varying recruitment of other actin-binding proteins (ABPs), culminating in specific cell migration characteristics. We further optimized our cryo-ET and STA approaches to obtain a 3.9 Å structure of F-actin within lamellipodia. Building on this, we can now analyze in more detail how ABPs interact with F-actin in a cell. Our work underscores the potential of combining reverse genetic approaches and in situ cryo-ET to allow for an integrated functional and structural characterization of molecular machinery in its cellular context.

SG105

Phosphoregulation of γ-Tubulin Directs Spindle Assembly Through Kinesin-5 and Microtubule Number

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The function of the mitotic spindle depends on the forces that are generated by microtubules (MTs), motor proteins such as kinesin-5, and chromatin which acts as a spring when attached to kinetochore MTs (kMTs). In metaphase, inward forces applied by kMTs balance outward forces applied by interpolar MTs (ipMTs) to prevent spindle collapse. The ipMTs are formed early in spindle assembly and must be maintained throughout mitosis. We recently showed that the precursors of ipMTs are present when the spindle poles separate to form a bipolar spindle in budding yeast. An open question is how many ipMTs must be formed to maintain spindle stability. Surprisingly, regulation of γ-tubulin, which nucleates MTs, may control the number of ipMTs and thus promote spindle stability. γ-Tubulin is phosphorylated during
spindle assembly at multiple residues in the globular domain and unstructured carboxyl terminus (γCT). A Y445D mutation in the γCT causes spindle instability and is co-lethal with a null mutation in Ase1, a PRC1 homolog that maintains the ipMTs. These phenotypes suggest a defect in the kinesin-5 Cin8, which has important roles in both forming and maintaining ipMTs in budding yeast and mammalian cells. Using confocal microscopy, we find that Cin8 is mislocalized in the Y445D mutant. In WT metaphase cells Cin8 is associated with the MTs of both spindle poles. In Y445D cells, Cin8 is sequestered to the MTs of the spindle pole inherited from the previous cell cycle. In contrast, Ase1 is properly localized to the spindle midzone in both WT and Y445D cells. We hypothesize that in the Y445D mutant 1) sequestration of Cin8 on MTs of the pre-existing pole inhibits the formation of ipMTs and 2) Ase1 can compensate for the defect in Cin8 function. Dephosphorylation of Ase1 plays an important role in maintaining the ipMTs. We find that a null mutation of the M-phase cyclin Clb2 partially restores spindle stability in the Y445D mutant. The clb2Δ mutation does not restore WT Cin8 localization and the cin8-3A mutation exacerbates the defect in Cin8 localization in Y445D cells. Currently, we are investigating if inhibiting the phosphorylation of Ase1 restores Cin8 function in Y445D cells. It is unclear how γ-tubulin can influence the activity of Cin8. We speculate that early in spindle assembly, Cin8 tends to walk towards the (+) ends of individual MTs. An intriguing possibility is that phosphorylation of the γCT might regulate how proteins interact with the (-) ends of MTs. Using nuclear magnetic resonance, we demonstrated that the Y445D mutation causes the γCT to adopt unique extended conformations. We hypothesize that changes in the conformation of the γCT may regulate the exchange of Cin8 between anti-parallel MTs within a newly formed bipolar spindle and thus specify the number of ipMTs in the spindle midzone.

SG106

Myogenin controls non-centrosomal microtubule organizing center formation at the nuclear envelope.

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The formation of non-centrosomal microtubule-organizing centers (ncMTOCs) is characteristic for differentiated cells types and microtubule arrays generated by ncMTOCs are often pivotal for cell type-specific functions. Despite the importance of ncMTOCs, it remains elusive how the switch from a centrosomal MTOC to ncMTOCs is regulated in most cell types. Myogenic differentiation is an optimal experimental system to study ncMTOC formation in the context of differentiation, as it is controlled by a small number of transcriptional master regulators, the myogenic regulatory factors (MRFs). Additionally, individual MRFs are sufficient to induce skeletal muscle phenotypes in non-muscle cells. Here, we find that the MRF myogenin is required in differentiating myoblasts for localization of MTOC proteins to the nuclear envelope. Moreover, myogenin is sufficient in fibroblasts for ncMTOC formation as well as attenuation of the centrosomal MTOC. A bioinformatic analysis identified the scaffold protein AKAP6 as a potential myogenin downstream target to be involved in ncMTOC formation at the nuclear envelope. Depletion experiments revealed that AKAP6 is a novel component of the ncMTOC in skeletal muscle cells and is essential for the recruitment of MTOC proteins downstream of the nuclear envelope anchor protein nesprin-1α. In myotubes, AKAP6 depletion resulted in mispositioning of nuclei, confirming that AKAP6 is required for a functional MTOC at the nuclear envelope. Promoter studies indicated that myogenin preferentially induces the transcription of muscle- and ncMTOC-specific isoforms of AKAP6.
and nesprin-1. Overexpression of these isoforms, AKAP6β and nesprin-1α, was sufficient to recruit endogenous MTOC proteins to the nuclear envelope of myoblasts in the absence of myogenin. Taken together, our results illuminate a differentiation-dependent transcriptional control of ncMTOC formation in a mammalian cell type and identify AKAP6 as a novel component of the skeletal muscle ncMTOC.

SG107

Precise control of microtubule disassembly in living cells
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Microtubules (MTs) are components of the evolutionarily conserved cytoskeleton, which tightly regulates various cellular activities. Our understanding of MTs is largely based on MT-targeting agents, which, however, are insufficient to dissect the dynamic mechanisms of specific MT populations due to their slow effects on the entire pool of MTs in cells. To address this limitation, we have used chemogenetics and optogenetics to disassemble specific MT subtypes by rapid recruitment of engineered MT-cleaving enzymes. Acute MT disassembly swiftly halted vesicular trafficking and lysosome dynamics. We also used this approach to disassemble MTs specifically modified by tyrosination and several MT-based structures including primary cilia, mitotic spindles, and intercellular bridges. These effects were rapidly reversed by inhibiting the activity or MT association of the cleaving enzymes. The disassembly of targeted MTs with spatial and temporal accuracy enables to uncover new insights of how MTs precisely regulate cellular architectures and functions.

SG108

Cryo-EM structures illuminate the mechanisms of Arp2/3 complex activation and inhibition
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Actin filament nucleators catalyze the rate-limiting step in actin polymerization, i.e. nucleation. Arp2/3 complex is the only nucleator capable of generating branched actin networks. Arp2/3 complex consists of seven proteins, including two actin related proteins (Arp2 and Arp3) that act as a pseudo-actin dimer during nucleation, and five scaffolding subunits (ArpC1-ArpC5) that hold the Arps in place. A family of Arp2/3 complex activators, known as nucleation promoting factors (NPFs), recruit and activate Arp2/3 complex at specific subcellular locations to generate dendritic actin networks, i.e. networks of branched actin filaments. These networks exert forces on membranes during processes such as cell motility, vesicular trafficking, and membrane scission. Activated by NPFs, Arp2/3 complex undergoes conformational changes that prompt the complex to bind to the side of a pre-existing (mother) filament and nucleate the formation of a new (branch) filament. The activity of Arp2/3 complex is additionally regulated by inhibitors. A major inhibitor of Arp2/3 complex, Arpin, controls the persistence of lamellipodial protrusions and cell migration. Despite intensive interest, the mechanisms that control Arp2/3 complex activation by NPFs and inhibition by Arpin have remained poorly understood. Several cryo-EM structures of Arp2/3 complex have been recently obtained in our lab, including structure of Arp2/3 complex with bound NPFs and actin and with the inhibitor Arpin. These structures reveal the conformational changes that the complex undergoes during activation, as well as the binding sites of NPFs and Arpin on the complex. Strikingly, despite their opposite functions, NPFs and Arpin use similar
ORAL PRESENTATIONS

structural mechanisms. The structures combined with biochemical and cellular data help illuminate the mechanisms that control Arp2/3 complex activation and inhibition.

SG109
**Evolutionary diversification of *Drosophila* Arp2 for specialized actin branching**
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The actin cytoskeleton, which is composed of force-generating polymers, often forms branched networks that are critical in many fundamental cellular processes, including cell motility, cell division and vesicular movement. Branched actin networks are generated by the Arp2/3 complex, a 7-membered protein complex including actin-related proteins (Arps) 2 and 3. Similar to actin and most Arps, Arp2 is evolutionarily ancient among eukaryotes and under stringent sequence conservation, yet we surprisingly discovered two clade-specific gene duplications of Arp2 in *Drosophila*: Arp2D in the obscura clade and Arp2D2 in the montium clade. Our targeted sequencing and phylogenetic analyses of Arp2D and Arp2D2 show these duplicates have evolved independently and arose ~14 million years ago at the origin of their respective clades. The two duplicates exhibit distinct sequence diversification from canonical Arp2, and unlike the ubiquitously expressed Arp2, both duplicates are testis-enriched in expression. Why would evolution recurrently select for a divergent Arp2 for potential roles in the male germline? To elucidate the function of these duplicates, we investigated whether both duplicates can polymerize branched actin networks similar to canonical Arp2 despite their sequence divergence. We replaced canonical Arp2 in *D. melanogaster* with *D. pseudoobscura* Arp2D or *D. auraria* Arp2D2 and surprisingly found they can both rescue the Arp2 knockout lethality phenotype, though to varying degrees, and cytological analyses confirmed the two duplicates localize to branched actin networks. Due to the testis-enriched expression of the duplicates, we are currently comparing male fertility and the integrity of germline actin structures in the Arp2D and Arp2D2 *D. melanogaster* transgenics to determine how Arp2 divergence impacts actin polymerization and testis biology. Based on our findings, we hypothesize that diversified *Drosophila* Arp2 duplicates have retained the ability to polymerize branched actin networks yet modified this reaction for specialized physiological processes, including sperm development.

Mechanoimmunology: Molecular Mechanisms and Biological Function

SG110
**Mechanical Control of Cytotoxic Secretion**
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The secretory output of cell-cell interfaces must be tightly controlled in space and time to ensure functional efficacy. This is particularly true for the cytotoxic immune synapse (IS), the stereotyped junction formed between a cytotoxic lymphocyte and the infected or transformed target cell it aims to destroy. Cytotoxic lymphocytes kill their targets by channeling a mixture of granzyme proteases and the pore forming protein perforin directly into the IS. The synaptic secretion of these toxic molecules constrains their deleterious effects to the target cell alone, thereby protecting innocent bystander cells in the surrounding tissue from collateral damage. Despite the importance of this process for immune specificity, the molecular and cellular mechanisms that establish secretory sites within the IS remain
poorly understood. Here, we identified an essential role for integrin mechanotransduction in cytotoxic secretion using a combination of single cell biophysical measurements, ligand micropatterning, and functional assays. Upon ligand-binding, the αβ₂ integrin LFA-1 functioned as a spatial cue, attracting lytic granules containing perforin and granzyme and inducing their fusion at closely adjacent sites within the synaptic membrane. LFA-1 molecules were subjected to pulling forces within these secretory domains, and genetic or pharmacological suppression of these forces abrogated cytotoxicity. We conclude that lymphocytes employ an integrin-dependent mechanical checkpoint to enhance both the potency and the security of their cytotoxic output.

SG111
Dynamic interactions of the actin and microtubule cytoskeleton during T cell activation and cytolytic function
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Activation of T cells leads to the formation of the immunological synapse (IS) with antigen presenting cells. Synapse formation is driven by extensive rearrangement of the actin and microtubule (MT) cytoskeleton and force generation that is critical for T cell activation. The interactions between these two cytoskeletal components during T cell activation are not well understood. We have previously demonstrated that dynamic microtubules modulate actin-based force generation via a Rho-mediated pathway, indicating a mechanical coupling between the actomyosin and MT systems during activation. We present our recent findings on how actomyosin generated forces in turn affect MT growth and shape dynamics and the distinct roles of different actin nucleating proteins on MT dynamics. The intricate spatiotemporal patterns of cytoskeletal forces are instrumental in promoting cytolytic activity at the IS of Cytotoxic T lymphocytes (CTLs). Centrosome polarization is required for transport of lytic granules along MT towards the target cell while actin forces facilitate granule secretion, and promote target cell death by mediating mechanical force exertion at the IS. While most studies of CTL activation have been in the context of T-cell receptor activation by peptide-MHC complexes and co-stimulation via the CD28 receptor (two signals), recent studies have indicated that inflammatory cytokines produced by APCs, such as interleukin-12 (IL-12), act as a third signal for CTL activation and enhance cytolytic activity. We have used live cell imaging to study actin and microtubule dynamics at the IS of murine CTLs activated in the presence of two signals, or additionally with IL-12 (three signals). We show that three-signal activated cells have altered actin flow dynamics compared to two-signal activated CTLs. Moreover, three-signal activation differentially regulates lytic granule accumulation and dynamics at the IS. Finally, we use traction force microscopy to study CTL force exertion patterns at the IS. We show that three signal-activated CTLs exert greater traction forces than two signal-activated CTLs. Collectively, our results demonstrate that activation of CTLs in the presence of IL-12 leads to differential modulation of the cytoskeleton, thus indicating a mechanochemical pathway via which the third signal can augment the T cell response.
SG112

Cooperative ectodomain interaction among TCRαβ, CD3γε and CD3δε

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The T-cell receptor (TCR) complex comprises of the ligand-binding subunit TCRαβ, and the signaling subunits CD3γε, CD3δε and CD3ζζ. TCRαβ-ligand binding triggers CD3 phosphorylation to activate the T cell. Direct measurements of the ectodomain interactions had not been successful, although they are believed to be important for TCR stability and functionality. Mechanical force has been shown to modulate TCR-ligand interactions. The TCR mechanosensor hypothesis predicts that force-encoded information may transmit from pMHC to CD3 via TCRαβ-CD3 interaction. Evaluating ectodomain interactions among TCRαβ, CD3γε and CD3δε can help elucidate the TCR triggering mechanism and further guide the design of TCR-based immunotherapy. Using two mechanical based assays, we were able to measure the weak two-dimensional (2D) affinities among ectodomains of human TCRαβ (2B4-LC13), and human CD3γε or CD3δε and showed catch bond formation where lifetimes of TCRαβ-CD3γε and TCRαβ-CD3δε bonds are prolonged by forces <15 pN. Remarkably, CD3γε and CD3δε bind TCRαβ cooperatively, forming more bonds that last longer when both CD3s interact with TCRαβ as a whole than the sum of either CD3 interacting with TCRαβ individually. Using free molecular dynamics (MD) simulations and steered MD simulations based on a published CryoEM structure, we further compared the contact areas, binding energies, bond lifetimes and rupture forces of bimolecular TCRαβ-CD3γε and TCRαβ-CD3δε bonds and trimolecular CD3γε-TCRαβ-CD3δε bond. These results further showed the cooperativity in TCRαβ-CD3 ectodomain interactions. Lastly, with a novel occupancy analysis to quantifying the interface interacting residues, we identified the formation of long-lasting CD3γε-TCRαβ-CD3δε trimolecular bonds as the structural mechanisms of the cooperativity and reviewed the functional relevance of the critical interacting residues. Our work helps explain TCR function under force and suggests strategies for engineering of TCR for immunotherapy applications.

SG113

Dynamics of B cell synapse formation on viscous substrates

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B cell activation is triggered by specific interactions between the B cell receptor (BCR) and antigen displayed on the surface of an antigen-presenting cell. These binding events trigger intracellular signalling leading to actin reorganisation, B cell spreading, and the formation of an immune synapse, which is required for antigen acquisition leading to full B cell activation. This dynamic cellular response promotes B cell discrimination of antigen affinities and is thought to be important for the affinity maturation of antibodies. In recent years it has become clear that B cells can sense and respond to mechanical properties of their environment. BCR binding to membrane-bound antigens generates forces from the cytoskeleton that influence early signalling events and immune synapse formation. Forces transmitted to the BCR depend upon physical properties of the antigen-presenting cell, including stiffness and viscosity. The effect of stiffness on B cell activation has been investigated using elastic hydrogels as antigen-presenting surfaces, revealing that BCR clustering and signalling increase with substrate stiffness. Less well understood is the effect of membrane viscosity on B cell responses to antigen. Here, we investigate B cell interactions with antigens presented on planar lipid bilayers, which
are purely viscous surfaces that lack an elastic component. By altering the bilayer lipid composition, we can tune the mobility of presented antigens by an order of magnitude. This modification affects both BCR clustering and force loading on the BCR, enabling us to investigate the contributions of these critical early events to BCR signalling and evolution of the contact region between the B cell and antigen-presenting membrane. Based upon our data, we propose a model where BCR signalling and force transmission regulate coupling of BCR-antigen complexes with the actin cytoskeleton and therefore cell spreading and activation of downstream signalling.

SG114

**Exerting and sensing forces during integrin-mediated phagocytosis**

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Professional phagocytes are innate immune cells specialized in the uptake and clearance of large particulate materials including infiltrated microbes, apoptotic cells and debris. αMβ2 integrins (also called complement receptor 3) are highly expressed in macrophages and neutrophils. These integrins are thought to be the main phagocytic receptors for many pathogens, and participate in the clearance of dead cells and cancer cells. However, internalization of these different targets involves various physical constraints that professional phagocytes must overcome, including cortical and membrane tensions. In other contexts, such as cell adhesion and migration, cells deform and move using surface-attached integrins, which are coupled to mechanical forces generated by the actin cytoskeleton. By analogy, we asked whether phagocytosis required mechanical coupling of αMβ2 integrins to the actin cytoskeleton. Using quantitative live cell imaging, we found that particle internalization was driven by formation of Arp2/3 and formin-dependent actin protrusions that wrapped around the particle. Focal complex-like adhesions formed in the phagocytic cup, which contained β2 integrins, focal adhesion proteins and tyrosine kinases. Perturbation of talin and syk demonstrated that a talin-dependent mechanical link between integrins, and actin and a syk-mediated recruitment of vinculin independently of myosin II, enabled force transmission to target particles and promoted phagocytosis. Altering target mechanical properties demonstrated more efficient phagocytosis of stiffer targets, which could enable the discrimination of different targets based on their mechanical properties. Thus, macrophages build a myosin II-independent mechanosensitive molecular clutch, which couples integrins to cytoskeletal forces to control particle engulfment.

SG115

**Phosphatidylserine-mediated phagocytosis involves coreceptors TREM2, CD14 and integrin αMβ2 and a sinking engulfment mechanism**

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Efferocytosis, phagocytic uptake of apoptotic cells, is critical for tissue homeostasis. Failure in this process is a hallmark in disease such as auto-immune disorders and atherosclerosis. Whereas the mechanisms and regulators of antibody-mediated phagocytosis of pathogens are well-established, mechanisms of efferocytosis have remained more obscure. Phosphatidylserine (PS) is the best-described ligand for efferocytosis, as this phospholipid is normally sequestered in the inner leaflet of the plasma
membrane but becomes exposed on the outer leaflet of apoptotic cells. Here, we use murine J774 macrophage-like cells and PS-functionalized hydrogel microparticles, designed to have similar size and rigidity as apoptotic cells, to investigate the mechanisms of efferocytosis. We show that PS-mediated uptake follows a unique progression, characterized by cups appearing sunken deep into the cytoplasm before cup closure, and by lack of F-actin accumulation at the cup rim, both dramatically different from antibody-mediated phagocytosis of physically similar particles. We then used magnetized hydrogel microparticles in a comparative genome-wide screen for genes involved in PS- and antibody-mediated phagocytosis. This approach identified a core of shared regulators common to both uptake pathways, but also many new regulators unique to PS-mediated uptake. This specific subset includes actin-binding proteins likely involved in the unique cup-shaping dynamics as well as a substantial fraction of the enzymes involved in GPI-anchoring of cell surface proteins. Importantly, whereas many putative PS-receptors have previously been implicated in efferocytosis, our unbiased screening approach identifies CD14, integrin αmβ2 and neurodegenerative disease-associated TREM2 as the most critical co-receptors in PS-mediated phagocytosis as distinct from antibody-mediated phagocytosis. High-resolution immunofluorescence confirms the accumulation of these receptors in the phagocytic cup for PS-coated particles, each with a unique distribution and with remarkable clustering of TREM2 at the rim of late-stage cups. The importance of CD14, a GPI-anchored protein, is further illustrated by the observation that enzymatic removal of other GPI-anchored proteins does not affect phagocytic efficiency, hence suggesting that the GPI-synthesis pathway is solely important because of CD14 involvement in efferocytosis. Together, these findings reveal that PS-mediated efferocytosis involves recognition by coreceptors CD14, TREM2 and ITGαM and a distinct cup morphology. More generally, we establish many novel players in the phagocytic pathway mediated by PS.

SG116

**Invasion and locomotion of immune cells in dense environments**

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During metazoan development, immune surveillance and cancer dissemination, cells migrate in complex three-dimensional microenvironments. These are crowded by cells and extracellular matrix, generating mazes of differently sized spaces typically smaller than the diameter of the migrating cell. Most mesenchymal and epithelial cells actively generate their migratory path using pericellular tissue proteolysis and transmit traction forces via specific adhesion receptors. On the contrary, amoeboid cells such as leukocytes and some metastatic cancers employ non-destructive strategies of locomotion and do not hold on to extracellular substrates. This raises the question how these usually extremely fast cells negotiate dense tissues. We find that amoeboid cells are able to migrate in the total absence of transmembrane force coupling, making the cell entirely autonomous and independent of the composition of the extracellular environment. Instead, active deformations of the cell body can impose normal forces on the substrate and thereby generate propulsion. Whenever the cell has to passage through areas that are too narrow to allow unrestricted passage, they respond generate pushing forces that dilate the local microenvironment. We investigate the molecular mechanisms triggering such cytoskeletal responses.
The BAR domain protein SH3BP1 regulates plasma membrane tension to control immune cell migration

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For efficient polarization and spreading, key aspects of motility, cells need to regulate the magnitude of tension in their plasma membrane and efficiently respond to its changes. However, the molecular players in this process remain poorly understood. Neutrophils display one of the most dynamic modes of migration among all vertebrate cells, and are emerging as a key model system to investigate the role of membrane mechanics for cell migration. Membrane tension affects the landscape of membrane deformations, which, in turn, could alter the kinetics of membrane-binding proteins. A particularly interesting group for cell motility is curvature-sensitive and membrane shaping BAR domain proteins. To understand whether and how BAR domain proteins regulate membrane mechanics, we first quantitatively screened candidates from this family for membrane binding at various tension regimes using TIRF microscopy. By developing an image analysis pipeline, we could show that SH3BP1, a protein enriched at the cell front, localizes to the plasma membrane in a tension-dependent manner. Next, we generated a CRISPR/Cas9 knockout cell line, and measured its membrane tension using Atomic Force Microscopy. These knockout cells have higher membrane tension than their wild-type counterparts, thus, SH3BP1 not only responds to membrane tension changes, but also contributes to its magnitude. Finally, to test the interplay between membrane mechanics and a key immune-cell function, we quantitatively assessed directed cell migration using microfluidic devices. SH3BP1 knockout cells migrate faster and are more efficient at choosing the path of the least resistance, which is particularly important for navigating complex environments. In summary, we show that the curvature-sensitive BAR domain protein SH3BP1 is key to control directed cell migration by affecting plasma membrane tension.

Impact of Mechanical stress on dendritic cell migration and fate

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In response to mechanical stress, cells assemble a pool of actin around their nucleus, which results in nucleus compression and facilitates cell migration in confined environments. How this phenomenon is regulated and whether it impacts on the fate and function(s) of cells is unclear. Here, we show that actin nucleation at the nucleus of dendritic cells (DCs), sentinels of the immune system, is negatively regulated by Arpin, a recently described inhibitor of the Arp2/3 complex. Limitation of actin polymerization by Arpin protects immature DCs from initiating an Arp2/3-dependent “maturation-like” gene expression program in response to mechanical stress. Activation of this program requires cytosolic phospholipase 2 (cPLA2)-dependent NFKB activation and leads to the expression of ~350 genes including CCR7, i.e. the chemokine receptor that drives DC migration to lymph nodes. Consistent with these results, we observed that Arpin KO DCs exhibit enhanced migration to lymph nodes even in the absence of inflammation. Remarkably, we found that this transcriptional program also includes anti-inflammatory genes and, accordingly, that mechanically challenged-DCs harbor tolerogenic properties. These results show that by controlling cPLA2 and NFKB activation, Arpin prevents DC spontaneous maturation and migration to lymph nodes in response to physical confinement. They further suggest that the mechanical stress experienced by DCs in peripheral tissues might constitute one of the so far
unknown signal(s) that trigger CCR7 expression and DC migration to lymph nodes in homeostasis for maintenance of peripheral tolerance.

**Mitochondrial-Nuclear Crosstalk**

**SG119**
The mitochondrial outer membrane carrier MTCH2 regulates mitochondrial fusion in response to lipogenesis
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Mitochondrial function is integrated with cellular status through the regulation of mitochondrial dynamics. MTCH2 is a modified outer mitochondrial membrane carrier protein implicated in the regulation of fatty acid metabolism and intrinsic cell death. Our data indicate that MTCH2 is a selective effector of starvation-induced mitochondrial hyperfusion, a cytoprotective response to nutrient deprivation. MTCH2 stimulates mitochondrial fusion in a manner dependent on the bioactive lipogenesis intermediate lysophosphatidic acid. We propose that MTCH2 monitors flux through the de novo lipogenesis pathway and transmits this information to the mitochondrial fusion machinery to promote mitochondrial elongation, enhanced energy production, and cellular survival under homeostatic and starvation conditions. These findings will help resolve the roles of both MTCH2 and mitochondria in tissue-specific lipid metabolism in animals.

**SG120**
Mitochondrial stress is relayed to the cytosol by an OMA1-DELE1-HRI pathway
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Through transcriptional profiling, we and others identified that mitochondrial dysfunction in mammalian cells triggers the integrated stress response (ISR). The ISR converges on the phosphorylation of eukaryotic translation initiation factor 2α (eIF2α) by four different kinases, which results in attenuation of global protein synthesis, but increasing the translation of certain mRNAs with uORFs like ATF4. However, how mitochondrial stress triggers the ISR is unknown. Here we show that HRI is the eIF2α kinase that is necessary and sufficient for this relay. Taking advantage of the regulatory mechanism of ATF4 translation, we established an ATF4 translational reporter to perform an unbiased CRISPR interference screen. From this screen, we identified factors upstream of HRI: OMA1, a mitochondrial stress-activated protease; and DELE1, a little-characterized protein that we found was associated with the inner mitochondrial membrane. Mitochondrial stress stimulates OMA1-dependent cleavage of DELE1 and leads to the accumulation of DELE1 in the cytosol, where it interacts with HRI and activates the eIF2α kinase activity of HRI. In addition, DELE1 is required for ATF4 translation downstream of eIF2α phosphorylation, of which we are dissecting the mechanism currently. In addition, we are investigating how this newly identified pathway regulates mitochondria under stress conditions.
SG121

**Nuclear-based quality control of non-imported mitochondrial proteins**

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Mitochondria are protein-rich organelles, with ~1000 proteins in yeast, and ~1500 in mammals. The majority of mitochondrial proteins (~99%) are encoded in the nucleus, synthesized in the cytoplasm, and imported into mitochondria post-translationally. Mitochondrial import deficiency causes cellular toxicity due to the accumulation of non-imported mitochondrial precursor proteins, termed mitoprotein-induced stress. Despite the burden mis-localized mitochondrial precursors place on cells, our understanding of the systems that dispose of these proteins is incomplete. To increase our understanding of how cells deal with unimported mitochondrial proteins, we cataloged the sub-cellular localization and steady-state abundance of mitochondrial precursor proteins during mitochondrial import failure using *S. cerevisiae* as a model system. We found that a number of non-imported mitochondrial proteins localize to the nucleus, where they are subjected to proteasome-dependent degradation through a process we term nuclear-associated mitoprotein degradation (mitoNUC). Recognition and destruction of mitochondrial precursors by the mitoNUC pathway requires the presence of an N-terminal mitochondrial targeting sequence and is mediated by combined action of the E3 ubiquitin ligases San1, Ubr1, and Doa10. Impaired breakdown of precursors leads to alternative sequestration in nuclear-associated foci, the nature and composition of which are currently unclear. Overall, these studies identify an important and unexpected role for the nucleus in combating mitoprotein induced stress, and enhance our understanding of the crosstalk between these two cellular organelles. Our future studies are focused on understanding whether any of the newly identified nuclear mis-localized mitochondrial proteins, many of which are metabolic enzymes, perform retrograde signaling functions under conditions of mitochondrial impairment.

SG122

**A developmental mitophagy resets the mitochondrial proteome to facilitate germline mtDNA quality control**

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Mitochondrial DNA (mtDNA) is subject to high mutation rates and undergoes little recombination, making it susceptible to the accumulation of deleterious mutations. Left unchecked, over generations the increased genetic load would ultimately result in the decline of the species. To prevent this, the female germline has evolved a quality control mechanism to purge itself of mutant mtDNA. Through a large screen, we uncovered a programmed mitophagy that is essential for mtDNA quality control. We find that germline mitophagy is developmentally regulated and triggered upon entry into meiosis. We identify the RNA binding protein, Ataxin-2, as a master regulator of germline mitophagy, which activates mitophagy by inhibiting TORC1. Once induced, germline mitophagy works in a piecemeal fashion to reset the mitochondrial proteome so that each mitochondrion’s proteome matches its genome, allowing for mitochondria harboring mutant genomes to be selected against. This work sheds new light onto the mechanism of germline mtDNA inheritance and posits a generalizable strategy for mtDNA quality control applicable across tissues and disease states.
Mitochondrial Stress Induced Activation of Nuclear Encoded Innate Immune Genes
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Mitochondrial stress can lead to the release of damage-associated molecular patterns (DAMPs) that can activate nuclear genes encoding for innate immune responses. This may occur through the cGAS STING pathway and through NFkB signaling. Parkin and PINK1 are proposed to function to prevent inflammation and neurodegeneration by clearing damaged mitochondria by autophagy to prevent increases in cytosolic and circulating mtDNA and perhaps other mitochondrial DAMPs. New cell culture models of mitochondria damage offer ways to map the pathways of mitochondrial damage and explore how different DAMPs may be involved in nuclear response to mitochondria. Furthermore, new animal models reveal unexpected feedback between innate immunity and mitochondrial damage.

Mitochondrial DNA replication stress activates mitochondrial retrograde signaling via a novel mtDNA-release pathway

Mitochondria contain their own DNA (mtDNA), which was originally derived from bacteria and is normally housed in the matrix of the organelle. However, when it escapes the confines of mitochondria, mtDNA can trigger innate immune responses and inflammatory pathology, including cardiovascular and Parkinson’s disease. We previously discovered that depletion of the mtDNA-packaging protein, TFAM, leads to enhanced release of mtDNA into the cytosol, where it initiates cGAS-STING signaling, thereby upregulating interferon-stimulated gene (ISG) expression in the nucleus and enhancing anti-viral innate immunity. More recently, we found that chemotherapeutic DNA-damaging agents also stimulate mtDNA release, resulting in expression of a specific ISG subset that promotes nuclear DNA damage signaling and repair. The mechanism by which mtDNA damage leads to its release is unknown, but is associated with enlarged nucleoids (mtDNA/protein complexes analogous to chromatin), as well as elongated mitochondria. First, using live cell imaging, we found that these enlarged nucleoids are released in their entirety from mitochondria and co-localize with the innate immune sensor cGAS soon after escape. Next, we performed 5-ethynyl-2’-deoxyuridine (EdU) incorporation experiments and found that enlarged nucleoids have a diminished rate of mtDNA replication. Consistent with mitochondrial/ER contacts coupling mtDNA replication to mitochondrial fission, we found that the ER forms extensive contacts at mitochondria around the sites of enlarged nucleoids, and that blocking mitochondrial fission in wild-type cells drives the formation of enlarged mtDNA nucleoids and stimulates mtDNA release. These results indicate that stalled or incomplete mtDNA replication alters mitochondria/ER contacts, inhibits mitochondrial fission, and increases mtDNA release. Finally, evidence for a novel pathway for mtDNA release downstream of this type of mtDNA stress will be discussed that we propose is also relevant in the context of viral infection.
Systematic analysis of human mitochondrial translatomes reveals points of mitonuclear balance

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Oxidative phosphorylation (OXPHOS) complexes consist of nuclear and mitochondrial DNA-encoded subunits. Their biogenesis requires cross-compartment gene regulation to mitigate the accumulation of disproportionate subunits. To determine how human cells coordinate mitochondrial and nuclear gene expression processes, we re-engineered ribosome profiling to comprehensively capture translating mitoribosomes with subcodon resolution and quantified mitoribosome density across all transcripts in five human cell types. The resultant detailed view of mitochondrial translation across the 13 canonical open reading frames revealed features of translation initiation on leaderless mt-mRNAs. We also discovered a small ribosome-engaged ORF in the 3’ UTR of MT-ND5. Investigating mitonuclear coregulation across cell types revealed a modest correspondence between the average RNA abundances of nDNA- and mtDNA-encoded OXPHOS subunits. Remarkably, we found that translational control tightened the correspondence substantially, such that average mitochondrial and cytosolic subunit synthesis for each OXPHOS complex demonstrated a near perfect correlation. Balanced mitochondrial and cytosolic synthesis did not rely on rapid feedback between the two translation systems. By contrast, LRPPRC, a gene associated with Leigh syndrome, is required for the reciprocal translatomes and maintains cellular proteostasis. Based on our findings, we propose that human mitonuclear balance relies on the coregulation of OXPHOS subunit translation across cellular compartments, which may represent a proteostasis vulnerability.

A tRNA processing enzyme is a central regulator of the mitochondrial unfolded protein response

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Mitochondria-nuclear crosstalk is critical for maintaining healthy mitochondria. This inter-organelle communication is exemplified by the activation of mitochondrial unfolded protein response (UPRmt) which results in the expression of hundreds of genes that aim to restore mitochondrial function. The transcription factor ATFS-1 has emerged as the crucial player in the induction of UPRmt. However, cellular components that communicate mitochondrial dysfunction to ATFS-1 are not known. Here, we sought to identify such components. In C. elegans, we discovered that the tRNA processing enzyme HOE-1 (for homolog of ELAC2) is required for UPRmt. While HOE-1 localizes to mitochondria and the nucleus, we find that nuclear-localized HOE-1 is required for UPRmt. Consistent with this observation, we show that mitochondrial stress triggers accumulation of HOE-1 in the nucleus. We also show that preventing HOE-1 from leaving the nucleus is sufficient to robustly induce UPRmt, even in the absence of mitochondrial stress. Our data suggest that HOE-1 is necessary and sufficient for UPRmt. Mechanistically, we find that HOE-1 promotes nuclear accumulation of ATFS-1. We propose that HOE-1 does so via tRNAs because we find that blocking tRNA export from the nucleus inhibits HOE-1-induced UPRmt. Taken together, we have identified a new player and a novel mechanism by which mitochondrial stress triggers UPRmt, revealing molecular underpinnings important for mitochondria-nuclear crosstalk.
SG127

Mapping the genetic landscape of complex cellular phenotypes with genome-scale Perturb-seq

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Mapping the genetic landscape of complex cellular phenotypes is a central goal in genetics. Perturb-seq (pooled single-cell RNA-sequencing CRISPR screens) represents a unique tool for surveying this landscape by combining high-dimensional genetic perturbations with high-content phenotyping in human cells. Here we present the first genome-scale Perturb-seq screens which reveal fundamental principles of genetics and regulatory networks. By profiling millions of cells bearing thousands of distinct genetic perturbations, we show that transcriptional signatures can be used to systematically cluster genes into complexes and subcomplexes. This clustering uncovers functions for uncharacterized genes in ribosome biogenesis, mitochondrial respiration, transcriptional regulation, and nucleosome positioning. Beyond classifying gene function, our Perturb-seq dataset enables the study of unexplored connections between phenotypes in single-cells. In this context, an examination of the relationship between the cell cycle and differentiation highlights a role for replication stress in driving altered differentiation of leukemia cells. Finally, we use Perturb-seq to examine the regulatory function of the mitochondrial genome. We find that cells respond to diverse mitochondrial perturbations with simple nuclear-encoded but multidimensional mitochondrial-encoded transcriptional changes. This supports a model where retention of the endosymbiont genome may enable perturbation-specific, local adaptations to stress. Our results establish Perturb-seq as a powerful, scalable tool for mapping complex pathways in human cells.

Quantum Biology

SG128

Radical pair based magnetic field effects on cellular photochemistry

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In the 1970s, it was established that weak magnetic fields could alter chemical reactions proceeding through the formation of reaction intermediates known as spin-correlated radical pairs. The radical pair mechanism (RPM) is fundamentally quantum mechanical in nature and allows magnetic fields even as weak as that of the Earth’s to measurably affect chemical reaction rates and yields, despite the field inducing a change in energy of much less than the thermal energy. The RPM is of particular interest in biology as it potentially lies at the heart of avian magnetoreception and similar abilities in many other animals. It has also been proposed as a potential mechanism to account for effects of environmental electromagnetic fields on human health. RPM based magnetic sensitivity constitutes a true quantum biological phenomenon and has many potential future applications in biological sensing and imaging. While magnetic field effect (MFEs) due to the RPM are manifold, examples in biology are few and there are no confirmed observations, for unmodified biological reactions in vitro. In this work, we used a custom designed fluorescence microscope to project a magnetic field on native HeLa cell samples, to investigate whether the natural autofluorescence of cells exhibits radical pair based magnetic responses. This idea is based on the fact that flavin photochemistry is well established to show RPM based MFEs in aqueous solution, even at physiological pH, and flavins are known to contribute to cellular autofluorescence. Furthermore flavin adenine dinucleotide is the blue light absorbing cofactor in
cryptochromes, which are the currently frontrunners as the source of the magnetic compass ability in migratory birds. We were able to demonstrate direct realtime changes in cellular autofluorescence under the application of a switched or ramped magnetic field and use the latter to determine the change in magnetic field response with increasing magnetic field strength. The field dependence provides strong evidence that the RPM is responsible for the observed effect. In addition, we were able to confirm that the fluorescence observed arose directly from flavins in the cells. This is the first observation of RPM based MFES in living cells. In this presentation, we provide an introduction to the radical pair mechanism for cell biologists and present details on our measurements and their implications.

SG129

_Cryptochrome - A Photoreceptor that Responds to Electromagnetic Fields._

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One of the ongoing mysteries of how organisms respond to their environment has been of whether and how biological receptors may detect electromagnetic fields. Birds and other organisms have long been known to use the earth’s magnetic field to find direction, whereas biological effects in man have been reported from exposure to both static and oscillating radiofrequency fields produced in telecommunications or by power grids. The cryptochrome photoreceptor has emerged as a promising candidate receptor for both magnetic fields and oscillating radiofrequency fields in organisms ranging from plants to man. Cryptochromes are flavoprotein receptors that can be activated by redox reactions in response to blue light. Physiological responses mediated by cryptochromes in many organisms have proven sensitive to applied electromagnetic fields, including plant growth and the entrainment of the circadian clock in flies. Hence, they have been proposed as biological magnetoreceptors. In this talk, using a combination of physiological, biochemical, and biophysical data, I will briefly summarize the evidence linking cryptochrome, to the perception of electromagnetic fields, with particular emphasis on avian and plant cryptochromes. Theory explains that weak forces in electromagnetic fields may subtly ‘speed up’ or ‘slow down’ the cryptochrome activation reaction, in this way altering its biological response. Possible magnetically sensitive radical pairs (reaction intermediates) will be identified by EPR spectroscopy. In addition, radiofrequency exposure in the MHz range directly alter the active state of the plant cryptochrome _in vivo_. Thus, cryptochrome to date is the only known biological receptor that responds both to static and radiofrequency electromagnetic fields, apparently by an underlying biophysical mechanism conserved in all cryptochromes. Finally, cryptochrome has been implicated in responses to a wide range of electromagnetic field exposure conditions in human cells, including static, pulsed (10 - 50 Hz) and radiofrequency (MHz to GHz range). These all induce ROS (reactive oxygen species) in the nuclear, perinuclear, and cytosolic compartments. Because small, localized changes in intracellular ROS can have profound physiological effects, these findings should better help to explain and control the consequences of magnetic field exposure in humans, including for novel beneficial therapeutic effects.
Excited Electrons in Human Disease
D. E. Brash; Therapeutic Radiology, Yale University School of Medicine, New Haven, CT.

One aspect of quantum biology is electron excitation. In an ordinary chemical reaction, supplying energy increases inter-nuclear vibration and stretches covalent bonds to their breaking point. In excited-state chemistry, much higher energies excite an electron to a new orbital - accessing not only higher energy levels but also new geometries needed for reactions otherwise forbidden by the Woodward-Hoffman rules of quantum chemistry. The cyclobutane pyrimidine dimer (CPD) in DNA - which leads to mutations and skin cancer - requires a [2+2] cycloaddition of two bonds at once, a geometry requiring electron excitation and usually provided by sunlight's UV radiation. Surprisingly, CPDs can be made in the dark by the biophysical process of chemiexcitation, aided by unusual electronic properties of melanin. This pigment, normally responsible for blocking UV from reaching the skin, contains electrons that are easily excited or oxidized and have easily flippable spins. UV activates, for hours, the enzymes NADPH oxidase and nitric oxide synthase, generating the radicals superoxide and nitric oxide. These combine to form peroxynitrite, which oxidizes melanin fragments to add a dioxetane - a high-energy 4-membered ring. The ring splits spontaneously and the energy goes to the resulting carbonyl, exciting an electron while also flipping that electron's spin to create a "triplet state". Triplet spins cannot easily flip back, so the state is long-lived; this allows time to transfer the energy to nearby molecules such as DNA by exchanging the high-energy triplet electron for a low-energy electron in the recipient. The discovery of a spin-dependent chemiexcitation process in mammalian cells raises several prospects. Sunburn features the same two activated enzymes, so might create a second wave of dark CPDs. Inflammation and melanin co-occur in other diseases, such as macular degeneration, noise-induced deafness, and Parkinson's disease. Several neurotransmitters have structures resembling melanin monomers, are easily oxidized, and can be electronically excited, so may also be substrates for chemiexcitation and pathogenesis. Conversely, Nature has devised triplet-state quenchers to protect, e.g., chloroplasts. Melanin's quantum structure confers further intriguing properties: it is one of the best sound absorbers known and can act as a proton conductor and a semiconductor. Identifying biological problems that have quantum underpinnings, and solving them, will be hastened by communication between quantum chemists or physicists - familiar with quantum phenomena and tools for studying them - and biologists familiar with the problems needing solving and accustomed to proving causal relationships in systems that have many parts, hierarchical organization, and feedback loops.

Using biological inspiration for chemical physics informed design of chromophores for shortwave infrared imaging and quantum measurement
J. R. Caram; University of California, Los Angeles, Los Angeles, CA.

The near and shortwave infrared spectral window provides fantastic contrast in complex environments, through skin, tissue, fog and foliage. However, there are few organic chromophores which absorb and emit in this window, and those that do have very low quantum yields. To systematically improve chromophores in the SWIR we turn to fundamental chemical physics, designing more efficient radiative emission, or designing out non-radiative loss pathways. These insights have led us to leverage new insight into chromophore self-assembly and J-aggregate design principles to make infrared emissive materials, in analogy to photosynthetic complexes.
SG132

Using QuantumPhenomena to Control ROS-Mediated Stem Cell Proliferation
W. S. Beane; Western Michigan University, Kalamazoo, MI.

Recent advances in quantum biology have highlighted the potential advantages of quantum approaches as future therapies. Our prior work identified weak magnetic fields (WMFs) as one such approach. Biological systems (from cells to animals) are constantly exposed to WMFs (<1 mT) in the form of the natural geomagnetic field (25-65 µT) as well as WMFs from technology. One theoretical model for how WMFs can affect biological systems is through quantum effects that alter electron spin states of free radicals (the radical pair mechanism), with different field strengths hypothesized to either promote or inhibit the formation of radical oxygen species (ROS). Here, I will discuss our investigations into the ability of WMFs to modulate stem cell activity in vivo, using the planarian regeneration model. Our data revealed that compared to both unexposed and earth-normal (45 µT) controls, planarians exposed to 200 µT WMFs had significantly decreased levels of: ROS accumulation, Hsp70 expression, adult stem cell numbers, stem cell proliferation, and blastema (new tissue) growth. Conversely, our studies found that 500 µT exposure had the opposite effects: resulting in significantly increased ROS levels, Hsp70 expression, stem cell proliferation, and blastema sizes. Furthermore, WMF exposure both phenocopied molecular-genetic ROS inhibition and was predictive of subsequent results obtained with traditional methods to increase ROS. Thus our results support the hypothesized action of WMFs on free radical concentrations and indicate that WMFs may be a potential non-invasive tool to modify cell proliferation and stem cell activity either positively (as in regenerative medicine) or negatively (as in cancer therapies). Together, these data suggest quantum approaches to controlling stem cells are an emerging research area. Funding: NSF #1652312 and 1644384.

The Interplay Between Lipids and Proteins in the Endoplasmic Reticulum (ER): How the ER is the Master Regulator of the Cell Universe

SG133

Endoplasmic reticulum stress sensor ire-1 is detrimental in young but essential in aged animals fed high glucose diet
G. Thibault; School of Biological Sciences, Nanyang Technological University, Singapore, SINGAPORE.

Ageing is one of the most critical risk factors for the development of metabolic syndromes. Prominent metabolic diseases have a strong association with endoplasmic reticulum (ER) stress. Upon ER stress, the unfolded protein response (UPR) is activated to limit cellular damage. However, adaptive genes upregulated from the UPR tend to decrease with age. Here, we show that 5-day-old Caenorhabditis elegans fed a bacteria diet with 2% glucose (high glucose diet, HGD-5) extend their lifespan while shortening the lifespan of 1-day-old (HGD-1) animals. We observed a metabolic shift in HGD-1 as glucose and fertility synergistically prolonged the lifespan of HGD-5, independently of DAF-16. Surprisingly, we identified that UPR stress sensors ATF-6 and PEK-1 extended the longevity of HGD-5 while the absence of IRE-1 drastically increased HGD-1 lifespan. Based on these observations, we hypothesise that HGD activates the otherwise quiescent UPR in aged worms to overcome age-related stress and restore ER homeostasis. In contrast, young adult animals subjected to HGD leads to unresolved ER stress, conversely leading to a deleterious stress response.
SG134

**Rhomboid pseudoproteases employ lipid distortion function for retrotranslocating ERAD membrane substrates**

S. Neal; University of California, San Diego, La Jolla, CA.

Nearly one-third of proteins are initially targeted to the endoplasmic reticulum (ER) membrane where they are correctly folded, assembled, and then delivered to their final cellular destinations. In order to prevent the accumulation of misfolded membrane proteins, ER associated degradation (ERAD) moves these clients from the ER membrane to the cytosol; a process known as retrotranslocation. Our recent work in *S. cerevisiae* has revealed a derlin rhomboid pseudoprotease, Dfm1, is involved in the retrotranslocation of ubiquitinated ERAD membrane substrates. We have sought to understand the mechanism associated with Dfm1’s actions and found that Dfm1’s conserved rhomboid residues are critical for membrane protein retrotranslocation. Specifically, we identified several retrotranslocation-deficient Loop 1 mutants that display impaired binding to membrane substrates. Furthermore, Dfm1 has retained the lipid thinning functions of its rhomboid protease predecessors to facilitate the removal of ER membrane substrates. We find this substrate engagement and lipid thinning feature is conserved in its human homolog, Derlin-1. Utilizing interaction studies and molecular dynamic simulations, this work reveals that rhomboid pseudoprotease derlins employ novel mechanisms of substrate engagement and lipid thinning for catalyzing extraction of multi-spanning membrane substrates.

SG135

**Order from disorder: COPII coat assembly driven by intrinsically disordered domains**

E. Miller, X. Li, V. Stancheva; MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM.

The COPII coat mediates export of secretory proteins from the endoplasmic reticulum (ER). It assembles on discrete domains of the ER known as ER exit sites (ERES) to recruit cargo proteins and bend the membrane into small, spherical transport carriers. We are interested in understanding how assembly of the coat is regulated to ensure that vesicle form at the appropriate site and capture appropriate cargo proteins. We use yeast genetics and biochemistry, coupled with correlative light and electron microscopy (CLEM) to visualize ERES and dissect coat assembly requirements. We find that coat assembly is driven locally by multiple weak interactions between the cargo adaptor proteins and the "outer" scaffolding layer. These weak interactions mutually reinforce each other to drive oligomerization. In the absence of scaffolding activity contributed by Sec13, larger more pleiomorphic vesicles form at ERES, and we see a corresponding increase in leakage of ER residents. We propose that coat oligomerization is favoured when cargo-bound adaptor layers trigger local scaffolding to form curved membranes. This curvature in turn creates physical pressure in the ER lumen as the vesicle forms, which serves to exclude non-specific cargo by steric effects. Thus specificity in ER export is driven in part by the biophysical effects of local membrane curvature and cargo capture.

SG136

**Apoe at the intersection of lipid storage and secretion**

S. Cohen, I. Windham; UNC, Chapel Hill, NC.

How do cells modulate the balance of lipid storage versus secretion? Lipid droplets (LDs) are the primary lipid storage organelle, while lipoprotein particles transport lipids between cells. Neutral lipid synthesis
for the formation of both LDs and lipoprotein particles occurs in the endoplasmic reticulum (ER). In the brain, astrocytes play a central role in lipid metabolism. Astrocytes can store triglycerides and sterol esters within cytoplasmic LDs, or package these neutral lipids into lipoprotein particles which are then secreted and taken up by neurons to build membranes and synapses. ApolipoproteinE (ApoE) is the primary protein component of lipoprotein particles secreted by astrocytes. The APOE4 allele is also the strongest genetic risk factor for developing late-onset Alzheimer’s disease. We have made the surprising discovery that under certain metabolic conditions, ApoE subverts translocation into the ER, and instead traffics to the cytoplasmic surface of LDs. ApoE moves from the ER to LDs via membrane contact sites. The C-terminal domain of ApoE is responsible for LD binding, while the N-terminal domain is necessary for subverting translocation into the ER. Once on the LD surface, ApoE modulates LD number, size, and function. Our results suggest that ApoE plays a novel role in lipid metabolism, independent of its function in lipid secretion. We propose that ApoE is at the intersection of lipid storage versus secretion in the ER, shifting the balance between the two in response to changing environmental or developmental conditions.

SG137

Multimodality structured illumination microscopy for super-resolution live-cell imaging

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Biological research is desired to characterize the intracellular dynamics at high spatiotemporal resolution with low photobleaching and phototoxicity effects, which therefore allow continuously resolve the delicate structures and behaviors of the engaged organelles over the whole biological process. However, the trade-offs between spatial and temporal resolution, and low phototoxicity/photobleaching always compromise the practical performance of current super-resolution imaging techniques. To achieve these normally opposing goals, in this talk I will discuss our latest developments in multi-modality structured illumination microscopy (Multi-SIM) and lattice light sheet SIM microscopy (LLS-SIM), which enable high-speed super-resolution live-cell imaging for thousands of time-points spanning over hours of time-lapse. Recently, we further developed a deep-learning algorithm for super-resolution image reconstruction, termed deep Fourier channel attention network (DFCAN), which further extends the applicability of Multi-SIM and LLS-SIM into more challenging imaging conditions. Moreover, we applied these techniques to characterize the ultra-dynamics of endoplasmic reticulum (ER) and microtubule dynamic instability, the association of mitochondrial fission and fusion sites with ER tubule, the phase separation process of typical membrane-less organelles, and the multifarious hitchhiking forms between different organelles, etc.

SG138

Pex30-like proteins function as adaptors at distinct endoplasmic reticulum membrane contact sites

J. P. Verissimo Ferreira, P. Carvalho; University of Oxford, Oxford, UNITED KINGDOM.

Membrane lipids and proteins synthesized in the endoplasmic reticulum (ER) are utilized for de novo assembly of organelles, like lipid droplets and peroxisomes. After assembly, the growth of these organelles is supported by ER-derived lipids transferred at membrane contact sites (MCS). How ER sites for organelle biogenesis and lipid transfer are established and regulated is unclear. Here, we investigate how the ER membrane protein Pex30 and its family members Pex28, Pex29, Pex31 and Pex32 target and function at multiple MCS. We show that different Pex30 complexes function at distinct ER domains and
MCS. Pex30 targets ER-peroxisome MCS when bound to Pex28 and Pex32, organizes the nuclear-vacuolar junction when bound to Pex29 and promotes the biogenesis of lipid droplets independently of other family members. Importantly, the reticulon-homology domain (RHD) mediates the assembly of the various Pex30 complexes. Given the role of RHD in membrane shaping, our findings offer a mechanistic link between MCS and regulation of membrane curvature.

SG139  
**Spartin is a receptor mediating the selective autophagy of lipid droplets**  
**J. Chung**¹,², J. Park³, Z. Lai¹,², R. C. Richards¹,², R. V. Fares Jr.¹,², T. C. Walther¹,²; ¹Department of Cell Biology, Harvard Medical School, Boston, MA, ²Department of Molecular Metabolism, Harvard T. H. Chan School of Public Health, Boston, MA, ³Department of Pharmacology, Department of Neurology, Wayne State University, Detroit, MI.

Lipid droplets (LDs) are cellular organelles involved in energy storage and lipid homeostasis. Selective autophagy of LDs is an important pathway for LD catabolism, but cellular receptors mediating lipophagy are unknown. Here we show that spartin, encoded by SPART, a causative gene of Troyer syndrome/hereditary spastic paraplegia, functions as a receptor mediating the autophagic degradation of LDs. Spartin localizes to LDs and is required to deliver them to lysosomes. Spartin targets to LDs through an array of amphipathic helices and interacts with autophagosomal ATG8/LC3 proteins using its ubiquitin-binding region. Spartin deficiency impairs LD mobilization in cells. Using a lipophagy-Keima flux reporter, we find that spartin deficiency abolishes lipophagic flux in cells. Moreover, interfering with spartin function in mouse cortical neurons resulted in neuronal LD accumulation, indicating a requirement for LD autophagy in these cells. Our findings thus identify a selective lipophagy receptor and suggest impaired LD turnover may contribute to the development of Troyer syndrome.

WEDNESDAY, DECEMBER 8, 2021  
Centromere Structure and Function  

SG140  
**DNA repair proteins moonlighting at the inner centromere**  
**K. Bloom**, J. Stanton, D. Cook, J. Lawrimore, E. Yeh; University of North Carolina, Chapel Hill, NC.

Several homologous recombination proteins (Rad51, Rad52) have been reported to contribute to centromere function and kinetochore clustering in the budding yeast *S. cerevisiae*. We have discovered that Rad51 and Rad52 are required for organization of the inner centromere. The centromere is defined at the DNA sequence level in *S. cerevisiae* and is the site of assembly of the kinetochore. The inner centromere (also known as the pericentromere) is the chromatin region between sister centromeres in mitosis. The 3D architecture of the kinetochore reveals a cylinder ~70 nm in length (N-terminal Ndc80 to Cse4) with a diameter slightly larger than a 25 nm microtubule. The inner centromere is ~800 nm length x ~ 500 nm dia. and is considerably less well-defined than the kinetochore. Using point fluorescence microscopy and statistical probability maps to deduce the two-dimensional mean position of representative components of the centromere we have mapped Slk19 and Sgo1 (shugoshin) to the inner centromere. Relative to the position of the centromere-specific histone variant (Cse4), Slk19 and Sgo1 are 62 nm and 68 nm toward the chromosome axis (away from the kinetochore). Slk19 is found at the...
SG141
The kinetochore protein KKT23 acetylates histone H2A to promote chromosome segregation in kinetoplastids.

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The kinetochore is the macromolecular protein machinery that drives chromosome segregation in eukaryotes. A hallmark of kinetochores in most eukaryotes is the presence of specialized nucleosomes containing the centromere-specific histone H3 variant CENP-A, which epigenetically specifies the position of kinetochore assembly and recruits other kinetochore proteins. However, CENP-A is absent in all sequenced kinetoplastids, an evolutionarily divergent group of eukaryotes. Essentially nothing is known about the nature of centromeric chromatin in kinetoplastids so far, and it remains a mystery how their kinetochores assemble specifically at centromeres. Kinetoplastids possess a unique set of kinetochore proteins (KKT1—20, 22—25 and KKIP1—12) that lack significant similarity to canonical kinetochore components present in other eukaryotes. One of these proteins, KKT23, is predicted to have an acetyltransferase domain at the C-terminus and is essential for cell growth and chromosome segregation in Trypanosoma brucei. Interestingly, no known kinetochore protein has an acetyltransferase domain in other eukaryotes, thus highlighting KKT23 as a unique feature of kinetoplastid kinetochores. So far, nothing is known about its molecular function or its substrate. Using X-ray crystallography and NMR spectroscopy, we show that KKT23 indeed has an acetyltransferase domain with close structural similarity to the histone acetyltransferase Gcn5. Moreover, we identify histone H2A as a substrate of KKT23. We propose that KKT23 acetylates histones at centromeres, which specifies the position of kinetochore assembly in kinetoplastids.

SG142
Human centromeres drift through cellular proliferation

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CENP-A is a heritable epigenetic mark that determines centromere identity and is essential for centromere function. Centromeres are the central genetic element responsible for accurate chromosome segregation during cell division, and as such, they are anticipated to be evolutionarily stable. How centromeres evolved to allow faithful chromosome inheritance on an evolutionary timescale despite their epigenetic maintenance is unclear. Here we sought to determine whether CENP-A is capable of precisely and stably specifying human centromere position throughout cellular
proliferation. To investigate the positional stability of human centromeres as cells proliferate, we used the PD-NC4 fibroblast cell line that harbors a neocentromere (epigenetic stable acquisition of a new centromere at a new chromosomal site) on chromosome 4. This non-repetitive neocentromere provides an advantage for centromere analysis by allowing precise mapping of CENP-A-bound DNA molecules to resolve CENP-A distribution at high, base pair resolution. CENP-A ChIP-sequencing in the parental PD-NC4 cell line and in nine single cell-derived clones reveals significant differences in patterns of CENP-A deposition. While parental cells show three major peaks of CENP-A binding at the neocentromere, three distinct patterns of CENP-A deposition are seen in the single-cell derived clones. Thus, the neocentromere position on chromosome 4 in PD-NC4 cells varies within a population. Our preliminary data reveals that each pattern of CENP-A deposition is relatively stable over cellular proliferation, i.e., the number of major CENP-A peaks is stable. Interestingly, we observe significant drift for CENP-A binding over cellular proliferation within each clone, but the total length of chromatin assembled with CENP-A does not change significantly. Our results suggest that while the deposition pattern of CENP-A may change, the number of CENP-A-containing nucleosomes remains constant over cellular proliferation, which is important for preserving centromere function.

SG143
Centromere Function in the Asymmetric Cell Division of Drosophila Germline Stem Cells
A. Kochendoerfer, E. Dunleavy; National University of Ireland Galway, Galway, IRELAND.

Germline stem cells divide asymmetrically to produce one new stem cell and one differentiating daughter cell. The daughter cell will subsequently undergo meiosis and differentiate to generate the mature gamete (egg or sperm). As a mechanism to explain how asymmetric cell division (ACD) can generate two cells with unequal fates, the silent sister hypothesis (SSH) proposes the selective inheritance of sister chromatids carrying specific epigenetic marks between stem and daughter cells. To facilitate this selective inheritance, the hypothesis specifically proposes that the centromeric region of each sister chromatid is distinct. In Drosophila germ line stem cells (GSCs), we and others have recently shown that the centromeric histone CENP-A (called CID in flies) - the epigenetic determinant of centromere identity - is asymmetrically distributed between pairs of sister chromatids. In addition to harbouring more CID, sister chromatids destined to end up in the stem cell assemble more kinetochore proteins (CENP-C, NDC80) and capture more spindle microtubules. Taken together these findings support a model of ‘mitotic drive’ that can bias chromosome segregation in asymmetric stem cell divisions. Here I will present our recent data in which we disrupt the asymmetric inheritance CID in Drosophila GSCs by the over-expression of centromere proteins and measure the subsequent impact on ACD and cell fate. We find that excess CID expressed together with its assembly factor CAL1 leads to a symmetric CID distribution between stem and daughter cells. Moreover, we measure a change the balance of stem and daughter cells in the niche, with an increased pool of GSCs. In contrast, excess CAL1 alone did not affect CID asymmetry and appears to drive cellular proliferation, increasing the overall size of the niche. Our results provide support for the SSH according to which biased sister chromatid inheritance via epigenetically distinct centromeres can impact on cell fate.
Within the pericentric regions of human chromosomes reside large arrays of tandemly repeated satellite sequences. Expression of the human pericentric satellite HSATII is normally prevented by extensive heterochromatin-mediated silencing within pericentric regions in normal cells, yet in cancer cells, HSATII RNA is aberrantly expressed and accumulates in large nuclear foci. Expression and aggregation of HSATII RNA in cancer cells is concomitant with recruitment and sequestration of key chromatin regulatory proteins including MeCP2, which binds to HSATII transcripts. While HSATII expression has been observed in a wide variety of cancer cell lines and tissues, the effect of its misregulation within the nuclear environment is unknown. Due to both its localization within pericentric regions and the observations that HSATII RNA accumulates in cis and binds key chromatin regulatory proteins, we tested the effect of stable expression of HSATII RNA within cells that do not normally express HSATII. Stable ectopic expression of HSATII leads to focal accumulation of HSATII RNA in cis and recruitment of MeCP2 to nuclear HSATII RNA bodies. Further, long-term ectopic expression of HSATII RNA leads to cell division defects including lagging chromosomes, chromatin bridges, and other chromatin defects.
ORAL PRESENTATIONS

SG146
A dynamic SUMO cycle ensures stable centromeric chromatin inheritance
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Centromeres are defined by a self-propagating chromatin structure based on stable inheritance of CENP-A containing nucleosomes. Using SNAP-tag based pulse-labeling of CENP-A, we previously showed that CENP-A nucleosomes have no appreciable turnover at the centromere in cycling cells, undergoing only replicative dilution. This is consistent with a role in epigenetic specification of the centromere. In a genetic screen coupled to pulse-chase labeling that allows us to identify proteins selectively involved in long-term transmission of chromatin-bound CENP-A we discovered a role for the SUMO protease SENP6. Using a temporally controlled degron allele we find that acute depletion of SENP6 results in rapid loss of CENP-A chromatin at any stage in the cell cycle. Loss of SENP6 results in hyper-SUMOylation of CENP-C and CENP-I but not CENP-A itself. SUMOylation not only controls CENP-A stability but virtually the entire centromere and kinetochore, suggesting that a dynamic SUMO cycle underlies a continuous surveillance of the centromere complex that in turn ensures stable transmission of CENP-A chromatin.

SG147
Aurora B activation by phosphorylation is a synergistic process inducing structural organization and synchronized internal motion of the enzyme
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Aurora B is a component of the chromosome passenger complex that controls multiple events during cell division. Aurora B, together with IN-box, the C-terminal region of INCENP, constitutes the catalytic component of the complex. In the cell, [Aurora B/IN-box] is activated by auto-phosphorylation while removal of the phosphoryl groups by phosphatases is inhibitory for the complex. However, the effect of phosphorylation on the structural and internal dynamic properties of the enzymatic complex is not clear. We use a combination of experimental (hydrogen-deuterium exchange and enzyme kinetics) and computational (molecular dynamics simulation) approaches to assess how the internal dynamics and enzymatic function of the [Aurora B/IN-box] complex change with phosphorylation. Our results provide evidences that the unphosphorylated inhibited form is more entropic with the active centre only partially assembled. Auto-phosphorylation is associated with structural organization in both the active centre and the allosteric parts of the enzyme complex. We used a chemical ligation approach to generate partially phosphorylated intermediates and assess the individual contribution of the two major phosphorylation sites: activation segment in Aurora B and TSS motif in IN-box, to the activation process. Our results clearly show that the two phosphorylations sites act synergistically to activate the enzyme complex resulting in a dominant productive breathing motion. This study provides a detailed mechanistic insight in the allosteric communication between Aurora B and IN-box and how this
communication is modulated by phosphorylation, thus resulting in a regulatory switch of Aurora B enzymatic activity.

SG148
Mislocalization of CENP-A contributes to chromosomal instability (CIN) and aneuploidy
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Aneuploidy resulting from chromosomal instability (CIN) contributes to tumorigenesis and karyotypic heterogeneity. Our studies focus on evolutionarily conserved histone H3 variant, CENP-A (Cse4 in budding yeast) which is essential for chromosomal stability. CENP-A overexpression (CENP-OE) contributes to its mislocalization to non-centromeric regions resulting in CIN in yeast and flies. CENP-A OE and mislocalization have been observed in several cancers and this correlates with poor prognosis. However, the consequences of CENP-A OE on CIN and aneuploidy have not been defined. We used a multi-organismal approach to define the causes and consequences of CENP-A mislocalization in yeast, human cells and mouse model. A genome-wide screen was used to identify mutants that exhibit growth sensitivity when Cse4 is overexpressed in yeast. The top hits of the screen encode the evolutionarily conserved ubiquitin ligase (SCF), replication dependent kinases (DDK) and the replication-independent histone chaperone (HIR) complexes. We defined a role for SCF-Met30, SCF-Cdc4, DDK and HIR proteins in proteolysis of Cse4 to prevent its mislocalization and CIN. For studies with human cells we used HeLa or stable pseudodiploid DLD1 cell lines overexpressing CENP-A and showed that CENP-A OE leads to mislocalization to non-centromeric regions and this contributes to CIN. Furthermore, mislocalization of CENP-A contributes to aneuploidy, karyotypic heterogeneity and increased invasiveness in DLD1 cell line and xenograft mouse model. We are using an image based high-throughput siRNA screen to identify gene deletions that show higher expression of YFP-CENP-A. We are pursuing in-depth studies with the top hits of the screen which include multiple components of the NuA4 complex (EP400, KAT5/TIP60, TRRAP), histone chaperone complexes (CHAF1B, CHAF1A, and HIRA) and a component of the SCF-ubiquitin ligase (SKP1). In summary, we provide the first evidence for a role of CENP-A OE in promoting aneuploidy with karyotypic heterogeneity and define a role for evolutionarily conserved pathways that prevent mislocalization of CENP-A for CIN in yeast and human cells. Our studies provide mechanistic insights into how mislocalization of CENP-A promotes aneuploidy with karyotypic heterogeneity and if this contributes to the poor prognosis of CENP-A OE cancers.

SG149
Molecular Dissection of the KMN network on the CENP-T pathway
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Chromosomes replicated during the S-phase must be divided into daughter cells during mitosis to transmit the genetic information to the progeny. The chromosome segregation process is ensured by a large protein complex called kinetochore on the centromere of each sister chromatid. Multiple proteins establish complicated networks to form functional kinetochores. CENP-C and CENP-T independently recruit the KMN (Knl1 complex (C), Mis12C, Ndc80C) network onto the kinetochores (CENP-C or CENP-T pathway). To clarify functions of the KMN network on the CENP-T pathway, we precisely evaluate its roles in chicken DT40 cell lines lacking the CENP-C-KMN network interaction. By analyzing mutants lacking the both CENP-T-Mis12C and CENP-C-Mis12C interactions, we demonstrate that Knl1C and Mis12C (KM) play critical roles in the sister chromatids cohesion or the recruitment of spindle-
checkpoint proteins onto kinetochores. Two copies of Ndc80C (N-N) exist on CENP-T via Mis12C or directly binding, and analyses of cells specifically lacking the Mis12C-Ndc80C interaction reveal that N-N are needed for proper kinetochore-microtubule interactions. However, using artificial engineering that enable the two copies of Ndc80C locate to CENP-T, we demonstrate that Mis12C does not necessarily interact with Ndc80C, and KM and N-N are separable in native kinetochores. We also demonstrate that Ndc80C binding to Mis12C is not necessary for microtubule binding in human cells. This study effectively demonstrates the mechanisms by which the complicated networks play their roles in native kinetochores.

SG150
Modulating and Rewiring Cell Division
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The macromolecular kinetochore structure plays an essential role in chromosome segregation by mediating chromosome-microtubule interactions. One of the most fascinating features of the kinetochore is its molecular and functional plasticity. Most essential molecular machines, such as the ribosome or proteasome, are functionally and structurally conserved amongst eukaryotes, but the kinetochore is remarkably flexible in its composition and properties. Although a subset of kinetochore proteins is required across most eukaryotes, kinetochore composition, protein sequences, and properties can vary dramatically between species. Interestingly, the kinetochore is also modulated within the same organism, with kinetochore composition and function changing over the cell cycle, during meiosis and development, and across diverse physiological situations. This plasticity appears to be critical for the kinetochore machine to flexibly adapt to diverse situations and functional requirements. I will discuss specific examples of how kinetochore function is modulated under different physiological contexts.

Cytoskeletal Dynamics in Health and Disease

SG151
Membrane and Cytoskeletal Dynamics During Muscle Progenitor Migration, Proliferation and Differentiation
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Defects in cytoskeletal dynamics during cell migration and morphogenetic movements are associated with many pathogenic processes. Here we explore the role of cytoskeleton regulation during cell migration, proliferation and differentiation of mesodermal precursors in the roundworm nematode, C. elegans. The C. elegans sex myoblasts (SMs), descendants of the Mesoblast (M) lineage, migrate anteriorly during larval development, arrive at the center of the gonad, re-enter the cycle and undergo three rounds of division before they differentiate into the adult vulval and uterine muscles, which are required for egg laying. The signaling pathways and guidance cues that lead the migration of SMs have been established. However, the genetic and kinetic mechanisms underlying their cell migration remain largely unknown. Using high-resolution sub-cellular microscopy, we analyzed the migration, proliferation
and differentiation of these cells. Using an M lineage specific membrane marker, we show that these cells are highly protrusive during migration and proliferation. Furthermore, using a cell cycle state sensor we correlate cell cycle state with cell morphology and protrusion dynamics during migration and proliferation. From live imaging of tagged endogenous proteins via CRISPR/Cas9 genome engineering, we show that these dynamic protrusions are actin rich and contain actin regulators such as Arp2/3/ARX-2, CDC-42, Ena-Vasp/UNC-34 and coflin/UNC-60. Additionally, we show that the SM cells express high levels of the cytoskeletal protein, septin/UNC-59, compared to surrounding cells and tissues. Using a combination of RNAi and the auxin/AID system, we are currently investigating the SM-specific depletion of these key cytoskeletal regulators. Thus, we can take advantage of the unique attributes of the SM cell lineage, allowing us to mechanistically dissect in vivo how reorganization of the actin cytoskeleton is required for switching between migratory, proliferative and differentiated states.

SG152
Towards a mechanistic understanding of motile and primary cilia with CLEM and cryo-electron tomography
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We combine EM and cell biology methods to obtain a mechanistic understanding of the molecular processes involved in the assembly and function of motile and primary cilia: conserved organelles that are fundamental for most eukaryotic cells and for human health. Cilium assembly requires the rapid bidirectional intraflagellar transport (IFT) of ciliary components to and from the site of assembly at its tip. IFT-trains are moved by the anterograde motor kinesin-2 and the retrograde motor dynein-1b, together with a large complex of about 25 IFT-proteins. By imaging the flagella of Chlamydomonas cells with cryo-electron tomography we showed how IFT protein complexes organize to assemble anterograde trains and how the competition between kinesin and dynein that are on the same train is avoided, as there is no tug-of-war during IFT. We show that the IFT trains and even their constitutive protein complexes rearrange their architecture completely when switching from anterograde to retrograde motility at the ciliary tip. We recently also started investigating the role of tubulin posttranslational modification for ciliary assembly and function. We have identified the position of modifications such as tubulin polyglycylation and polyglutamylation on the microtubules of motile cilia/flagella, investigated their role for the regulation of IFT and flagellar beating, and showed how mutations of the corresponding enzymes can affect human health.

SG153
Coordination of alpha-tubulin acetylation and mitochondrial transport by MFN2
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Acetylated microtubules (MTs) are a subset of stable MTs with key roles in the regulation of axonal transport and mitochondria dynamics: in addition to providing preferential tracks for kinesin-1 and dynein dependent transport, two properties of acetylated MTs, i.e., stability and flexibility, make them uniquely adapted to sustain mechanical stress caused by organelle/organelle interactions. It is thanks to these properties that also mitochondria fusion and ER/mitochondria contacts occur selectively on acetylated MTs. Mitofusin-2 (MFN2), a large GTPase residing in the mitochondrial outer membrane that
is mutated in an axonal form of Charcot-Marie-Tooth (CMT2A), is a critical regulator of mitochondrial fusion, transport and mito/ER tethering. Despite defects in mitochondria dynamics are associated with the pathogenesis of CMT2A, how mutations in MFN2 underlie the onset of this axonal neuropathy is not completely understood. Here we show that mitochondrial contacts with MTs are preferential sites of α-tubulin acetylation, which occurs through the recruitment of the ATAT1 to mitochondrial outer membranes by MFN2. Importantly, we find that this novel MFN2 function is critical for mitochondrial motility and distribution but not fusion or functional mito/ER tethering. Furthermore, while ectopic expression of the functional homolog MFN1 completely rescues loss of acetylated tubulin in MFN2 KO cells, MFN2 mutations associated with CMT2A affect the binding affinity of MFN2 with ATAT1. Our findings reveal a novel role for mitochondria in regulating acetylated tubulin through MFN2 and suggest that disruption of the α-tubulin acetylation/deacetylation cycle may contribute to the onset of CMT2A caused by mutations in MFN2.

SG154
The integrin-actin linker Tensin1 contributes to focal adhesion disassembly at mitosis to relieve an integrin-inactivation G2-M checkpoint.

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Cell rounding at mitosis is required for proper spindle positioning, orientation and stability, and thus is critical to proper cell division. For a mitotic cell to round up, it must reduce its contact area with the extracellular matrix (ECM). This can be achieved by increasing cell cortical tension beyond the cell-ECM adhesion strength, reducing cell-ECM adhesion, or both. Here, we tested the hypothesis that mitotic cell rounding is mediated by reducing cell-ECM adhesion through timely regulation of focal adhesions (FA) dynamics by the mitotic promoting factors. Using high resolution live TIRF, spinning-disc confocal and differential interference contrast (DIC) microscopy of cells expressing FA and cell cycle markers, we found that cell de-adhesion is an early prophase event that is accompanied by a rapid drop in traction forces with no apparent pre-de-adhesion peak. This suggests that mitotic cells don’t de-adhere simply by increasing cell contraction beyond the adhesion strength but undergo a regulated de-adhesion process. Analysis of FA dynamics showed that during mitosis, the number of assembling FA decreased while the FA disassembly rate increased. Inhibition of mitotic kinases showed that at mitotic onset, PLK1 activity was required for stopping FA assembly, while FA disassembly required CDK1 activation. Manipulation of the regulators of FA disassembly known from studies of cell migration, including myosin II, FAK, calpain and integrins showed that neither calpain, myosin II nor FAK activity were required for cell de-adhesion during mitosis, suggesting that FA disassembly at mitosis requires a mitotic-specific mechanism. Accordingly, we found that the integrin-actin linker tensin1 exhibits rapid and mitotic-specific serine and threonine phosphorylation, downstream of CDK1 activation. Analysis of tensin1 dynamics showed that at mitotic entry, tensin1 leaves disassembling FA earlier than Talin1 and paxillin. Knocking down tensin1 using siRNA leads to an increase in cell rounding time, a delay in mitotic entry and a defect in cell proliferation suggesting a role in cell de-adhesion and G2/M transition. Similarly, locking integrins in their active/high affinity ECM-binding conformation using either manganese or conformation-specific antibodies showed that integrin inactivation was required for mitotic cell de-adhesion and effective G2/M transition. Taken together, our data suggest that the mitotic promoting factors CDK1 regulates cell de-adhesion at mitosis by post-translationally modifying the integrin actin
linker, tensin1, which potentially drives its early removal from FA leading to integrin inactivation and FA disassembly. Our data further suggest that cell de-adhesion at mitosis is required for cell cycle progression in adherent cells.

SG155
Trim9 and VASP ubiquitination regulate actin dynamics and neuronal morphology
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Actin-rich filopodia are critical at numerous stages of neuronal morphogenesis, including neuritogenesis, axon guidance, and dendritic spine formation. Defects in these critical developmental processes can result in improper synaptic connectivity, neurodevelopmental disorders, and psychiatric syndromes. The actin polymerase VASP is a component of the filopodial tip complex, where it influences actin dynamics and filopodial stability and maintenance. In developing neurons, filopodia extend outward from the axonal growth cone, detecting cues in the local environment and influencing growth. Previously, the Gupton lab showed that VASP transiently co-localizes with the E3 ubiquitin ligase TRIM9 at the tips of growth cone filopodia. TRIM9 was required for the reversible, non-degradative ubiquitination of VASP and this modification was associated with decreased filopodia number and stability. Furthermore, the axon guidance cue netrin promoted deubiquitination of VASP. Although the dynamic actin cytoskeleton, VASP, and netrin are appreciated to play important roles in the postsynapse, regulation of VASP activity in dendritic filopodia and maturing dendritic spines is unclear. Likewise, Trim9-null mice have dramatic spatial learning and memory deficits, yet the role of TRIM9 in synapse formation and maintenance is unknown. Here we show VASP, TRIM9, and ubiquitinated VASP (VASP-Ub) are enriched in the postsynaptic density following differential centrifugation, suggesting a role for all three proteins in dendritic spines. While netrin increases dendritic filopodia density and promotes synaptogenesis, this response is abrogated in neurons lacking Trim9 at seven days in vitro (DIV7). At DIV12, neurons overexpressing TRIM9 show defects in spine maturation but not dendritic spine number. In vivo, we demonstrate that loss of Trim9 alters the proteome of the post-synaptic density; ongoing work is examining the consequences of Trim9 deletion on synaptic plasticity. Finally, we recently identified several lysine residues that are ubiquitinated in VASP. We are currently testing the mechanistic impact of ubiquitination on actin-VASP interactions through in vitro biochemical reconstitution assays, as well as the impact of VASP ubiquitination on dendritic filopodia and spine number in cultured neurons.

SG156
Untangling the interdependent actin filament nucleation and elongation activities of formin
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Actin filament assembly is tightly regulated in cells, both at the initial step of nucleation and during subsequent elongation. Whereas many proteins that regulate actin assembly do so by influencing either filament nucleation or elongation, formins promote both processes with isoform-specific activity. Aberrant formin function gives rise to defects in actin dynamics that disrupt fundamental cellular processes and lead to diseases including cancer, cardiomyopathy, and neurological disorders. To determine how formins influence the physical properties of the actin networks they assemble, we
quantified the contributions of their nucleation and elongation activities to the process of polymerization. Using kinetic modeling and in vitro assays, we found that the period over which nucleation and elongation occur is a key determinant of the number of filaments that are ultimately assembled, as well as their equilibrium lengths. Inclusion of formin in polymerization reactions speeds nucleation, thus increasing the number and shortening the lengths of filaments that are assembled. Variations in elongation rates produce modest changes in the lengths of assembled actin filaments, but these effects are limited by the number of filament ends generated via nucleation. Sustained elongation of formin-bound filaments therefore requires inhibition of nucleation via monomer sequestration and a low concentration of activated formin. Our results underscore the mechanistic advantage for keeping formin’s nucleation efficiency relatively low in cells, where unregulated actin assembly produces deleterious effects on cytoskeletal dynamics. This strategy also maximizes differences among actin filament networks assembled by formin isoforms with differing elongation activities.

SG157

ALS-linked PFN1 mutants cause mitochondria defects through loss and gain of function
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Mutations in the actin-binding protein profilin 1 (PFN1) are associated with a hereditary form of amyotrophic lateral sclerosis (ALS), though the exact disease-causing mechanisms have remained elusive. In this study, we screened PFN1 knockout cells for novel functions of PFN1 to help pinpoint its role in ALS. RNA-seq analysis identified that PFN1 deficient cells had a significant upregulation of autophagy-related signaling pathways. Subsequent experiments revealed that it was mitophagy, a form of selective autophagy that removes defective or damaged mitochondria from the cell, that was activated. This was notable given that mitochondrial dysfunction is common to multiple types of ALS and is one of the early pathological events in motor neuron degeneration. Super-resolution microscopy, live-cell imaging, electron microscopy, and functional assays confirmed that the loss of profilin significantly affected mitochondria morphology, motility, and function. PFN1 mutants associated with ALS failed to rescue PFN1 knockout mitochondrial phenotypes. PFN1 regulation of mitochondria requires actin-binding, and the ALS-associated PFN1 mutants were all found to be defective in regulating actin. Furthermore, mutant profilin 1 protein aggregates colocalized with mitochondria and this association was correlated with increased mitochondria fragmentation and decreased membrane potential. We propose that ALS-linked PFN1 mutants cause mitochondria dysfunction through a two-hit mechanism. First, through early dysregulation of the actin cytoskeleton then later through gain of function effects caused by the formation of PFN1 mutant protein aggregates. This work identifies a novel role for PFN1 in regulating mitochondria and helps explain why the expression of PFN1 mutants causes progressive degeneration of motor neurons in ALS.

SG158

Forces and integrin activity in cancer cell migration
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Tissue homeostasis is dependent on the spatially controlled localization of specific cell types and the correct composition of the extracellular stroma. Integrin mediated adhesions, in conjunction with the
actin cytoskeleton, allow cells to sense the stiffness of the surrounding extra-cellular matrix (ECM). Conversely, cells exert acto-myosin and integrin dependent forces to remodel and organize the surrounding ECM. In cancer, stiffening of the tumor stroma is an instrumental contributor to tumor progression. Tissue stiffness acts as a migration cue in durotaxis and supports cell proliferation in cancerous tissue. However, in vivo, disseminating cancer cells are likely to encounter a broad range of variable tissue rigidities and it remains poorly understood how mechanosensory inputs tune adhesion signaling over a range of rigidities to regulate cell migration, proliferation and cell fate decisions. Another key regulator of cell migration is spatially regulated integrin activity. Integrins switch between active and inactive conformations on the plasma membrane and intracellular adhesome components control the balance between activation and inactivation. I will describe our recent findings on integrin activity regulation in distinct adhesion types and how adhesion dynamics regulate cell migration in cell type and context dependent manner.

**Immune Cell Biology and Immunotherapy**

**SG159**  
**Size-dependent activation of chimeric antigen receptor**  
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Chimeric antigen receptor (CAR)-T therapy has achieved unprecedent success in treating B cell cancers and its application has been extending towards solid tumors, autoimmune diseases, viral infections, and fibrosis. As the pool of CAR-T targets expands, there is an increasing demand on identifying new antigens and designing new CARs that can be effectively activated. However, the rational selection of antigens and design of CARs are limited by a lack of knowledge on the molecular mechanism by which CARs are activated by antigens. Here we present data supporting a “size-exclusion” model explaining how antigen signals are transmitted across the plasma membrane to activate the intracellular domains of CARs. In this model, antigen engagement with CAR results in a narrow intermembrane space that excludes CD45, the bulky phosphatase on the T cell membrane, out of the CAR zone, thus favoring CAR phosphorylation by kinases, which further triggers downstream pathways leading to T cell activation. Increasing the size of CAR extracellular domains diminished CAR T activation. Membrane-proximal epitopes activated CAR-Ts better than membrane-distal epitopes. On the other hand, increasing the size of CD45 enhances the activation of CARs that recognize membrane-distal epitopes. Together, our work revealed CAR size and epitope position as critical factors for selecting new antigens and developing corresponding CARs. We also identified the size of CD45 as a new target for improving CAR T activity.

**SG160**  
**Novel mechanisms controlling secretion from immune cells**  
G. Griffiths; University of Cambridge, Cambridge, UNITED KINGDOM.

Recent advances in immunotherapy have highlighted the powerful anti-cancer potential of Cytotoxic T lymphocytes (CTLs). Understanding the mechanisms that determine and regulate killing by these cells becomes increasingly important with their increased role in immunotherapies. CTLs are remarkably polarized cells that use their centrosome to direct secretion as they rapidly kill one target after another. We have combined different single-cell approaches to understand the cell biology of CTL killing from single cell gene analysis to high resolution imaging, allowing us to determine how the strength of T cell
receptor (TCR) signaling controls polarization of the secretory machinery within these cells. Although many of the genes involved in immune function have been identified, there are more to be discovered. This presentation will focus on a functional screen of single gene knockout mice which has allowed us to identify genes with previously unknown roles in the immune cell secretion. We have now characterized several of these mutants using a combination of imaging, biochemical, functional and proteomic approaches to identify the molecular mechanisms that allow these novel immune genes to regulate CTL killing.

SG161

**Nano-optogenetic immunotherapy**

Y. ZHOU; Institute of Biosciences and Technology, Texas A&M University, HOUSTON, TX.

Chimeric antigen receptor (CAR) T cell-based immunotherapy approved by FDA shows promising curative potential in patients with CD19-positive hematological immunotherapy. CAR T-cell therapy, nevertheless, lacks precise control over the location and duration of the anti-tumor immune response, and therefore, is fraught with devastating side effects in some patients. Herein, we present the design of light-switchable CAR (designated “LiCAR”) T-cells that enable photo-tunable activation of therapeutic T cells to induce CD19-positive tumor cell killing both *in vitro* and *in vivo*. When coupled with imaging-guided, surgically removable upconversion nanoplates (UCNPs) that have enhanced near infrared (NIR)-to-blue upconversion luminescence as miniature deep tissue transducers, LiCAR T-cells enable precise spatiotemporal control over T cell-mediated anti-tumor therapeutic activity with greatly mitigated side effects. This remotely controllable nano-optogenetic device will not only provide a unique tool for interrogating CAR-mediated anti-tumor immunity, but also set the stage for developing smart immunotherapy to deliver personalized anti-cancer therapy.

SG162

**Mucins form a nanoscale physical barrier against Natural Killer cell cytotoxicity**

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Cancer cells construct a cellular glycocalyx with immunomodulatory glycans to evade immune detection and establish a permissive microenvironment. Whether the structural properties of the glycocalyx also physically shield cancer cells from immune recognition has not been fully resolved. Here, we combine genetic approaches and interference-based imaging tools to edit and measure the nanoscale physical dimensions of the cellular glycocalyx. Glycocalyx thickness and density were manipulated through titratable expression of native and engineered mucins, as well as through overexpression and CRISPR/Cas9-mediated knockout of glycosyltransferases that govern mucin-type O-glycosylation. We resolved how the surface density, polypeptide backbone length, and glycosylation of cancer-associated mucins dictate the physical structure of the cellular glycocalyx. We uncovered a strong reciprocal relationship between the thickness of the glycocalyx and immune cell killing. Natural Killer (NK) cell-mediated cytotoxicity demonstrated a nearly perfect inverse correlation with the glycocalyx thickness of target cells regardless of the specific glycan structures present, suggesting that the physical properties of
glycocalyx may be key determinants of cancer immune evasion. Similar relationships were found for chimeric antigen receptor (CAR) NK cells against target cells with engineered glycocalyces. Enzymatic digestion of the glycocalyx dramatically improved the efficiency of NK cell-mediated killing. Thus, our results suggest that targeting the biophysical structure of the cell-surface glycocalyx might improve the efficiency of immune therapeutics.

SG163

**Competition between activation and inhibition in macrophage phagocytosis**  
D. Fletcher; University of California, Berkeley, Berkeley, CA.

Macrophages engaging with antibody-opsonized tumor cells through their Fc receptors will often encounter ligands that activate inhibitory receptors, shutting down effector function. Understanding how activating and inhibitory receptors compete to decide macrophage response is important both for a basic understanding of signal transduction at complex interfaces and for efforts to control effector function for immunotherapeutic purposes. This talk will describe recent work investigating how macrophages decide whether to phagocytose target particles decorated with both activating and inhibitory ligands, as well as ongoing work understanding how receptor design impacts phagocytic decision making. Using endogenous as well as chimeric receptors and an in vitro assay, we show that activating:inhibitory ligand ratios of at least 10:1 are required to promote phagocytosis of target particles coated with activating IgG antibodies and inhibitory CD47. We also show that lowering this ratio reduces FcγR phosphorylation due to inhibitory phosphatases recruited to CD47-bound SIRPα, and that receptor-ligand height plays a role in the competition. These results suggest that balancing activating and inhibitory signaling may be important for controlling macrophage phagocytosis in cancer immunotherapy.

SG164

**Myd88 oligomer size functions as a physical threshold to trigger IL1R Myddosome signaling**  
R. Deliz-Aguirre; Visualization of Immune Signaling, Max Planck Institute for Infection Biology, Berlin, GERMANY.

A recurring feature in innate immune receptor signaling is the self-assembly of signaling proteins into oligomeric complexes. The Myddosome is one such oligomeric complex that is required to transmit inflammatory signals from TLR/IL1Rs. However, its assembly mechanism remains poorly understood. We developed a novel assay to analyze the spatiotemporal dynamics of IL1R and Myddosome signaling in live cells. We found that MyD88 oligomerization is inducible and initially reversible. Moreover, the formation of larger, stable MyD88 oligomers trigger the sequential recruitment of IRAK4 and IRAK1. Notably, genetic knockout of IRAK4 enhanced MyD88 oligomerization, indicating that IRAK4 controls MyD88 oligomer size and growth. MyD88 oligomer size thus functions as a physical threshold to trigger downstream signaling. These results provide a mechanistic basis for how protein oligomerization might function in cell signaling pathways.
SG165

**Bioengineering approaches to study the phagosome**

**B. D. Bryson;** Massachusetts Institute of Technology, Cambridge, MA.

Phagocytes, sentinel cells of the immune system, are critical in the regulation of tissue homeostasis and responses to infection. The phagosome is a dynamically formed organelle that is generated upon phagocyte encounter with cargo. Following phagosome formation, a dynamic series of steps proceed involving organelle trafficking and fusion. Ultimately, these collective molecular events influence and shape phagosome function. While many of the stereotyped features of phagosome maturation and biochemistry have been studied, there have been relatively few studies that take an integrated systems-level view from signaling to phagosome biochemistry. Here, we present a synthetic biology inspired toolkit to perform systems-level molecular characterization of phagosome biology. Specifically, we describe the engineering of phagocytic cargoes with the capacity to perform proximity biotinylation of specific phagosomes. By chemically modifying the cargo or tuning the stimulation environment in which phagocytosis occurs, we can specifically evaluate how phagosome biochemistry changes in response to diverse signal inputs or other perturbations. Collectively, we hypothesize that this approach will provide a platform for high-throughput colocalization studies with phagosomal cargo and phagocyte proteins to define the determinants of phagosome biochemistry.

SG166

**Deep-sea microbes as tools to refine the rules of innate immune pattern recognition.**

**A. Gauthier;** Harvard University, Boston, MA.

All multicellular organisms face the threat of microbial infection. As such, a common feature associated with multicellularity is the ability to detect microbes and their products as a means to initiate host defenses. These defense strategies are classically referred to as innate immune responses. They are initiated by the detection of conserved pathogen associated molecular patterns (PAMPs) by host-encoded pattern recognition receptors (PRRs). The assumption of near-universal bacterial PAMP detection by PRRs is a foundation of modern immunology. The limits of this pattern recognition concept, however, remain undefined. As a test of this hypothesis, we determined whether mammalian cells can recognize bacteria that they have never had the natural opportunity to encounter. These bacteria were cultivated from the deep Pacific Ocean, where the genus *Moritella* was identified as a common constituent of the culturable microbiota. Most deep-sea bacteria contained cell wall lipopolysaccharide (LPS) structures that were expected to be immunostimulatory, and some deep-sea bacteria activated inflammatory responses from mammalian LPS receptors. However, LPS receptors were unable to detect 80% of deep-sea bacteria examined, with LPS acyl chain length being identified as a potential determinant of immunosilence. The inability of immune receptors to detect most bacteria from a different ecosystem suggests that pattern recognition strategies may be defined locally, not globally.

SG167

**Sting mediates immune responses in the closest living relatives of animals**

**A. Woznica;** UT Southwestern Medical Center, Dallas, TX.

Animals have evolved unique repertoires of innate immune genes and pathways that provide their first line of defense against pathogens. To reconstruct the ancestry of animal innate immunity, we have
developed the choanoflagellate *Monosiga brevicollis*, one of the closest living relatives of animals, as a model for studying mechanisms underlying pathogen recognition and immune response. We found that *M. brevicollis* is killed by exposure to *Pseudomonas aeruginosa* bacteria and selectively avoids ingesting them. Moreover, *M. brevicollis* STING, which, in animals, activates innate immune pathways in response to cyclic dinucleotides during pathogen sensing, is upregulated in response to *P. aeruginosa*. By developing transgenics and genetics for *M. brevicollis*, we found that STING increases the susceptibility of *M. brevicollis* to *P. aeruginosa*-induced cell death and is required for responding to the cyclic dinucleotide 2′3′ cGAMP. Furthermore, similar to animals, autophagic signaling in *M. brevicollis* is induced by 2′3′cGAMP in a STING-dependent manner. This study provides evidence for a pre-animal role for STING in antibacterial immunity and establishes *M. brevicollis* as a modelsystem for the study of immune responses.

**Making Big Data Work: Generating, Mining, and Integrating Massive Microscopy Datasets and Atlases**

SG168

*Whole cell organelle segmentation in volume electron microscopy*

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Cells contain hundreds of organelles and macromolecular assemblies. Obtaining a complete understanding of their intricate organization requires nanometer-level, three-dimensional reconstruction of whole cells which is only feasible with robust and scalable automatic methods. To support the development of such methods, we annotated up to 35 different cellular organelle classes - ranging from endoplasmic reticulum to microtubules to ribosomes - in diverse sample volumes from multiple cell types imaged at a near-isotropic resolution of 4 nm per voxel with focused ion beam scanning electron microscopy (FIB-SEM). We trained deep learning architectures to segment these structures in 4 nm and 8 nm per voxel FIB-SEM volumes, carefully validated their performance, and showed that automatic reconstructions can be used to directly quantify previously inaccessible metrics including spatial interactions between cellular components. We further demonstrated that such reconstructions can be used to automatically register light and electron microscopy images for correlative studies. We created an open data and open source web repository, OpenOrganelle, to share the data, computer code, and trained models, enabling scientists everywhere to query and further improve automatic reconstruction of these datasets.

SG169

*Robust integrated intracellular organization of the human iPS cell: where, how much, and how variable*

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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. As an initial step towards this goal we created a benchmark for mean cell organization and the natural range of cell-to-cell variation.
This benchmark can be used for comparison to other normal or abnormal cell states. We developed a reproducible microscope imaging pipeline to generate the hiPSC Single-Cell Image Dataset (cfe.allencell.org), a high-quality public resource dataset of 3D, high-resolution images of over 200,000 live cells from 25 isogenic human induced pluripotent stem cell (hiPSC) lines from the Allen Cell Collection (allencell.org/cell-catalog). Each line contains one fluorescently tagged protein, created via endogenous CRISPR/Cas9 gene editing, representing a key cellular structure or organelle. We previously used these images to develop a new multi-part, generalizable analysis approach to create a data-driven set of quantitative rule-building constraints for the locations, amounts, and variation of these 25 cellular structures within the hiPSC. We found that both the extent to which a structure’s individual location varied and the extent to which the structure localized relative to all the other cellular structures were robust to a wide range of cell shape variation. We took advantage of this benchmark to develop a new analysis approach to quantify differences in integrated cellular organization between distinct cell state populations in a cell shape agnostic manner. We compared integrated cellular organization for cells at the edge vs. cells located throughout the colony and found that both the cytoskeleton and the major organelles were polarized in edge cells in the direction of the edge of the colony. We also quantified the changes in integrated organization of cells in early mitosis with cells in interphase using these approaches. The quality, breadth, and scale of this dataset permitted us to develop new quantitative approaches to analyze cell shape and organization and to explore this dataset to gain novel biological insights into changes in cell organization between several different cell states found naturally within this normal population of cells.

SG170

Jump-cell painting - powering drug discovery and development with images
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JUMP-Cell Painting - Powering drug discovery and development with images
Cell microscopy images contain a vast amount of information about the status of the cell: whether it is diseased, how it is responding to a drug treatment, or whether a certain pathway has been disrupted, for example. These profiles can be analyzed to identify subtle cellular patterns, potentially biologically meaningful but undetectable to the human eye. The JUMP-Cell Painting Consortium, a group of pharmaceutical companies and non-profits, aims to create a critical mass of such cellular imaging data to empower discoveries about cell biology that can inform drug discovery and development. The goal is to create the world’s largest public Cell Painting image set of chemical and genetic perturbations. The community will use it to identify the effect of each gene or compound on the cell’s shape or activity — creating a morphological atlas that can be referenced as a baseline in further studies. With a large reference of image-based cellular profiles, scientists could computationally compare their images to determine a drug’s likely mechanism of action or a gene variant’s impact, accelerating basic biology research and drug discovery alike.

SG171

Image-based spatiotemporal dissection of the human proteome
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Biological systems are functionally defined by the nature, amount and spatial location of the totality of their proteins. Resolving the spatiotemporal distribution of the human proteome at a subcellular level
increases our understanding of human biology and disease. In the Human Protein Atlas project, we are systematically mapping the human proteome in a multitude of human cells and organs using microscopy. We have generated a high-resolution map of the subcellular distribution of the human proteome and have shown that as much as half of all proteins localize to multiple compartments. Such proteins may have context specific functions and ‘moonlight’ in different parts of the cell, thus increasing the functionality of the proteome and the complexity of the cell from a systems perspective. Recently, we performed a single cell spatiotemporal dissection of the transcriptome and proteome over the cell cycle. We could identify 20% of the human proteome to display cell-to-cell variability, and present the first evidence of cell cycle association for 301 proteins. Our results show that cell cycle progression explains less than half of all cell-to-cell variability, and that most cycling proteins are regulated post-translationally, rather than by transcriptomic cycling. All this work is critically dependent on computational image analysis, and I will discuss machine learning approaches for classification and embedding of spatial subcellular patterns, including the results from two recent Kaggle competitions. In summary, I will demonstrate the importance of spatial proteomics data for single cell biology and present how the freely available Human Protein Atlas database (www.proteinatlas.org) can be used as an image resource for life science.

SG172
Opencell and cytoself: integrating genome engineering, live imaging, proteomics and deep learning for the cartography of human cellular architecture

Synergistic technological advances are transforming our ability to comprehensively interrogate the architecture of the human cell. In parallel, the generation of large multi-modal datasets requires the development of new computational approaches to analyze, integrate and share this data. Here, we will present our effort to characterize the human proteome by integrating genome engineering, confocal imaging, mass spectrometry and data science. Our OpenCell dataset builds upon a library of >1,300 CRISPR-edited human cell lines harboring fluorescent tags that also serve as handles for affinity capture, and systematically maps protein localization in live cells and protein interactions under endogenous expression. This rich dataset allowed us to develop cytoself, a fully self-supervised deep learning framework that encodes the localization pattern of each protein without the need of pre-existing annotations. Our approach provides a data-driven description of the molecular and spatial networks that organize the human proteome. We show that unsupervised clustering of these networks delineates functional groups and catalyzes biological discovery. Furthermore, our analysis demonstrates that the localization pattern of each given protein is remarkably specific and can be interpreted using deep learning to derive functional insights for proteins on the sole basis of their localization. For example, a direct comparison of imaging and proteomics data reveals that localization patterns often contain enough information to predict molecular interactions. This opens exciting avenues for the use of imaging as an information-rich readout for functional genomics. Finally, both our data and analyses are publicly accessible through an interactive website (opencell.czbiohub.org) and open-source repositories (Github: czbiohub/opencell and royerlab/cytoself).
SG173
**Machine learning methods for the integration of spatio-temporal microscopy data with transcriptomics data**
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To understand how an organized multi-cellular embryo forms from a single fertilized egg, we need to examine all the intermediate steps between the one-cell stage and the adult stage which involve individual cell spatial positioning, cell proliferation dynamics and morphogenesis together with cell differentiation. To study these different aspects, developmental biology has grown into a data intensive science with the development of high-throughput imaging and multi-omics technologies leading to very large datasets that cannot be explored by hand. Machine learning is a versatile set of techniques that can help make sense of these large datasets with minimal human intervention. We propose new machine learning methods for data integration of two complementary and intrinsically incompatible measurement techniques: microscopy images and transcriptomics data; leading to morpho-transcriptomics datasets. Our approaches rely mainly on public databases and therefore the quality and organization of these resources will greatly influence the success of our prediction.

**Mechanical Forces and the 3D Sculpting of Cell-matrix Interactions and Tissues**

SG174
**STReTCh: a strategy for detection of mechanical forces across proteins in cells**
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The ability of living cells to generate and sense molecular-scale mechanical forces is crucial to embryonic development, cell motility, and other biological processes. Existing genetically encoded sensors for measuring these forces in intact cells primarily rely on Förster Resonance Energy Transfer (FRET). While such sensors have yielded numerous biological insights, acquiring and interpreting these FRET measurements requires specialized equipment and analysis techniques. Here, we present a molecular tension sensor, which we term STReTCh (Sensing Tension by Reactive Tag Characterization), that is sensitive to forces in the single piconewton (pN) range and whose use follows typical fix-and-stain protocols. To generate the STReTCh sensor module, we embedded the 13-amino acid SpyTag sequence in the I10 immunoglobulin-like domain of human titin. Mechanical force unfolds the STReTCh sensor, increasing the accessibility of SpyTag to covalent labeling by fluorescently tagged SpyCatcher, its cognate binder. Using magnetic tweezers, we demonstrate that the STReTCh module is unfolded at forces greater than ~2 pN, providing sensitivity to forces in the single pN range. We validated the ability of STReTCh to visualize forces in biological systems by culturing human foreskin fibroblasts on surfaces functionalized with STReTCh fused to a fibronectin-mimetic RGD peptide. In this system, we observed ~6:1 preferential localization of SpyCatcher to focal adhesion complexes, where individual integrin-RGD bonds are known to bear mechanical tension. To more directly evaluate the utility of STReTCh as a genetically encoded sensor, we also introduced the STReTCh module into vinculin, an intracellular protein that links integrin-based adhesions to the actin cytoskeleton and bears pN loads. When expressed in vinculin-null mouse embryonic fibroblasts, vinculin-STReTCh recruited fluorescently labeled SpyCatcher in a tension-dependent manner. These results suggest that STReTCh is suitable for detection
of biologically relevant mechanical forces and offers an accessible alternative to existing FRET-based force sensors.

SG175

Nuclear and chromatin mechanics in cell state regulation

Mechanical forces and the nucleus: regulation of cell faCellte asdfdsfnd integrity

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Cell survival and tissue integrity depend on the ability to maintain functional integrity, even under conditions of extreme stress. One source of physiologically and clinically relevant stress are mechanical forces that act on cells and their genetic material. It is thus critical to understand how cells sense and integrate mechanical and chemical information from their environment to control cell state and behavior to adapt to new force environments. We have shown that short-term acute mechanical stress triggers nuclear deformation and calcium release from the ER to remodel heterochromatin state and protect the genetic material from physical damage. We thus identified chromatin as an active rheological element of the nucleus contributing to nuclear stiffness and its viscoelastic properties to ensure stress dissipation and genome integrity in high force environments. Importantly, cellular mechanical environment is often progressively altered with various pathologies, such as cancer, as well as during aging with profound effects on chromatin accessibility and landscape, as well as transcription. We have further observed that long term mechanical stress alters gene expression through effects on transcription and chromatin architecture, thereby impacting cell states and tissue dynamics in altered mechanical environments.

SG176

The Ras-MAPK pathway senses 3D matrix structure to regulate hydraulic pressure and the mode of cell migration

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Primary human fibroblasts can use at least two distinct mechanisms or modes of migration through three-dimensional (3D) matrices. In these cells, actomyosin contractility pulls the nucleus forward like a piston to increase cytoplasmic hydraulic pressure and switch cells from using low-pressure lamellipodia to high-pressure lobopodial protrusions. Though this nuclear piston mechanism of migration is triggered specifically within 3D matrices that act as a barrier to the passage of the bulky nucleus, how it is activated remains unclear. Here, we show that there is a significant increase in nuclear mechanical stress when the piston is activated, as measured by nuclear deformation and pressure. However, these physical changes are not sufficient to activate the piston mechanism. We performed a phosphoproteomics screen to identify proteins differentially phosphorylated immediately following piston activation in 3D collagen. Our data identified 10 unique phospho-peptides that either increased or decreased their phosphorylation after triggering the nuclear piston mechanism. Critically, these phospho-peptides corresponded to proteins within the Ras-MAPK signaling pathway. To confirm whether the Ras-MAPK pathway regulated activation of the nuclear piston mechanism, we inhibited ERK1/2 in primary fibroblasts migrating with the piston mechanism off and measured hydraulic pressure and the extent of lamellipodia formation. Our results show that ERK1/2 activity acts as a negative regulator of the piston mechanism by suppressing myosin II activity and hydraulic pressure in both primary human fibroblasts and Ras-transformed fibrosarcoma (HT-1080) cells. Further, we found
endogenous ERK1/2 signaling is downregulated following activation of the nuclear piston mechanism in 3D collagen in response to matrix metalloproteinase (MMP) inhibition. This result suggests ERK1/2 activity is downstream of the MT1-MMP activity that helps to govern activation of the piston. Finally, we found that overexpression of H-Ras prevented piston activation and promoted lamellipodia-based 3D migration in primary human fibroblasts. Together, these findings show that the Ras-MAPK pathway acts as a molecular switch in response to the physical structure of the 3D extracellular matrix to activate the nuclear piston mechanism of 3D migration and prevent the formation of lamellipodial protrusions.

**SG177**

**Mechanical worrying drives cell migration in crowded environments**

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Migratory cells navigate through crowded 3D microenvironments in vivo. Amoeboid cells, such as immune cells and some cancer cells, are thought to do so by deforming their bodies to squeeze through tight spaces. Yet large populations of nearly spherical amoeboid cells migrate in microenvironments too dense to move through without extensive shape deformations. We used high-resolution light-sheet microscopy to visualize metastatic melanoma cell migration through dense collagen matrices. We observed that cells indeed maintain a round morphology as they migrate and are capable of remodeling the extracellular matrix to tunnel without the need for proteolytic degradation via matrix metalloproteinases. Using 3D image analysis, we found that cells not only use blebs to push collagen fibers out of their path, but to mechanically break up fibers via repeated agitation. Retracting blebs also pull collagen fragments to the cell surface where they are endocytosed in a macropinocytic manner. We termed the process of mechanically digging a tunnel via abrasion, worrying, which means to wear away or tear repeatedly at something. Membrane blebs are short-lived relative to the timescale of migration, and thus persistence in their polarization is critical for productive ablation of the extracellular matrix. Using a combination of 3D image analysis, genetic and small molecule perturbations and optogenetic manipulations, we found that interactions between small but long-lived cortical adhesions and collagen at the cell front induce PI-3 Kinase signaling that drives bleb enlargement via branched actin polymerization, specifically via the Rac1 - Arp2/3 pathway. Large blebs in turn abrade collagen, creating a feedback between extracellular matrix remodeling, cell morphology, and cell polarization that results in both ECM tunneling and persistent cell movement. Our findings highlight the coupling between extracellular matrix remodeling and migration persistence, even for the case of amoeboid migration, a mode not known to rely on remodeling, and suggest the possibility that extracellular remodeling is critical to all major migration modes.

**SG178**

**Invadopodia enable cooperative invasion and metastasis of breast cancer cells**

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Multiple clones of cancer cells can seed metastases via collective invasion and dissemination. While it is known that cancer clones can cooperate during invasion, the events leading to it and the effects it may have on metastasis are not known. In this study, we demonstrate that, when mixed in 3D spheroids, the
isogenic 4T1 and 67NR breast cancer cells sort from each other, followed by their cooperative invasion. By time-lapse microscopy of heterogenous spheroids embedded in collagen I, we show that the invasive 4T1 cells that reach the spheroid edge remain there, while the non-invasive 67NR cells move randomly. This results in cell sorting and enrichment of invasive 4T1 cells at the spheroid periphery. Following cell sorting, 4T1 cells lead the 67NR cells in cooperative invasion. Elimination of invadopodia in 4T1 cells, by knockdown of the protein Tks5, blocks invasion and demonstrates that invasion requires invadopodia only in leader cells and not in follower cells. Importantly, using syngeneic mouse model, we demonstrate that cells with and without invadopodia can also engage in cooperative metastasis. Altogether, our results suggest that a few clones with invadopodia could drive the metastasis of cell clusters from heterogeneous tumors.

SG179
A tumor-derived type III collagen-rich ECM niche regulates tumor cell dormancy
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Background. Metastasis is the primary cause of death in cancer patients. However, metastasis occur years after primary tumor removal. This delay is a consequence of tumor dormancy, a reversible growth arrest that can be regulated by the interaction of disseminated tumor cells with the microenvironments. Extracellular matrix (ECM) is a key component of the microenvironment that provide signals to the tumor cells and regulates tumor progression. Here, we investigate the role of tumor-derived ECM and tumor cell/ECM interactions in dormancy. Methods. We used dormancy models of human head and neck squamous cell carcinoma as well as murine mammary carcinoma coupled with multiphoton intravital imaging to define how assembly of ECM by dormant cells sustain their quiescence. Results. SHG imaging showed that ECM of dormant cells is mainly formed of a curly ECM whereas proliferative tumors set up collagen fibers straight. Transcriptome analysis of dormant and proliferative tumors revealed that dormant ECMs are highly enriched in collagens. Interestingly, depleting COL3A1 in dormant cells results in their awakening and restoration of tumor growth in vivo. We identified DDR1, a collagen receptor for these collagens, as upregulated in dormant cells upon dormancy onset (i.e TGFb2). Col III-rich microenvironments induce DDR1 expression and the entrance in dormancy. DDR1 downregulation leads to the reactivation of dormant cells in vivo. ECM-proteomic analysis revealed that DDR1 is required to sustain tumor cell-derived COL3A1 expression in dormant cell, ECMs suggesting that DDR1 sustain dormancy by regulating the assembly of a pro-dormant ECM enriched in COL3. Moreover, we further identify a transcription factor network activated downstream DDR1, involving STAT1, that contribute to dormancy of cancer cells and to the assembly of this tumor self-made pro-quiescence ECM. Conclusion. We demonstrated that upregulation of DDR1 prime cancer cells to secrete and assemble a pro-quiescence ECM through STAT1 signaling.
SG180

**Cancer associated fibroblasts actively compress cancer cells and modulate mechanotransduction**

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During tumor progression, cancer-associated fibroblasts (CAFs) accumulate in tumors and produce excessive extracellular matrix (ECM), forming a capsule that enwraps cancer cells. This capsule is a barrier that restricts tumor growth leading to the buildup of intratumoral pressure. Combining genetic and physical manipulations *in vivo* with microfabrication and force measurements *in vitro*, we found that the CAFs capsule is not a passive barrier but instead actively compresses cancer cells using actomyosin contractility. Cancer cells mechanosense CAF compression, resulting in an altered localization of the transcriptional regulator YAP. Abrogation of CAFs contractility *in vivo* leads to the dissipation of compressive forces and impairment of capsule formation. By mapping CAF force patterns in 3D, we show that compression is a CAF-intrinsic property independent of cancer cell growth. Supracellular coordination of CAFs is achieved through fibronectin cables that serve as scaffolds allowing force transmission. Our study unveils that the contractile capsule actively compresses cancer cells, modulates their mechanical signaling, and reorganizes tumor morphology.

SG181

**Fibrillar collagen in tumor microenvironment determines the mode of tumor progression**

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Extracellular matrix (ECM) of tumor microenvironment (TME) plays an important role in tumor progression. In particular, stiffness and the porosity of ECM are known to affect the migration rate of cancer cells dissociated from primary tumors and invading the stroma. Such invasion initiates the metastatic cascade which eventually leads to the deadly spread of cancer. However, relatively little is studied about whether and how compositional heterogeneity of ECM in TME is involved in the development and progression of cancer, especially in terms of the matrix architecture determined by fibrillar ECM proteins including collagen I, collagen II and fibronectin, among others. In our *in vitro* reconstituted TME model, we observed that when aligned, by either contractile cancer cells or cancer-associated fibroblasts (CAFs), fibrillar ECM proteins served as conduits to facilitate efficient diffusion of exosomes and macromolecules secreted by cancer cells and CAFs, thereby transforming nearby bystander cells such as adipocytes, immune cells, endothelial cells, into accomplices of tumor progression. Suppressing the alignment of ECM fibrils either by moderate crosslinking, or by inhibiting the contractility in CAFs and cancer cells, stalled cancer invasion, decreased cancer cell proliferation and enhanced killing by chemotherapeutic drugs. Moreover, we found that the fibrillar structure itself, not availability of ligands to engage integrin receptors on cancer cells, is responsible for a faster mode of tumor progression. We constructed two TME models, one consisting of fibrillar collagen I, the other gelatin which is derived from hydrolyzed collagen I but no longer fibrillar. We found that tumor growth was significantly higher and cancer cells invaded stroma using focal adhesion-based motility in TME consisting of collagen, whereas cancer cells invaded using amoeboid motility in TME made of gelatin. Moreover, cancer cells in TME made of collagen I were more resistant to drug treatment. It has been
reported that the content of fibrillar ECM in individual patients vary, our finding indicates that the content of these ECM proteins might contribute to different treatment outcomes and prognoses.

SG182
Exploring cell mechanisms of tissue fluidity by optogenetic manipulation of Rho activity
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Epithelial tissue sheets can be shaped into complex forms through series of stretching, flowing, and folding events that are driven by myosin-generated forces that are patterned in space and time. In Drosophila development, a planar polarized pattern of myosin II regulated by the conserved Rho/Rho-kinase signaling pathway is required for the rapid convergent extension tissue flow that elongates the embryonic body. However, it is not well understood how specific patterns of Rho1 activity and actomyosin contractility influence the ability of tissues to remodel and flow—tissue fluidity. Here we use a collection of optogenetic tools to manipulate Rho1 activity and actomyosin contractility by controlling the localization of RhoGEF and RhoGAP regulatory exchange factors in the germband epithelium during Drosophila body axis elongation. The ability to flexibly induce changes in myosin patterns across the tissue allows us to analyze the effects of myosin patterns on cell shapes, tissue tensions, and tissue fluidity. We find that optogenetic activation of Rho1 at the apical cell membrane transforms the endogenous planar polarized myosin pattern, increasing apical myosin accumulation, altering tissue tensions, and enhancing active cell shape fluctuations. In contrast, optogenetic deactivation of Rho1 decreases apical myosin accumulation, tension, and cell shape fluctuations. Our results indicate that changes in the distribution of medial and junctional myosin caused by different Rho1 perturbations alter tissue tension anisotropy, cell shape fluctuations, and cell packings in distinct ways, leading to disruptions to cell rearrangements and reduced tissue flow. Interestingly, analyses of cells shape and alignment predict reductions in tissue fluidity to varying degrees in each of these Rho1 perturbations compared to wild-type controls, suggesting that Rho1 activity influences both the driving forces for tissue flow and the tissue mechanical properties that resist flow. Taken together, these results directly link Rho1 activity to myosin-generated force patterns and show how Rho1 activity patterns control the contractile cell behaviors that promote cell rearrangement and tissue flow during convergent extension.

SG183
Tissue hydraulics in early mammalian development
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Many developmental processes involve the emergence of intercellular fluid and luminogenesis. This often results in a build up of hydrostatic pressure and signalling molecules in the lumen. However, the potential roles of lumina in cellular functions and tissue morphogenesis have yet to be fully explored. Using mouse blastocyst as a model, we show that hydraulic force during lumen expansion leads to robust control of blastocyst size and cell fate specification. We further showed that lineage segregation of the inner cell mass (ICM) can be guided by biochemical signalling cues from within the lumen. Interestingly, during mammalian folliculogenesis, a similar process occurs where a fluid-filled cavity emerges in the antral follicle. We are currently studying the dynamics and mechanisms of antral follicle
development, with the goal of understanding how lumen expansion impacts the oocyte quality and the eventual ovulation. We propose that the interplay between lumen hydraulics, signalling and tissue mechanics provides a unified framework in understanding reproductive biology, with important implications in ovarian ageing and infertility treatment.

SG184

A tubule tension switch regulates the physical packing geometry of epithelial branch tips in the developing kidney


Developing organs must progressively increase their structural complexity while adhering to physical rules and tissue geometric boundaries. During embryonic kidney development, branching epithelial tubules blueprint the collecting duct network responsible for transporting urine from between 200,000 and 2 million nephrons to the bladder. Each nephron is induced at the tip of a branching epithelial tubule and branching tips are located at the kidney’s surface. This creates a packing conflict where the available surface area per branching tip decreases with increasing kidney size. How the kidney navigates this challenge - through chemorepellent-based mutual avoidance or by rearranging tubules through physical forces - remains unclear. Since a role for physical forces has not been fully explored here, we developed computational and physical models of kidney tip packing based on imaging-based measurements of mouse kidneys collected between embryonic day (E) 13.5 and E18.5. Our models indicate that developing kidneys visit only a narrow range of possible tubule packing solutions and highlight two classes of potential packing defects - tip burial within deeper tissue layers or “short circuits” through adjacent tip fusion - examples of which can be found in published literature. Furthermore, they suggest that internal, radially oriented forces on tubules are required to avoid these defects. During the “node retraction” phase starting at E15.5 - where branch point nodes undergo retrograde motion towards the renal pelvis - tubules enter a “vertically packed” state that orients tips towards the kidney surface. We used embryonic kidney explants to test whether tubule tension is responsible here by enzymatically severing the basement membrane between tips and the surrounding mesenchyme. Loss of basement membrane attachment promoted rapid (~10 mins) tip retraction, which was accelerated by activation of myosin II and nearly completely ablated upon myosin II inhibition, consistent with contractility-based radial tension and basal traction forces on tubules. We also tested the model prediction that radial tensions prevent tip burial by dissecting cortex “cap” explants and assaying tip height distribution after overnight culture. Compared to uncut controls where tips were distributed evenly at the outside surface, ~12% of tubules within caps were buried within deeper surface layers. Taken together, these results indicate that radial tensions are necessary for the kidney to navigate this packing conflict at later developmental stages. Our results have implications for understanding the wide variability in nephron count reported for human kidneys and for modeling congenital kidney defects.
Pressure and curvature control of contact inhibition in epithelia growing under spherical confinement


During morphogenesis, the final tissue shape and size ultimately depend on the spatiotemporal regulation of cell shape and cell division, which are regulated by biochemical, mechanical and geometrical cues propagating across the tissue. However, evaluating the contribution of tissue geometry and intrinsic forces to the regulation of cell proliferation remains challenging. Here, by using an in vitro encapsulation technique, we show that epithelia growing under spherical confinement accumulate pressure which inhibits proliferation above a threshold value, which depended on the beta-catenin pathway. Interestingly, because folding of the epithelium through buckling is part of the pressure accumulation process, we observed that folding partially reactivates cell cycle dynamics, concomitantly with transient reactivation of the YAP/TAZ pathway. But the major transition correlating with YAP/TAZ inhibition was the abrupt increase of cell aspect ratio upon reaching confluency. This shape transition did not depend on pressure, but rather on density and cell contractility. Altogether, our results support a spatiotemporal separation of mechanosensitive pathways, YAP/TAZ being involved in local, transient changes of the cell environment, while the beta-catenin pathway being involved in sensing sustained, tissue-level changes of pressure.

Remodelling and Reshaping Membranes (RRM)

Large self-assembled clathrin lattices spontaneously disassemble without sufficient adaptor proteins

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Clathrin-coated structures must assemble on cell membranes to internalize receptors. These structures can grow surprisingly large, containing over 20 clathrin seemingly locked in a hexagonal lattice, yet often fail to form productive vesicles. We show that these abortive events happen spontaneously when adaptor availability is low, despite abundant clathrin. Here, we combine recent in vitro kinetic measurements with stochastic reaction-diffusion simulations and theory to differentiate mechanisms of stable vs unstable clathrin assembly on membranes. While in vitro conditions drive stable assembly, we show that concentrations, geometry, and dimensional reduction in physiologic-like conditions do not support nucleation if only the adaptor AP-2 is included. Nucleation requires a stoichiometry of adaptor to clathrin that exceeds 1:1, meaning additional adaptor types are necessary to form lattices successfully and efficiently. We show that the critical nucleus contains ~25 clathrin, remarkably similar to sizes of abortive structures observed in vivo. We find that the critical concentration of adaptors needed to nucleate lattices can be lowered by the addition of cross-linking proteins (Fcho1, eps15) that have been shown to initiate droplet-like dynamics of AP-2 clusters on the membrane, helping to catalyze nucleation. Last, we quantify the cost of bending the membrane under curved clathrin lattices using a continuum thin-film model, finding that the energy gained from flat to curved structures largely offsets the bending energy. Our model predicts how adaptor density controls stabilization of clathrin-coated structures against spontaneous disassembly, and shows ATPases are not required for lattice remodeling, which is a critical advance towards predicting productive vesicle formation.
Adaptation of clathrin- and actin-mediated endocytosis in animal development

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In animal development, cells produce complex form through an astounding choreography of division, differentiation and remodeling events, and continuously adapt their cellular functions to their environment. What remains unknown is if cells also adapt their most basic “housekeeping” processes, which are generally assumed to occur stereotypically across cell types and tissues. One such process is Clathrin-Mediated Endocytosis (CME), which is essential for molecular uptake and membrane recycling in eukaryotes. It has been widely studied in cell culture, where assembly dynamics of CME and the required proteins vary between cell types. However, cultured cells lack the tissue context. Thus, it is unclear how this heterogeneity relates to CME in cells in their natural environment. We aimed to understand how CME functions in native tissues, and whether the dynamics, lifetimes and the machinery of CME is adapted to tissues and during development. We used Drosophila melanogaster, and CRISPR to endogenously tag endocytic proteins. Optimized fluorescence microscopy of CME in non-motile pupae allowed us to image individual endocytic events with a similarly high spatiotemporal resolution as in cell culture. First, we focused on the surface epithelium of the pupal notum, where specialized mechanosensory bristles develop extremely stereotypically. We used these bristles as model system to image CME throughout cell differentiation. We found that CME is fast and regular in bristle cells. However, during bristle development, endocytic dynamics are progressively adapted following a stereotypic pattern. Specifically, we found that coat assembly and disassembly phases remain stereotypic, but the duration of a variable delay phase in between is modulated. This might indicate that CME is very robust at first, but can become stalled before vesicle scission due to high mechanical requirements. We also found that endocytic events are associated with dynamic actin networks, and that this association changes during development. In bristles, actin is organized into thick bundles at the plasma membrane, and local, dynamic assemblies between the bundles. Imaging of AP-2 and actin markers showed that CME is corralled between the bundles, and in certain developmental states associated with highly dynamic actin networks. Using genetics and inhibitors, we revealed that actin polymerization was required for efficient CME throughout development of the bristle. Our data suggest that the adaptation of endocytic dynamics and the involved actin networks may be in response to changing mechanical constraints during development. Our findings underscore the importance of understanding the adaptation of cellular processes such as CME in the native tissue context.

Clathrin’s Essential Role During Endocytosis is to Stabilize Cell Membrane Curvature

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During clathrin-mediated endocytosis (CME), flat plasma membrane is rapidly remodeled to produce a nanometer-scale spherical vesicle. The mechanisms underlying this shape change are not known, but it has been hypothesized that the clathrin coat stabilizes membrane curvature. Here, we used nanopatterning to produce glass-like substrates with U-shaped features mimicking membrane shapes induced by fibrillar materials such as collagen. These substrates bend the ventral plasma membranes of cells grown on them into shapes characteristic of the energetically-unfavorable U-shaped intermediate
stage of CME. This induced plasma membrane curvature recruits the endocytic machinery and promotes productive endocytosis. Curvature-based localization of the endocytic machinery is unaffected by clathrin disruption, and induced curvature partially bypasses clathrin’s role in cargo uptake, supporting the conclusion that clathrin’s essential endocytic function is to stabilize membrane curvature. Disruption of the adaptor protein complex AP2, however, results in a complete abrogation of endocytic sites, which are not rescued by induced curvature, indicating that the curvature-sensing capabilities of the endocytic machinery are downstream of adaptor-receptor binding and clustering.

SG189
Regulation of Clathrin-Mediated Endocytosis in the Context of Huntingtin Aggregates
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Huntington’s disease, an inherited neurodegenerative disorder, is characterized by the formation of neuronal intracellular inclusions. The underlying cause of this disease is due to an abnormal expansion of the CAG tract (responsible for encoding glutamine, Q) in Exon 1 of the Huntingtin gene, resulting in the formation of intracellular aggregates. Previous studies have shown that Huntingtin aggregates affect endocytosis in yeast and in human cells. However, the underlying mechanism for this remains unknown. Using Drosophila melanogaster larval hemocytes we aimed to investigate the effect of PolyQ repeat-containing Huntingtin protein aggregates on clathrin-mediated endocytosis (CME). Our results demonstrate altered movement of clathrin-coated vesicles (CCVs) and CME in the presence of pathogenic Huntingtin aggregates. Further, these mutant cells also exhibit modified organization of the actin cytoskeleton and physical properties of the cell. Our study reveals a novel relationship between the endocytic pathway, the actin cytoskeleton and physical properties of the cell in the context of neurodegenerative diseases such as Huntington’s disorder, which may be exploited in development of therapeutic interventions.

SG190
Caveolae control intercellular tension during cytokinesis and promote successful abscission
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During cytokinesis, the intercellular bridge (ICB) connecting the daughter cells experience pulling forces, which delay abscission by preventing the assembly of the ESCRT scission machinery. Abscission is thus triggered by tension release, but how ICB tension is controlled is unclear. Here, we report that caveolae, which are known to buffer membrane tension upon mechanical stretch in interphase cells, are dynamically located at the midbody, the abscission site and at the ICB/cell interface in dividing cells. Functionally, depletion of the key caveolae components caveolin-1 or Cavin1 delays ESCRT-III recruitment during cytokinesis and impairs abscission. This is the consequence of a 2-fold increase of ICB tension measured by laser ablation, associated with locally increased myosin II activity at the ICB/cell interface. We propose that caveolae limit intercellular tension during cytokinesis to promote ESCRT-III assembly and abscission. The unexpected coupling between caveolae and acto-myoosin contractibility in cell-cell mechanics during cytokinesis reveals the first role of caveolae in cell division.
SG191

Theoretical modeling of membrane reshaping by membrane proteins

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Membranes physically interact with a myriad of membrane proteins, which, collectively, have the ability to reshape it and trigger signaling events. The theoretical modeling of membrane reshaping by proteins is challenging because molecular models can hardly access the required time and length-scales whereas mean field continuum models lack the molecular specificity. In this talk, I will discuss our recent efforts to develop models of the latter type, to map molecular mechanisms for protein-membrane interaction to mean-field models, and to use these models to understand caveolar structures and membrane reshaping by BAR proteins.

SG192

The ESCRT-III / PspA / Vipp1 superfamily of membrane remodelling proteins is conserved across the tree of life

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Membrane repair and remodelling are important for all cells. Proteins that carry out these functions include PspA in bacteria and some groups of archaea, Vipp1/IM30 in photosynthetic organisms, CdvB in TACK archaea and ESCRT-III in eukaryotes. In this work, employing a combination of structural and evolutionary analyses, we demonstrate that these protein families have similar primary, secondary, tertiary and quaternary structures and, therefore, are homologous and share a common ancient evolutionary origin. Phylogenetic data is in line with the hypothesis that a gene ancestral to ESCRT-III, PspA and Vipp1 was present within the Last Universal Common Ancestor (LUCA). The similarities of those protein families are noticeable when analysing new structures solved by cryo-electron microscopy (Cryo-EM) of cyanobacterial Vipp1 polymers presented over a variety of symmetries. Each polymer is assembled from rungs that stack and progressively tilt to create dome-like assemblies with 1.6 - 3.4 megaDaltons (MDa). The dome assembly is helped by conserved hinges found within the Vipp1 monomer and also present in ESCRT-III, which permit the formation of flexible polymers. The Vipp1 N-terminal amphipathic helix is found at the inner lumen and is the main region able to bind and deform membranes. Overall, these data reveal conserved mechanistic principles that regulate ESCRT-III, PspA and Vipp1 membrane remodelling across all domains of life.

SG193

Does membrane transformation point to hidden functions of active processes in bacteria?

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Our research aims at the assembly of a synthetic cell division system with a minimal number of functional components. In the past years, we successfully reconstituted key divisome elements from E.coli, such as the Min proteins and FtsZ, in giant membrane vesicles that could serve as cell-mimicking compartments. Not surprisingly, several of the described physiological functions of these active proteins fail to unfold outside their cellular context, with a drastically limited number of interaction partners. At
the same time, however, completely new and unexpected protein functions can be observed in these minimalistic settings in vitro, many of them actively transforming cell membrane mimics. These “hidden functions” of active systems may inform us about potentially simpler designs of cell division machinery and could generally be of great use for building minimal biological systems.

SG194
The herpesviral nuclear egress complex deforms and buds membranes by lipid ordering and protein scaffolding
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Herpesviruses are large viruses that infect nearly all vertebrates and some invertebrates and cause lifelong infections in most of the world’s population and cause lifelong infections in most of the world’s population. During replication, they export their capsids from the nucleus into the cytoplasm by a non-canonical mechanism in which capsids bud at the inner nuclear membrane. This process is mediated by the viral nuclear egress complex (NEC) that oligomerizes into a hexagonal, membrane-bound scaffold and deforms the membrane around the capsid. But how this membrane deformation is achieved is unclear. Here, we discovered that clusters of positive charges in the NEC from herpes simplex virus 1, a prototypical herpesvirus, alter lipid order by inserting into the lipid headgroups. We also found that the electrostatic interactions between the NEC and the membranes are essential for membrane deformation. Reducing the positive charge or introducing negative charges weakens the membrane deforming ability of the NEC. NEC is phosphorylated by a viral kinase during infection, and the corresponding phosphomimicking mutations block capsid nuclear egress. We found that the same phosphomimicking mutations disrupt the NEC/membrane interactions and inhibit NEC-mediated budding in vitro, providing a biophysical explanation for the in-vivo phenomenon. Our data suggest that the NEC generates negative membrane curvature by both lipid ordering and protein scaffolding and that phosphorylation acts as an “off” switch that inhibits the membrane-budding activity of the NEC to prevent unproductive budding in the absence of the capsid.

SG195
Intrinsically disordered protein networks drive membrane remodeling
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Membrane curvature is required for many cellular processes, from assembly of highly curved trafficking vesicles to extension of needle-like filopodia. Consequently, defects in membrane curvature play a role in most human diseases, including altered recycling of receptors in cancer and diabetes, targeting of filopodia by pathogens, and hijacking of vesicle traffic during virus replication. Therefore, understanding the basic molecular mechanisms that drive membrane remodeling is essential to our knowledge of cellular physiology and human disease. Research on membrane curvature has primarily focused on individual protein domains with specialized structures, such as crescent-shaped scaffolds and wedge-like amphipathic insertions. While this work has provided invaluable insights, it overlooks two essential elements. First, most membrane remodeling proteins contain large intrinsically disordered domains in addition to structured domains. And second these disordered domains drive assembly of large, multivalent protein networks. Recent work in our group supports the hypothesis that disordered protein
networks are essential drivers of membrane remodeling in the cell. Specifically, using clathrin-mediated endocytosis as a model pathway, we have shown that intrinsically disordered domains generate steric pressure at membrane surfaces. This pressure provides a surprisingly potent driving force for membrane bending, especially when coupled synergistically to the contributions of structured domains. Additionally, we have recently found that disordered domains within endocytic proteins drive assembly of liquid-like protein networks which efficiently initiate endocytosis. Importantly, this liquid-like behavior has the potential to resolve a long-standing paradox by explaining how curved membrane structures can be simultaneously highly interconnected, yet dynamic and flexible.

**Systems Reconstitution: Emergent Cellular Functions from the Assembly of Component Parts**

SG196  
**Structural Mechanism for Bi-Directional Actin Crosslinking by T-plastin**  
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The actin cytoskeleton, a network of actin filaments, myosin motor proteins, and hundreds of associated partners, governs cell shape, powers motility, and facilitates the generation and detection of mechanical forces by cells. To carry out these varied tasks, actin filaments must be incorporated into higher-order assemblies with diverse architectures, dictated by specialized actin crosslinking proteins which bridge filaments to control their relative orientations and spacings. Mechanisms of actin crosslinkers are poorly defined at the protein structural level due to a lack of methods for visualizing them actively bridging filaments at high-resolution. This work focuses on the plastin/fimbrin family of tandem calponin-homology domain-containing proteins, an evolutionarily ancient group of calcium-regulated crosslinkers which contain two actin-binding domains (ABDs) within a single polypeptide chain. Mammals feature three plastin isoforms expressed in a tissue-specific manner, which localize to diverse cytoskeletal networks ranging from linear actin bundles in filopodia/stereocilia to branched actin networks at the leading edge of migrating cells. When isolated, single plastin isoforms can promote the formation of both parallel and anti-parallel actin bundles, suggesting individual plastin molecules must possess the capacity to bridge actin filaments in radically different geometries. Here we introduce a deep-learning enabled cryo-electron microscopy (cryo-EM) workflow for structural analysis of crosslinked actin filaments, which we applied to dissect the mechanisms of full-length human T-plastin (plastin 3). We find that T-plastin initially engages actin through a single ABD, resulting in its other ABD adopting a flexible, conformationally frustrated state where it is available to engage a second filament. This intermediate can resolve into two distinct stable bridging conformations, producing either a parallel or anti-parallel geometry. In both orientations, T-plastin bridges a distance similar to the width of a single actin filament, suggesting the protein is optimized for crosslinking dense networks. In addition to revealing a sequential mechanism that enables T-plastin’s bi-directional bridging activity, the cryo-EM analysis pipeline we have developed will broadly enable dissection of structural mechanisms underlying the construction of actin networks.
Waves and spirals in contracting actomyosin networks

Contracting actomyosin networks have essential roles in many processes including cell division, intracellular transport and cell motility. To fulfill these functions, the networks must undergo extensive reorganization facilitated by rapid turnover of actin subunits. Despite substantive efforts, the contractile network behavior in the presence of turnover is still not well understood. To address this issue, we rely on an in-vitro system based on cytoplasmic Xenopus egg extracts encapsulated into cell-sized water-in-oil droplets. Thanks to the presence of physiological turnover rates, our system exhibits contractile flows that persist for hours and self-organize into a wide array of spatiotemporal patterns, which can be modulated by varying the system’s composition and boundary conditions. Interestingly, we observe a size-dependent transition in the contractile behavior of the system, going from continuous contraction in smaller droplets to periodic contraction in the form of waves and spirals in larger droplets. In the periodic regime, we observe occasional transitions between waves and spirals characteristic of excitable media. The periodicity increases with network contraction rates while the characteristic length-scale for the appearance of waves decreases. We complement the experiments with computational modeling, which suggests that the coupling of the contractile gel mechanics with turnover is indeed the key to the pulsatile behavior in large droplets.

Bottom-up reconstitution of focal adhesion complexes
N. Mizuno; National Institutes of Health, Bethesda, MD.

Focal adhesions (FA) are large macromolecular assemblies relevant for various cellular and pathological events such as migration, polarization and metastatic cancer formation. At FA sites at the migrating periphery of a cell, players gather and form a network to respond to extra cellular stimuli transmitted by the integrin receptor, the most upstream component within a cell, initiating the FA signaling pathway. The FA is an assembly of more than 200 types of proteins and the resulting intricate network formation hampers unraveling the precise molecular actions of individual players. We use, in vitro bottom-up reconstitution to precisely understand the molecular crosstalk of the components and elucidate the hierarchical crosstalk of involved cellular players using a combination of biophysical, and structural biological approaches.

Mechanism of active dynein complex assembly
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Cytoplasmic dynein-1 is the major motor that moves cargos towards the minus ends of microtubules. Active dynein complexes are composed of two dynein dimers, the dynactin complex and an activating adaptor. These giant complexes contain ~50 proteins and are over 4 MDa in size. We and others recently discovered that Lis1, which is genetically linked to the dynein pathway, plays a role in assembling these active dynein complexes. We are using a combination of cryo-electron microscopy, in vitro
reconstitution of human dynein complexes, and in vivo experiments in *S. cerevisiae* to determine how Lis1 assembles active dynein complexes.

SG200

**Dead-box ATPases are global regulators of phase-separated organelles and RNA flux**

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The ability of proteins and nucleic acids to undergo liquid-liquid phase separation has emerged as an important molecular principle of how cells rapidly and reversibly compartmentalize their components into membrane-less organelles such as the nucleolus, processing bodies or stress granules. How the assembly and turnover of these organelles are controlled, and how these biological condensates selectively recruit or release components are poorly understood. We found that members of the large and highly abundant family of RNA-dependent DEAD-box ATPases (DDXs) are global regulators of RNA-containing phase-separated organelles in prokaryotes and eukaryotes. Using *in vitro* reconstitution and *in vivo* experiments, we demonstrate that DDXs promote phase separation in their ATP-bound form, whereas ATP hydrolysis induces compartment turnover and release of RNA molecules. This mechanism of membrane-less organelle regulation reveals a principle of cellular organization that is conserved from bacteria to humans. Furthermore, we show that DDXs control RNA flux into and out of phase-separated organelles, and thus propose that a cellular network of dynamic, DDX-controlled compartments establishes biochemical reaction centres that provide cells with spatial and temporal control of various RNA-processing steps, which could regulate the composition and fate of ribonucleoprotein particles.

SG201

**Mechanism and function of T cell signaling Condensates**

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Linker for activation of T cells (LAT) serves as a key adaptor protein to transduce the T cell receptor (TCR) signaling. Following TCR activation, phase separation drives the formation of LAT condensates on the plasma membrane, which further recruit and organize a plethora of enzymes and adaptors to trigger downstream pathways including calcium, Ras, and actin. Although the components of LAT condensate have been largely identified, how individual proteins orchestrate LAT condensate formation remains incompletely understood. Combining a supported lipid bilayer-based reconstitution system with TIRF microscopy, we discovered that phospholipase Cγ1 (PLCγ1) cooperates with Grb2 and Sos1 to promote LAT condensate formation. PLCγ1 directly cross-links LAT through its two SH2 domains to enhance LAT oligomerization. PLCγ1 also facilitates LAT condensate formation by protecting LAT from dephosphorylation by phosphatase CD45. Live cell imaging and mutagenesis analysis confirmed PLCγ1’s structural function in promoting LAT clustering and LAT-dependent SLP76 phosphorylation and ERK activation. Interestingly, PLCγ1 also regulates LAT condensate size in a non-monotonic manner. A 2D coarse-grained computer model revealed that the size control was achieved by influencing the coalescence likelihood and the compactness of LAT condensates. Leveraging the above knowledge, we designed new chimeric antigen receptors (CARs) by implementing the LAT-PLCγ1 module. These new CARs induced superior cytotoxicity and cytokine secretion as compared to the conventional CARs. Taken
together, our work revealed new regulatory mechanism of T cell signaling condensates which led to the development of new CAR-Ts with improved signaling function.

SG202

**Force generation by protein-DNA co-condensation**

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Interactions between liquids and surfaces generate forces that are crucial for many processes in biology, physics and engineering, including the motion of insects on the surface of water, modulation of the material properties of spider silk and self-assembly of microstructures. Recent studies have shown that cells assemble biomolecular condensates via phase separation. In the nucleus, these condensates are thought to drive transcription, heterochromatin formation, nucleolus assembly and DNA repair. Here we show that the interaction between liquid-like condensates and DNA generates forces that might play a role in bringing distant regulatory elements of DNA together, a key step in transcriptional regulation. We combine quantitative microscopy, in vitro reconstitution, optical tweezers and theory to show that the transcription factor FoxA1 mediates the condensation of a protein-DNA phase via a mesoscopic first-order phase transition. After nucleation, co-condensation forces drive growth of this phase by pulling non-condensed DNA. Altering the tension on the DNA strand enlarges or dissolves the condensates, revealing their mechanosensitive nature. These findings show that DNA condensation mediated by transcription factors could bring distant regions of DNA into close proximity, suggesting that this physical mechanism is a possible general regulatory principle for chromatin organization that may be relevant in vivo.

**FRIDAY, DECEMBER 10, 2021**

**Bacterial Cell Biology**

SG211

**Multiscale investigation of photo sensing in non-photosynthetic bacteria**

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Bacteria must detect and respond to a broad range of physical and chemical stimuli in their environment to survive, both in natural and medical settings. One stimulus that pervades our planet is light. Light is detected by photoreceptor proteins in all domains of life. Despite the prevalence of photoreceptors in bacteria, the role of light in bacterial physiology and the photo-sensing signal transduction cascades are largely undefined. Historically, the characterization of photoreceptors from non-photosynthetic bacteria appears to have proceeded in a piecemeal fashion, driven by the lure to obtain better optogenetic tools. Phytochromes are the most abundant photoreceptors in bacteria. Bacteriophytochromes typically possess an amino-terminal biliverdin-binding domain and a carboxy-terminal histidine kinase domain. Surprisingly, very few bacteria encode a cognate response regulator in close proximity to the gene specifying the bacteriophytochrome, leaving the systems mostly undefined. To close this knowledge gap, we are combining bacterial genetics, molecular biology, biochemistry, microfluidics and fluorescence microscopy, mathematical modeling and genome-scale studies to systematically characterize photo-sensing systems in non-photosynthetic bacteria. We have identified a
photoreceptor-signal transducer pair in the human pathogenic bacterium \emph{Pseudomonas aeruginosa} that is widely conserved in diverse non-photosynthetic bacteria. Specifically, in a genetic screen for biofilm regulators in \emph{P. aeruginosa}, we found that the bacterio-phytochrome BphP is the kinase that, in response to light, phosphorylates and activates the transcription factor AlgB. Phospho-AlgB then acts to repress biofilm formation and virulence factor production. We went on to demonstrate that AlgB is the cognate response regulator for BphP in diverse bacterial phyla including other human pathogens. Thus, this work identified an entire cascade for light driven behavioral response — light as the input, BphP as the detector, AlgB as the signal transducer, and biofilm formation and virulence factor production as the outputs — enabling crucial insight into light-driven control of bacterial behaviors relevant to human disease. We aim to leverage this innovative model system to address major unanswered questions in bacterial light sensing - why have non-photosynthetic bacteria evolved to sense light? what role does light play in the physiology of these bacteria? how is light signal transduced in multicellular communities called biofilms? And can light be used in treating bacterial infections? Understanding these regulatory mechanisms that govern bacterial physiology is a prerequisite to the manipulation and control of bacterial growth and infection in clinical settings.

SG212
\textbf{Six ParAs, six cargos, one cell}
L. Tran, A. G. Vecchiarelli; MCDB, University of Michigan, Ann Arbor, MI.

Eukaryotic motor proteins are well known for governing intracellular transport and organization. In bacteria, where linear motor proteins are absent, it is poorly understood how the ParA/MinD family of ATPases spatially organize an array of genetic- and protein-based cellular cargos. ParAs are well-known to “partition” plasmids and segregate chromosomes, and MinDs are well-known for positioning the cell division machinery in bacteria. Less studied is the growing list of ParA/MinD-like ATPases found across prokaryotes that spatially organize diverse protein-based complexes, such as Bacterial Microcompartments (BMCs), flagella, chemotaxis arrays and conjugation machinery. The subcellular organization of these cargos has been individually studied to varying degrees, but largely in model bacteria encoding just one or two ParA/MinD ATPase(s). Here we find that a third of sequenced bacteria encode multiple ParA/MinD-like ATPases. How multiple ParA/MinD ATPases spatiotemporally coordinate the positioning of such a diverse set of cellular cargos in the same cell is unknown and has never been studied. Among the third, we identified the non-pathogenic and experimentally tractable bacterium Halothiobacillus neapolitanus encoding six ParA/MinD-like ATPases. Gene neighborhood analysis implicated the putative cargos: the chromosome, the divisome, the carboxysome BMC, the flagellum, the chemotaxis cluster, and the conjugation machinery. Using genetics and in vivo fluorescence microscopy, we used H. neapolitanus as a model to determine how multiple ParA/MinD family ATPases function and coordinate with each other to spatially organize these diverse cellular cargos. We find that each ParA/MinD-like ATPase is dedicated to the positioning of a specific cellular cargo without crosstalk. Our systems biology approach to the study of multiple ParA/MinD-based positioning reactions in a single organism will allow us to build integrative networks of subcellular organization in bacteria.
SG213

Inhibiting BamA to understand outer membrane protein folding

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The folding and insertion of integral β-barrel proteins into the outer membrane is critical for viability and pathogenesis in Gram-negative bacteria. The central component of the β-barrel assembly machine (BAM), BamA, is an essential outer membrane protein that interacts with four lipoproteins, BamBCDE. While genetic and structural information has provided a framework for understanding β-barrel protein folding, the fundamental mechanism by which BAM engages substrates and catalyzes folding is still unresolved. We have discovered novel BamA inhibitors and employed these antibacterial molecules to characterize outer membrane protein folding. Our structural and physiological studies provide novel insight into β-barrel protein folding and further validate BamA as a potential antibacterial target.

SG214

Hopanoid Lipids Regulate Membrane Fluidity in the Legume Root-Nodulating Bacterium

Bradyrhizobium diazoefficiens

E. Lawrence, S. Talamantez-Lyburn, B. Belin; Embryology, Carnegie Institution for Science, Baltimore, MD.

Hopanoids are a taxonomically widespread group of bacterial lipids that are structurally similar to cholesterol. The precursor for hopanoids is squalene, which is cyclized by Shc to form diploptene, the simplest hopanoid. Diploptene can be further modified by either HpnH or HpnP to form extended or methylated hopanoids, respectively. In the legume symbiont Bradyrhizobium diazoefficiens, knockouts of hopanoid biosynthesis genes lead to symbiotic deficiencies including decreased motility, delayed nodulation, and increased susceptibility to environmental stress [1-3]. We hypothesized that these phenotypes may stem from the ability of hopanoids to modulate membrane fluidity, which can impact the efficiency of membrane-based processes. **Our goal was to investigate how hopanoids influence membrane fluidity patterns on a subcellular level.** We stained cultures of wild type and three hopanoid mutants (ΔhpnP, ΔhpnH, and Pcu-shc, a cumate inducible shc knockout) with DiIC12, a fluorescent membrane probe with an affinity for thin, fluid membrane regions, and NADA-green, a fluorescent marker of newly incorporated peptidoglycan. Cells were analyzed in both exponential and stationary phase and imaged on a STED super-resolution microscope. We discovered that B. diazoefficiens exhibits unipolar growth and that all strains had relatively high fluidity at the growing pole and septa. In wild type, ΔhpnP, and shc overexpression (Pcu-shc plus cumate) strains, we identified high fluidity bands circling the non-growing pole in a helical pattern. The ΔhpnH and shc depletion (Pcu-shc without cumate) mutants had high overall fluidity and lacked this helical pattern. Preliminary proteomics of high-fluidity membrane domains identified the Min protein complex in strains exhibiting helical fluidity, and this complex helps guide cell division and forms membrane-associated helices in other microbes [4]. These observations suggest a new link between hopanoids and cell cycle regulation, bringing us closer to a molecular-level understanding of hopanoid mutant phenotypes. [1] Belin B.J. et. al, Nat Rev Microbiol, 16, 304-315, (2018) [2] Belin B.J. et. al, IS-MPMI, 32, 1415-1428, (2019) [3] Schmerk C.L. et. al, J Bacteriol, 193, 6712-6723, (2011) [4] Aguilar J. et. al, PNAS, 107, 3758-3763, (2010)
A Framework to Assess Liquid-Liquid-Phase-Separation in Bacterial Cells
Y. Hoang, A. Vecchiarelli; University of Michigan, Ann Arbor, MI.

Liquid Liquid Phase Separation (LLPS) has emerged as a mechanism for the assembly of membraneless organelles in eukaryotes, but little is known about this process in bacteria. LLPS refers to the ability of macromolecules to demix into a dilute phase and a dense phase, called a ‘biomolecular condensate’, which can be observed as clusters or foci in the cell. The major challenge for the study of LLPS in bacteria is the poor spatial resolution of foci in such tiny cells. As a result, it is very difficult to demonstrate the liquid-like nature of a focus in bacterial cells using the conventional approaches for studying large condensates in massive eukaryotic cells. Here, we developed a rigorous experimental framework for the characterization of LLPS in bacteria, using Escherichia coli as the host organism and the protein McdB, which robustly forms liquid-like droplets in vitro. McdB is a protein that coats a bacterial organelle called the carboxysome. This coating demarcates the carboxysome as cargo for its positioning system, which equally distributes carboxysomes along the cell length of rod-shaped cyanobacteria. We developed a suite of experiments to investigate the LLPS activity of McdB in vivo, based on the ability of biomolecular condensates to tune their size and shape, fuse, dissolve, and transition between phase states. We used both overexpression and tunable promoters to express fluorescent fusions of McdB and cIEP8, a well-known aggregator protein. We found that fluorescent fusions of McdB formed nucleoid-excluded foci in E. coli, but also maintained a soluble phase in the cytoplasm, consistent with LLPS theory. The aggregator protein cIEP8, on the other hand, lacked a soluble fraction in the cytoplasm. Condensates form at a saturation concentration threshold, called C_{sat}. A hallmark of LLPS is that condensates will dissolve if the concentration drops below C_{sat}, while insoluble aggregates should remain as stable foci even after dilution. We decreased protein concentration in vivo by increasing cell volume and by generational dilution via cell division. In both methods, McdB foci dissolved while cIEP8 foci remained intact as insoluble aggregates in response to decreased concentration in the cell. Finally, we also discovered that a well-established marker for insoluble protein aggregates in vivo, LbpA, does not colocalize with McdB foci. The result suggests that the colocalization of LbpA foci can be used as a broad-use sensor for the material state of protein complexes in bacterial cells. Our results provide multiple lines of evidence in support of LLPS of McdB in vivo. More broadly, our experimental framework for studying LLPS in bacteria overcomes current limitations in the field and can be used to assess the LLPS activity of other proteins of interest in bacterial cells.

Pseudomonas aeruginosa directs twitching motility by mechanotaxis
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The opportunistic pathogen Pseudomonas aeruginosa migrates over surfaces using twitching motility powered by retractile extracellular filaments called type IV pili (T4P). Single cells sequentially extend, attach and retract T4P, thereby driving incremental displacements. However, how P. aeruginosa
coordinates T4P to navigate surfaces remains unclear. Here, we demonstrate that *P. aeruginosa* actively directs twitching in the direction of mechanical input from T4P, in a process called mechanotaxis. A chemotaxis-like system called Chp controls the balance of forward and reverse twitching in response to the mechanical signals. Cells reverse spontaneously but more frequently following collisions with other cells or abiotic obstacles. As a result, wild-type cells colonize surfaces uniformly while non-reversing Chp mutants jam and form clusters, demonstrating a function for mechanosensing in regulating group behavior. On surfaces, Chp senses T4P attachment at one pole, therefore resolving a spatially-defined signal. As a consequence, the Chp response regulators PilG and PilH control the polarization of the extension motor PilB. PilG stimulates polarization favoring forward migration, while PilH inhibits polarization and induces reversal. Subcellular segregation of PilG and PilH efficiently orchestrates their antagonistic functions, ultimately enabling rapid reversals upon perturbations, such as collisions. The distinct localization of response regulators establishes a signaling landscape known as local-excitation, global-inhibition in higher order organisms, identifying a conserved strategy to transduce spatially-resolved signals. https://doi.org/10.1073/pnas.2101759118

**SG217**

Micro-crowdsourcing: how swarming bacteria integrate signals, recognize friends, and assemble communities

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Cells can use kin (self) recognition to achieve cooperative behaviors such as swarming, a collective mode for movement. Recognition genes principally evolve in tandem with partner alleles. Yet other constraints on protein evolution could exist. Here, we have identified an interaction outside of recognition loci that could constrain a recognition protein’s sequence variation. We show that recognition signaling co-opts SdaC, a serine transporter, during collective swarm expansion in *Proteus mirabilis*. Serine uptake is crucial for bacterial survival and colonization. Single-residue variants of SdaC reveal that kin recognition requires an open conformation of the protein; serine transport is dispensable. A distant ortholog from *Escherichia coli* is sufficient for kin recognition; however, a homologous serine transporter, YhaO, is not. In *P. mirabilis*, the SdaC protein couples kin recognition and serine transport, likely through a shared molecular interface. Further, signals from the identity proteins, through SdaC, of neighboring cells alter the receiving cell’s fate, removing its ability to reengage in collective motility. Understanding the molecular and ecological constraints on kin recognition mechanisms can provide insights into collective behaviors.

**SG218**

Spatio-temporal control of DNA replication by the pneumococcal cell cycle regulator CcrZ

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All living organisms need to duplicate and transfer their DNA to new daughter cells to survive. Eukaryotic cells have established checkpoints to separate each step of the cell cycle (*i.e.*, chromosome replication, segregation, cell elongation and division) to ensure that each process is completed before the next proceeds. In bacteria, however, these processes occur simultaneously. Work in *Escherichia coli* and *Bacillus subtilis* showed a correlation between DNA replication initiation and cell mass, but the exact molecular mechanisms for this control remain elusive. These models however do not apply for non-rod-shaped bacteria that do not perform multifork replication. This is the case of the human pathogen *Streptococcus pneumoniae* for which it was shown that DNA replication initiation and cell division are intimately correlated. Looking for mutants with defective DNA replication / segregation using CRISPRi we identified CcrZ (Cell Cycle Regulator protein interacting with FtsZ), a conserved and essential protein in pneumococci and related Firmicutes such as *Bacillus subtilis* and *Staphylococcus aureus*. We show that CcrZ controls the activity of DnaA, the master initiator of DNA replication. Absence of CcrZ causes mis-timed and reduced initiation of DNA replication, resulting in cells with no DNA and subsequently aberrant cell division. We also show that CcrZ from *S. pneumoniae* interacts directly with the cytoskeleton protein FtsZ, which places CcrZ in the middle of the newborn cell where the DnaA-bound origin is positioned. This rather simple system allows the cells to start a new round of DNA replication only once per cycle when the cell division machinery is being assembled in the daughter cells. Our work describes for the first time a mechanism that couples cell division with initiation of DNA replication to control the bacterial cell cycle in time and space.

SG219

**Structural biology of prokaryotic cell surfaces**

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My laboratory studies how molecules on the surface of prokaryotic cells mediate cellular interaction with the environment, enabling cellular motility, initiating cellular adhesion to surfaces, and facilitating biofilm formation. For our work, we leverage our expertise in electron cryotomography (cryo-ET) *in situ* imaging, together with ongoing method development in subtomogram averaging approaches for structure determination of macromolecules in their native context. We combine cryo-EM with FIB milling of specimens and cryo-light microscopy to study molecules on prokaryotic cells.

SG220

**Many hands, light work: multiple operonic regulatory elements modulate transcription and translation of a cyanobacterial RNA helicase during cold stress**

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RNA helicases, molecular motors which interact with, and modify, RNA and ribonucleoprotein secondary structures, are integral to all levels of RNA metabolism; positioning them as ideal regulators of gene expression. In bacteria, DEAD-box RNA helicases are often expressed in response to particular abiotic stress(es), which allows them to act as key coordinators of the cell’s genetic response to changes in environmental conditions. The *Synechocystis* sp. PCC 6803 DEAD-box RNA helicase, *crhR*, is encoded within the dicistronic *rimO-crhR* operon and its expression regulated by multiple cis-regulatory elements: operonic (*P_{rimO}*) and internal (*P_{crhR}*) promoters, two 5’ untranslated regions (UTRs), a 3’ UTR, and an RNA processing site. Unlike most bacterial DEAD-box helicases, *crhR* is expressed during a variety of abiotic stresses, including low temperature, high salinity, and heavy metal toxicity. While we have
characterized crhR transcript and protein accumulation during cold stress, the specific functions of each regulatory element within the operon remain to be dissected. To this end, plasmids were constructed in which one or more regulatory elements were deleted from the full-length operon, had substitution mutations introduced, or were inserted under the control of a constitutive promoter, and were then maintained in a crhR deletion strain. Plasmid-bearing cultures underwent cold stress (20°C) and return to standard growth temperature (30°C) before western blotting and qPCR were used to determine the elements’ role over protein and transcript accumulation, respectively. While we observed that PrimO is a cold-inducible promoter, the 5’ UTR of which robustly inhibited crhR transcript accumulation at 30°C but allowed readthrough during cold stress, PcrhR is active at a low, basal level at both temperatures. Deletion of the crhR 5’ UTR, however, had an insubstantial effect on transcript abundance but accumulated CrhR regardless of temperature, indicating that it functions to modulate translation such that protein remains basal at the non-permissive temperature, despite the presence of overabundant crhR transcripts. We therefore demonstrate that temperature-dependent crhR expression is complex, combining both cold-responsive transcript accumulation from the rimO promoter and 5’ UTR with upregulated translation from the crhR 5’ UTR, resulting in the rapid accumulation of CrhR.

SG221
Intracellular Growth of L. monocytogenes is Linked to Bacterial Density Within Host Cell
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Listeria monocytogenes is a bacterial pathogen that is able to spread intracellularly, traveling between adjacent cells without exposure to the extracellular space. When an epithelial monolayer is infected sparsely for several hours, many foci, or clusters, of L. monocytogenes are observed, where each focus originates from the infection of a single host cell. We found that growth rate of intracellular L. monocytogenes decreases over time. Moreover, the decrease occurs earliest and most sharply at the center of the focus and later and more moderately with distance from the originally infected cell. As the density of bacteria within the host cell is greatest at the center of the focus, we hypothesized that bacterial density and growth rate are anti-correlated. We found that growth rate of ΔActA L. monocytogenes, a mutant that is incapable of intercellular spread, is correlated with the volume of the infected host cell, suggesting that bacterial replication rate slows with increasing density. Then, we compared bacterial growth in control conditions with conditions where L. monocytogenes cell-to-cell spread is impaired. Restricting spread naturally increases the density of bacteria within each host cell. Not only did bacterial growth rate fall more quickly and severely when cell-to-cell spread was limited, the decrease occurred most acutely at the center of each focus and was delayed and reduced in magnitude farther from the initially infected host cell. Using an experimentally derived relationship between bacterial density and rate of replication, we also simulated focus growth while varying efficiency of cell-to-cell spread in silico. Lastly, we arrived at nutrient deprivation as a mechanism through which increasing bacterial density could reduce growth rate. As expected, initial growth rate of L. monocytogenes in spent or no glucose media was lower than that in fresh, high glucose media. Additionally, bacterial growth rate decreased earlier in nutrient-poor conditions, particularly at the densely populated center of the focus, as compared to control conditions. Therefore, we concluded that growth rate of intracellular L. monocytogenes begins to fall when the density of bacteria within the host cell becomes high enough to limit nutrient availability.
Faithful inheritance of a species’ complete genome during cell division is a requirement of cellular life. The task is complicated enough when daughter cells must inherit a single genome piece, as is the case for many bacteria. How can then a bacterium ensure complete inheritance of a segmented genome composed of 20 distinct replicons? *Borrelia burgdorferi* achieves this feat while cycling between a tick vector and a mammalian host, where it experiences vastly different environments that affect its growth rate and gene expression profile. To address this question, we visualized the *B. burgdorferi* genome by fluorescently labeling several chromosomal loci and most of its endogenous plasmids. While exponentially growing in culture, the chromosome and plasmids were polyploid and uniformly spaced. We also observed chromosome polyploidy in colonized ticks, indicating that polyploidy also occurs in the bacterium’s natural environment. Combined polyploidy, uniformly spaced distribution of the genome along the cell length, and mid-cell division readily ensure faithful genome inheritance. In many bacteria, the *par* locus controls chromosome organization and segregation. ParB simultaneously regulates genome organization through recruitment of SMC (structural maintenance of chromosomes) complexes at centromere-like *parS* site(s) and segregation through control of the ATPase activity and subcellular distribution of ParA. In *B. burgdorferi*, we show that SMC recruitment and ParA localization are independently regulated. We found that ParB retained its SMC-recruiting role, but that ParA localization is controlled by a novel centromere-recognizing protein, which we name ParZ. ParA forms waves of signal inside the cells, with maxima located in between chromosomal origins and ParA signal depletion around the origins. The ParA waves required ParZ but not ParB-ParS, consistent with the absence of an otherwise well-conserved ParA ATPase stimulating peptide from *Borrelia* ParB sequences. ParZ colocalized with ParB puncta but did not require ParB or *parS* for its subcellular localization and function. Our work provides single molecule-level description of the organization of the most segmented bacterial genome known to date as well as mechanistic insights into the regulation of segmented genome organization and segregation in the Lyme disease spirochete.

**Biological Size Control and Scaling**

Deep single-cell phenotyping uncovers general principles of cell size and cell cycle coordination in bacterial cells

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Bacterial proliferation requires the integration of a diversity of cellular processes, ranging from cell growth to DNA replication and cell division. The general principles that govern and coordinate growth, cell cycle events and cell morphogenesis remain poorly understood. To start addressing this fundamental gap of knowledge, we developed a high-throughput single-cell phenomics approach that enables the acquisition of a large number of cellular features related to growth, cell morphology,
nucleoid morphology, and cell cycle events across >800 mutants and multiple growth conditions. Using this approach, we have identified new governing principles and dependencies between different cell cycle- and cell size-related processes.

SG224

**Cytoplasmic organization promotes protein diffusion**

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Interphase Xenopus egg extracts can spontaneously self-organize into cell-like units. This gives us the opportunity to see how the organization of the cytoplasm influences protein dynamics. Using fluorescence correlation spectroscopy, we have show that the diffusion of a protein-sized probe is about twice as slow in a disorganized, cytoplasmic extract than it is in an organelle-depleted cytosolic extracts. Moreover, as the disorganized cytoplasmic becomes organized, the proteins diffuse faster over distance scales of a few hundred microns, approaching the speed at which proteins diffuse in cytosol. This shows that despite how crowded the cytoplasm is, cytoplasmic organization maximizes protein-protein interactions.

SG225

**Size Scaling and Repurposing of Cell Division Machinery in Early Xenopus Embryos**

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Amphibian eggs provide a useful system for investigating how the cell division machinery scales with cell size. Eggs start larger than 1mm and undergo at least 12 rounds of rapid, quasi-symmetric division with little change in their internal biochemistry as analyzed by proteomics. Undiluted cytoplasmic extracts prepared from eggs recapitulate most events of the cell cycle. Recent technical innovations allow us to visualize multiple rounds of mitosis in this cell free system, including decrease of spindle and aster size in successive divisions. In the first few cell cycles in eggs, mitotic spindles are small compared to cell size, but interphase microtubule asters grow to fill the whole egg and position cleavage furrows. Interphase asters grow by autocatalytic nucleation of microtubules. The boundary where sister asters meet recruits CPC and centraspinidlin signaling complexes which shape asters and position cleavage furrows. In recent work, we focused on how the morphology of the cytokinesis machinery changes with cell size and the role of cytoplasmic dynein and actomyosin in positioning asters and determining cleavage geometry. We also investigated re-purposing of proteins previously thought to be dedicated to cytokinesis. Anillin, which coordinates the location and activity of RhoA, actin, myosin-II and septins during cytokinesis, appears to play an important and unexpected role in controlling the shape of interphase cells during gastrulation. Other cytokinesis proteins may be similarly re-purposed. Repurposing of furrow-organizing proteins to control the shape and mechanics of interphase cells during morphogenesis of proliferating epithelial sheets could be relevant to organogenesis and tumorigenesis as well as gastrulation.
Mechanisms of mitotic chromosome scaling in Xenopus

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During the rapid and reductive cleavage divisions of a developing embryo, subcellular structures such as the nucleus and mitotic spindle scale with decreasing cell size. Mitotic chromosomes also decrease in size during development, but the underlying mechanisms are unknown. Here we have developed an in vitro approach to study mitotic chromosome scaling using egg extracts derived from *Xenopus laevis*. Using this approach, we have shown that mitotic chromosomes formed from blastula-stage embryo nuclei are 2-fold shorter than those formed using replicated sperm nuclei, due to differential recruitment of key mitotic compaction factors linker histone H1, condensin I and topoisomerase II. Polymer modeling of Hi-C data demonstrates that chromosome scaling occurs through dramatic changes in mesoscale DNA architecture. Finally, we show that chromosome scaling is regulated by importin α, a nuclear import factor that also scales the mitotic spindle and interphase nuclei to cell size by acting as a biochemical sensor of cell surface area: volume. Taken together, our data suggest a model in which during early embryogenesis, mitotic chromosomes scale alongside nuclei and mitotic spindles through a central pathway involving importin α, leading to the differential recruitment of mitotic compaction factors that rearrange DNA to form shorter chromosomes.

Coordination of Macromolecule Synthesis and Cell Volume

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The cytoplasm is a unique environment that allows thousands of chemical reactions to occur simultaneously. One of the defining features of the cytoplasm is the dense packing of macromolecules, which is close to its physical limit. Cytoplasm density varies very little in a given population of cells and alterations in cytoplasm density correlate with dramatic changes in cellular behaviour, for example during starvation or cell senescence. We and others have recently uncovered that macromolecule synthesis and cell volume increase can become uncoupled from each other and are thus to some degree regulated independent form each other. We are currently investigating how volume increase can become uncoupled from protein synthesis and how these two processes are normally coordinated to maintain cell density constant.

Nuclear growth and shape dynamics in growing hiPSCs

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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. We begin with a focus on the nucleus by developing a study of the size and shape of this key cellular structure over time. We took advantage of a mEGFP-tagged laminB1 line from the Allen Cell Collection (allencell.org/cell-catalog) of endogenous tagged hiPSC lines to capture nuclear dynamics during colony growth. We imaged live cells
growing in colonies in 3D for two days at 5 minute intervals at 20x magnification. We then developed an image analysis workflow to first transform these images into the higher magnification and resolution (100x, 1.4NA) needed for nuclear shape analysis via deep learning and then to track all the nuclei in the colony. Manual curation of nuclear volume trajectories over time yielded approximately 200 tracks spanning all of interphase. We analyzed the variation in timing and nuclear volume at three key points in these growth trajectories. We found that the nucleus undergoes two distinct phases of growth regulation, with an inflection point marking the transition between these early and late growth regimes occurring in G1 at a consistent time and nuclear size. During the early growth phase, smaller nuclei grow faster than larger nuclei, but both grow for a similar duration. During the late growth phase, however, nuclei approximately double their sizes with a remarkable size-independent linear growth rate but smaller nuclei grow for a longer duration. Furthermore, to determine the natural sources of shape variation throughout nuclear growth, we decomposed our highly accurate 3D nuclear segmentations into spherical harmonic coefficients used as inputs for principal component analysis to create a shape space. We interpret each shape space dimension as a shape mode, each corresponding to a different source of biological variation in hiPS cell colonies. We analyze how individual nuclei explore the shape space relative to the entire population, finding that shape modes could be characterized by two groups with distinctly different ergodic behavior. This general analysis framework will be extended to each of the key intracellular structures in an integrative fashion.

SG229

Cytoskeletal and osmotic regulation of cell size
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At short time scales (~minutes), mammalian cells use ion fluxes and cytoskeletal forces to modulate the cell cytoplasmic water content and the cell size. Using a quantitative microfluidic method of cell size measurement, we discover that 3T3 fibroblasts and HT1080 fibrosarcoma cells utilize the ionic and cytoskeletal modes of cell size control differently. Moreover, these different modes of cell size control can signal to each other through calcium dynamics, which ultimately allows the cell size to robustly adapt to hydrostatic and osmotic perturbations. These results suggest that cells utilize multiple interconnected mechanisms to achieve a homeostatic cell size. Implications of these results for cell growth control during the cell cycle are discussed.

SG230

Cell size-dependent G1/S transition controls stem cell size in epidermal and intestinal stem cells
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Cell size impacts cellular biosynthesis and stem cell fitness. How stem cell size is controlled, especially in the tissue context, is poorly understood. In unicellular eukaryotes and 2D culture models, cell size homeostasis is often achieved by coupling cell cycle progression to cell growth. We analyzed the growth of single stem cells in two mammalian epithelia: the basal layer epidermal stem cells growing in vivo and the Lgr5+ intestinal stem cells growing in an organoid. We find that cell size is controlled at the G1/S transition, where smaller-born stem cells spend longer and grow more during their G1 phase. We also find that as intestinal stem cells differentiate into transit-amplifying cells, their G1/S size-dependence remains invariant, suggesting that the molecular mechanism linking cell size to G1/S transition rate is
retained during fate specification. This G1/S cell size control is in contrast to how cell size is controlled in conventional 2D cell culture, where cells grow a constant volume per cell cycle. We are currently testing if Rb or p38 pathways, which were implicated in cell size control by previous in vitro work, may underlie the G1/S cell size control observed in vivo.

SG231

Cell size regulation in epithelial tissue

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To prevent epithelial tissue overgrowth cells sense density and mechanics to regulate growth and division, a process known as contact inhibition of proliferation. Contact inhibition is thought to act on the coupled process of cell growth and division. However, we observe that in confluent monolayers the process of growth and division become uncoupled with a large decrease in cell growth occurring before the cell cycle is arrested. This decoupling leads to a decrease in cell size which triggers a size dependent arrest of the cell cycle below a critical cell volume. Therefore, contact inhibition of proliferation involves two distinct transitions: A mechanical transition which limits cell growth as cells reach confluence and a cell size dependent transition which inhibits the cell cycle. By observing cell growth in subconfluent and confluent cell cultures, we see that cell growth is inhibited as cells in the monolayer become spatially confined. As cell size decreases the cell cycle becomes arrested with a strong dependence on cell volume. This cell cycle arrest occurs due to a reduction in the stability of cyclin D in small cells. Overexpression of cyclin D1 is sufficient to reinitiate the cell cycle causing additional cell division and size reduction. We see that size reduction beyond normal contact inhibition leads to increased nuclear to cytoplasmic ratio as cells approach a physical size limit based on volume occupied by the genome. Our work demonstrates that cell size regulation qualitatively changes during the transition to contact inhibition to facilitate cell cycle arrest through a cell size sensing mechanism. This shift in cell behavior may explain the discrepancy in cell size regulation previously observed between cell culture models and tissues in vivo and lead to new understanding of mammalian cell size regulation in tissue homeostasis and disease.

SG232

Natural Gradient of Cell Size Regulates Genome Activation Pattern and Vertebrate Early Development

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In early embryogenesis, embryos divide without growth. After this rapid reduction in cell volume, the embryos turn on nascent transcription in zygotic genome activation (ZGA), which is required for subsequent gastrulation. Our lab previously identified a spatial and temporal pattern of ZGA in Xenopus embryos that occurs initially in small cells at the animal pole and is delayed by two hours in cells of the vegetal pole. This spatiotemporal pattern tightly correlates with the gradient of cell sizes present in the blastula embryos. To understand the developmental importance of this evolutionally conserved cell size gradient and the resulting ZGA pattern, we developed an embryo temperature controller to eliminate or even reverse those patterns. It works by slowing down the division rate of the cells in the animal pole and speeding up the division of the vegetal cells. By generating Xenopus blastula embryos with reversed cell size gradients, we found that the spatial pattern of ZGA is also reversed; genome activation occurs first in the vegetal pole and is drastically delayed in the animal pole. Intriguingly, the ‘reversed’ embryos
still maintain a similar threshold of cell size for transcription onset. We hypothesized that the ectopic onset of genome activation could result in a mismatch of developmental timings in between different germ layers. Indeed, those ‘reversed’ embryos tend to arrest in gastrulation as they fail to close the blastopore before neurulation begins. For those ‘reversed’ embryos that go through gastrulation, they grow defective eyes and heads indicating abnormal neural induction. This is probably due to the delayed ectoderm misses the induction secreted by the premature mesoderm. Apart from that, applying temperature gradient in horizontal settings induces ectopic gradients of cell size, which could be employed to alter dorsal-ventral (D-V) patterning by specifically delaying the ZGA in the dorsal or ventral side. This work suggests that the natural gradient of cell sizes sets the timing of genome activation in different parts of embryos, which is important to coordinated tissue movement and proper germ layer specification during gastrulation.

Cell and Tissue Morphodynamics in Engineered Systems

SG233

Engineering epithelial shape and mechanics

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To perform their physiological functions, epithelial layers lining organs such as lungs, kidneys, bladder, and mammary glands fold into complex 3D shapes. We develop new experimental tools and models to understand how cellular forces and active material properties enable epithelial shape, fate and function. We engineer substrates with differential cell-matrix adhesion to sculpt three-dimensional epithelia of controlled size and shape. By subjecting these epithelia to pressure differences, we map epithelial tension under controlled deformations. These experiments reveal the constitutive properties of epithelial tissues and the impact of tension anisotropy on cellular shape. We also measure the three-dimensional cell-ECM and cell-cell forces in mouse intestinal organoids grown on soft hydrogels. These organoids exhibit a non-monotonic stress distribution that defines mechanical and functional compartments. Mechanical compartmentalization enables the intestinal crypt to fold through apical constriction and differentiated cells to move collectively towards the villus along a tensile gradient. Finally, I will discuss how the altered epithelial forces and material properties contributes to tumor shape and progression.

SG234

Self-organized morphogenesis of a human neural tube in vitro by geometric constraints

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Understanding how human embryos develop their shape is a fundamental question in physics of life with strong medical implications. However, it is not possible to study the dynamics of organ formation in humans. Animals differ from humans in key aspects, and in particular in the development of the nervous system. Conventional organoids are unreproducible and do not recapitulate the intricate anatomy of organs. Here we present a reproducible and scalable approach for studying human organogenesis in a dish, which is compatible with live imaging. We achieve this by precisely controlling cell fate pattern formation in 2D stem cell sheets, while allowing for self-organization of tissue shape in 3D. Upon
triggering neural pattern formation, the initially flat stem cell sheet undergoes folding morphogenesis and self-organizes into a millimeter long anatomically true neural tube covered by epidermis. Cell fate composition matches the in-vivo neural tube, including neural crest cells. In contrast to animal studies, neural and epidermal human tissues are necessary and sufficient for folding morphogenesis in the absence of mesoderm activity. Furthermore, we model neural tube defects by interfering with signaling that regulates tissue mechanics. Finally, we discover that neural tube shape, including the number and location of hinge points, depends on neural tissue size. This suggests that neural tube morphology along the anterior posterior axis depends on neural plate geometry in addition to molecular gradients. Our approach provides the first path to study human neural tube morphogenesis in health and disease.  

SG235
From averages to ensembles- a statistical mechanical perspective on structural distributions of self-organizing tissues

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Tissues exist in fluctuating environments and must actively maintain their structure through programs of self-organization. However, the fundamental limits of structural order at the tissue scale have not been defined. Here we combine theory and experiments to demonstrate that tissues exist as dynamic structural ensembles with properties that follow Boltzmann statistics, and therefore, are defined by a characteristic level of disorder. The statistical properties of tissue ensembles emerge from three parameters: underlying interfacial energies (enthalpy), geometric constraints (structural degeneracy or entropy), and mechanical fluctuations (activity) of the tissue. We measured the structural distribution for reconstituted organoids, comprising aggregates of human mammary epithelial cells derived from breast reduction mammoplasty surgeries. We show that distribution follow Boltzmann statistics, and engineer the tissue structural ensemble through a systematic perturbation of mechanics, geometry or dynamics. We further demonstrate how this structural ensemble becomes disrupted during breast cancer progression. Specific pathways downstream of breast cancer driver genes alter the structural ensemble by altering interfacial mechanics and consequently, increasing tissue structural disorder. Among 15 cancer-associated genetic changes, PIK3CA most significantly altered the interfacial mechanics of the breast cancer cell of origin—luminal epithelial cells—in a manner that promotes tissue configurations that are primed for invasion. We identify molecular perturbations that restore a normal structural ensemble by correcting luminal epithelial cell interfacial mechanics or decreasing global tissue activity. Overall, this statistical mechanical framework reveals that configurational entropy imposes a theoretical maximum limit to structural order and can promote the emergence of tissue structures that are primed for invasion in cancer.
The role of SGEF in tissue morphogenesis and organ development

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Most internal organs consist of a polarized epithelium surrounding a central lumen, which separates the inside of the cells from the external environment. The establishment of polarity is essential for epithelial cells’ function, and abnormalities in this process is a hallmark of many diseases, such as cancer. Polarization is initiated and maintained by coordinated action of three protein complexes: Crumbs, Par, and Scribble. The Scribble complex is comprised of Scribble, Dlg1 and Lgl, and was primarily identified as a critical regulator for polarity, but was later shown to be involved in other related cellular processes, including cell-cell adhesion, asymmetric cell division, and vesicular trafficking. In our recently published work, we identified a novel ternary complex that comprises two of the Scribble complex members: Scribble and Dlg1, as well as SGEF, a RhoG-specific guanine-nucleotide exchange factor. In three-dimensional MDCK cysts, our results show that SGEF’s scaffolding activity with Scribble and Dlg1 is required to regulate lumen opening. Meanwhile, its catalytic activity that is accomplished through RhoG activation is required for the regulation of lumen number. Our working model is that multiple lumens in SGEF knockdown cysts result from defects in vesicular transport or delivery, whereas closed lumens result from leakage as a result of impaired barrier function. Here, we have analyzed the progression of cyst development by fixing and staining cysts at various time points. Our results show that the differences between the control and SGEF knockdown cysts appeared at around 48 hours, and progressed until fully developed. SGEF knockdown cysts had a larger diameter, multiple lumens and reduced E-cadherin expression. In order to characterize this process in more detail, we optimized a system to follow cyst development in live cells imaging continuously for four days starting from a single cell using spinning-disk microscopy in MDCK cells stably expressing Scarlet-CAAX as a membrane marker. Surprisingly, our results show that SGEF knockdown cysts are more motile and prone to fusion with other cysts, suggesting the reduced E-cadherin expression may promote the enhanced motility. We plan to continue to use this system to perform a systematic quantitative analysis of different fluorescently-tagged junctional and cytoskeletal markers to identify which processes are being affected that lead to this motility, as well as multi-lumen cysts.

Disentangling the effect of confinement on keratocyte shape, size, and speed

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The larval zebrafish has emerged as a powerful system for studying wound cues that trigger lamellipodial-driven cell migration and subsequent stages of healing. Additionally, zebrafish basal epidermal cells can be isolated in tissue culture (frequently called “keratocytes”), where they migrate rapidly and persistently, and are amenable to various environmental perturbations. These systems offer an opportunity to carefully examine the role of the environment in epidermal cell migration. In this study, we expose isolated keratocytes to various environmental variables to test their relevance to in vivo cell shape and biophysical effects on cell migration. In vivo, basal cells appear to experience 2D confinement because they 1) reside between a basement membrane and an upper layer of epidermal cells, and 2) are much flatter than isolated keratocytes (~2-4 µm tall vs. ~7-9 µm). Additionally, these
cells in their native environment are typically oriented with their longest axis parallel to the direction of migration, while isolated keratocytes usually migrate parallel to their shortest axis. We attempted to mimic the natural physical environment of keratocytes by confining the isolated cells under either a hydrogel overlay or an elastomer ceiling. Under these conditions, isolated keratocytes maintain lamellipodial migration, but are significantly flatter and longer than unconfined cells, recapitulating the \textit{in vivo} shape phenotype. Significant elongation was only observed in cells < 4 µm tall, which corresponds to cell heights typically observed \textit{in vivo}. We conclude that 2D confinement is likely a major contributor to \textit{in vivo} basal epidermal cell shape and behavior. To investigate biophysical effects of a confining environment on lamellipodial keratocyte migration, we measured cell speed, size, and shape for a large number of keratocytes before and during geometric confinement. Confinement caused a 20% decrease in cell speed and, surprisingly, a 20% decrease in cell volume, while total cell surface area remained nearly constant. We speculate that volume change is one mediator of confinement-induced speed decrease and potentially other reported effects of confinement.

SG238

\textbf{Geometric control of intestinal organoid patterning}

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Organoids form through poorly understood morphogenetic processes in which initially homogeneous ensembles of stem cells spontaneously self-organize in suspension or within permissive three-dimensional extracellular matrices. Yet, the absence of virtually any predefined patterning influences such as morphogen gradients or mechanical cues results in an extensive heterogeneity. Moreover, the current mismatch in shape, size and lifespan between native organs and their in vitro counterparts hinders their even wider applicability. In this talk, I will present some of our ongoing work to develop next-generation organoids that are built by controlling cellular self-patterning in artificial stem cell microenvironments. In particular, I will present some recent work in which we highlight the role of tissue geometry in patterning intestinal organoids.

SG239

\textbf{In vivo study and synthetic engineering of mechanical rules for kidney tubule morphogenesis}

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The intricate branching of the adult kidney urinary collecting duct tree occurs in response to tightly choreographed cues passed between ureteric epithelial tubules and surrounding tissue layers during development. The tips of this epithelial network are the engines that drive formation of the 200,000-2 million nephron filters present in adult kidneys. However, the engineering principles governing the remarkable branching behavior of this tubule network in response to spatially and temporally dynamic signals are not well understood. The recent explosion of human kidney organoid systems has been highly enabling here, but these organoids do not yet mimic the long-range integration of nephrons within tree-like tubule systems. Thus, there is massive potential to study and apply new engineering rules that would shed light on the wide variability in nephron number between individuals, which correlates with adult disease, and to build large enough kidney tissue for functional replacement. We first study mechanical reorganization of tubule networks in the developing mouse kidney using organ...
explants and physical models. Our data indicate that developing kidneys visit only a narrow range of possible tubule packing solutions and highlight two classes of potential packing defects - tip burial within deeper tissue layers or “short circuits” through adjacent tip fusion - examples of which can be found in published literature. Furthermore, they suggest that internal, radially oriented forces on tubules are required to avoid these defects and for tubule trees to resolve into vertically packed arrays. We validate this by manipulating and measuring tubule tension within live kidney explants, and matching outcomes to our physical models. We next outline a route to synthetically create ureteric tubule networks by drawing parallels between programmed strain in micropatterned tissues and the kidney ureteric branching program. We reconstitute these mechanics in tissue scaffolds that progressively encode 3D extracellular microenvironments by programmed shape change. By integrating microfluidic cell and matrix patterning, kinematic models, and kidney tubule engineering, we describe a vision for encoding long-range developmental patterning in next-generation kidney organoids.

SG240
Kinome-wide Screen Identifies Raf-MAPK Pathway as a Regulator of Hair Follicle Stem Cell Plasticity
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The hair follicle stem cells (HFSC) undergo cyclic bouts of differentiation to fuel regeneration of the hair follicle throughout our life. Notably, dedifferentiation of a subset of hair follicle progenitors back to the stem cell state is requisite for long-term tissue maintenance. Signaling factors that regulate progenitor cell dedifferentiation have been identified, yet how their signals are transduced intracellularly is not clear. To identify signaling pathways controlling progenitor cell plasticity we established a HFSC organoid-based kinome-wide screen using a small molecule library of all known kinase inhibitors. Flow cytometry-based analysis of stem cell-to-progenitor ratio revealed two inhibitors, GDC-0879 and Dabrafenib, which target mutant B-Raf and are in clinical use to treat melanoma, to increase the proportion of HFSCs. We show that both inhibitors function to activate wildtype Raf signaling, as previously shown, resulting in an increase in active p42/44 Mitogen Activated Protein Kinase (p42/44) via MEK activity. Further, we found that the inhibitors attenuate stem cell differentiation while increasing progenitor dedifferentiation, indicating that p42/44 activity favors the stem state. p42/44 is known to influence cellular proliferation, but inhibitor treatment increased proliferation in both cell populations in a comparable manner, suggesting that mitosis does not underlie the increase in stem cells. Intravital imaging of ERK activity in the hair follicle using transgenic ERK-FRET mice revealed that p42/44 activity propagates along the hair follicle in the stem cell niche upon progenitor return to the niche, which resolves when the HFSC niche is repopulated. Together, our data reveals a novel regulatory function of p42/44 signaling in the hair follicle to repopulate the HFSC niche with stem cells. Ultimately, the ability to modulate cellular plasticity to regenerate stem cells from progenitor cells has major clinical implications.
SG241

Notch1 Regulates Ductal Morphogenesis, Adherens Junctions, and Mitogenic Signaling Through a Transcription-Independent Mechanism

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Spatial and temporal coordination of cell proliferation and movement shape developing tissues, drive regeneration and, when dysregulated, facilitate cancer progression. During epithelial growth and crowding, adherens junction complexes (AJs) that connect neighboring epithelial cells control behavioral and proliferative responses to soluble growth factors and mechanical stimuli, however the critical underlying control systems remain poorly understood. We recently identified a transcription-independent mechanism by which cortical Notch1 signaling at the plasma membrane regulates endothelial AJ integrity and vessel barrier function. Motivated by this finding and the central role of Notch receptors in a wide array of developmental and homeostatic processes, we investigated whether cortical Notch signaling influences the form and function of epithelial tissues. We have developed a three-dimensional (3D) organotypic model of a human mammary duct consisting of an extracellular matrix-embedded, lumenized tissue that permits high resolution observation of dynamic morphogenic behaviors. Applying our model, CRISPR-mediated deletion of NOTCH1 leads to occlusion of the lumen and dramatic ductal outgrowth, driven by changes in cell proliferation and organization. Stable expression of a dominant negative Notch transcriptional cofactor or truncation of endogenous Notch1 intracellular domain has no observable effect on ductal tissue architecture. Mechanistically, as cells reach confluence and growth arrest, Notch1 progressively localizes to cortical actin in the lateral domain. This transition is coincident increased proteolytic activation of Notch1, which occurs without a transcriptional response, and is dependent upon AJs. Loss of Notch1 cortical signaling leads to depleted cortical actin, defective apical-basal polarization, destabilized E-cadherin AJs, and elevated cell contractility. Further, we demonstrate the increase in cell proliferation and impaired contact inhibition in Notch1 knockout cells are driven by aberrant internalization and signaling of EGFR. Together, separation of cortical Notch signaling from transcriptional functions of Notch may illuminate how a single receptor might link transcriptional programs with adhesive and cytoskeletal remodeling during development and disease.

SG242

Trans-epithelial fluid pumping performance of renal epithelial cells and hydraulic pressure gradient transduction

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Using a novel microfluidic Organ-Chip platform to recapitulate fluid transport activity of kidney cells, we report that renal epithelial cells can actively generate hydraulic pressure gradients across the epithelium. The fluidic flux declines with increasing hydraulic pressure gradient (ΔP) until a stall pressure, at which the flux vanishes— in a manner similar to mechanical fluidic pumps. The developed pressure gradient translates to a force of 50-100 nanoNewtons per cell. Using live cell imaging, here we show that ΔP decreases baso-lateral localization of Na⁺/K⁺ ATPase (NKA), by disrupting the F-actin cortex, due to formation of asynchronous spatio-temporal cortical invaginations called pressure-cups. Each pressure-cup induces micron-scale hemispherical deformation of the baso-lateral plasma
membrane and diminishes within 15 seconds, and the dynamics depends on actomyosin contractility. Additionally, we found that ΔP increases the frequency of calcium oscillations, mediated by stretch activated channels. Furthermore, calcium overload caused Calpain-mediated degradation of Ankyrin-G, a major regulator of NKA polarization and vectorial fluid transport. These results, together with data from osmotic and pharmacological perturbations, implicate mechanical force and hydraulic pressure as important variables during morphological changes in epithelial tubules, and provide further mechanistic insights underlying the development and transduction of hydraulic pressure gradients.

Natural Products in Cancer Therapy in the Islands: Culturally Relevant Research Experiences from PEER Scientists

SG243
**Bioguided isolation of chemical constituents from *Simarouba tulae*, an endemic plant of Puerto Rico**

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Species of the genus Simarouba have been studied because of its anti-malarial, anti-inflammatory, anti-leukemic, anti-feedant and antiviral activities. A group of terpenes called quassinoids have been isolated from species of the *Simarouba* genus and are thought to be responsible for its therapeutic properties. We hypothesize that *Simarouba tulae*, endemic plant species, are a natural source rich on quassinoid compounds and, thus, will inhibit growth of cancer cells. The objective of this study is to test the biological activity of the secondary metabolites from *Simarouba tulae* against different cancer cells. The leaves were processed and extracted with solvents of different polarities. The crude extracts were screened for their antiproliferative activity showing that the chloroform extract was the most active extract. This extract was purified using different chromatographic techniques to afford the quassinoid Simalikalactone D (SKD) and a SKD derivative. In cancer cells, SKD showed high cytotoxicity activity with an IC₅₀ of 55 and 65 nm in A2780CP20 (ovarian) and MDA-MB-231 (breast) cell lines, respectively. Preliminary investigations of the anti-migratory activity in MDA-MB-231 cancer cells showed that SKD inhibits migration of MDA-MB-231 cells by 15%. In addition, SKD showed that can activate the cell death endpoints such as apoptosis induction, DNA fragmentation, and mitochondrial permeabilization. Based on our results, we demonstrate the strong anti-proliferative activity of the quassinoid SKD isolated from *Simarouba tulae*. As future work we want to isolate other compounds from this endemic plant and perform additional studies to investigate the anticancer potential of SKD and SKD derivatives.

SG244
**Effects of *Ganoderma spp.* extracts used as complementary therapy for aggressive breast cancers in Puerto Rico**

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Breast cancer (BC) is the leading cause of cancer among women in the United States and Puerto Rico. Women that are diagnosed with the triple-negative (TNBC) and inflammatory breast cancer (IBC) subtypes typically display a shorter overall survival and recurrence within 3y after diagnosis. Treatments for these subtypes are limited, and currently, there are no approved targeted therapies. Thus, a critical
need exists to discover effective therapies that selectively target tumor cells, not affecting normal cells, to reduce mortality rates associated with these diseases. In Puerto Rico, some naturopathic physicians use the medicinal mushroom *Ganoderma lucidum* (GLE) together with conventional therapies in cancer patients, however the mechanism of action is unknown. Herein, we aimed to test the anti-cancer effects of commercially available GLE on cancer cell viability, invasion, stemness, migration, and on the regulation of cell signaling events. Moreover, we also tested extracts from additional locally grown *Ganoderma* species: *Ganoderma multiplicatum* (GMu) and *Ganoderma martinicensis* (GMa) in our BC models. IBC and TNBC, as well as non-cancerous mammary epithelial cells were treated with various GLE, GMu or GMa concentrations for cell viability, washout assays, and cell signaling assays. IBC cells were also injected into mice to test *in vivo* GLE effects. Cancer stem cells (CSCs) were studied by CD44/CD24 markers and ALDH positivity, while FACS sorted cells were injected into mice to test GLE effects in CSCs *in vivo*. To study the effect of GLE on cell migration, cells were treated with vehicle or GLE, and wound healing, and invasion assays were conducted. Furthermore, the expression of proteins important in the dynamics of cell motility and cancer signaling pathways was tested. Results showed that GLE selectively decreases cancer cell viability, with no cell recovery after a 24h, while no toxic effects are seen in non-cancerous cells. GLE significantly decreases tumor volume by 50%, as well as CSCs both *in vitro* and in mice. TNBC cell migration, invasion, the expression of key cancer promoting proteins such as Rac, AKT, STAT3 and lamellipodin are all decreased. Finally, our locally grown *Ganoderma* displayed greater anti-cancer potency, by inhibiting cancer cell viability at lower inhibitory concentrations (IC₅₀), than GLE. In conclusion, our results suggest the potential of GLE as a natural anti-breast cancer progression agent, and a possible evolutionary advantage of tropically grown *Ganoderma* spp. against aggressive BC.

SG245

*Ganoderma lucidum* Enhances Carboplatin Effect by Inhibiting the DNA Damage Response and Stemness in Inflammatory Breast Cancer

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Inflammatory Breast Cancer (IBC) is a rare and aggressive type of breast cancer with a poor prognosis. Its management is challenging because of a lack of specific therapies, increased metastatic potential, and high recurrence rates. Interest in using platinum-based agents emerged from data suggesting frequent DNA repair defects in breast cancer. Studies show that *Ganoderma lucidum* (GLE) extract sensitizes cancer cells to radiation, tyrosine kinase inhibitors, and other anti-cancer drugs. Therefore, here we investigated the therapeutic potential of GLE, alone or in combination with carboplatin in IBC. We focused on the regulation of the DNA Damage Response (DDR) and cancer cell stemness. Carboplatin and GLE were tested in vitro using the IBC cell line, SUM-149, breast cancer non-IBC cells, MDA-MB-231, and *in vivo* using IBC xenograft models. Our results show that GLE or the combination of therapies decreased cell viability, induced cell, delayed the response to DNA damage, and downregulated the expression of damage repair proteins. Furthermore, the combination suppressed mammosphere formation and the expression of cancer stemness proteins. In xenograft models, the combination showed significant tumor growth inhibitory effects without systemic toxicity. Taken together, our results provide evidence of GLE’s potential in chemosensitizing TNBC cells to carboplatin therapy affecting the DDR. These results highlight GLE’s anti-breast cancer therapeutic potential.
SG246

Ergosterol peroxide effects, sub-localization and potential biological targets in aggressive breast cancer models

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Triple-negative breast cancer (TNBC) accounts for approximately 10-20% of the breast cancer population and is often associated with poor patient outcome. The increased poor prognosis in patients is due in part to the lack of availability of targeted therapies available for this intractable disease. Thus, a need exists to discover treatments that selectively target tumor cells, and that do not harm normal tissue. Accordingly, as an effort to provide effective therapeutic alternatives, we recently characterized the most bioactive compounds in the medicinal mushroom, Ganoderma lucidum (GLE), and tested them against aggressive breast cancer models. Our studies revealed that the steroidal compound, Ergosterol Peroxide (EP), is GLE’s main cytotoxic component. However, EP’s mode of action (MOA) remains unknown. We hypothesize that EP modulates cancer cell signaling affecting mitochondrial integrity to induce cell death. We evaluated EP’s anti-cancer potential using immunoblotting, cell death, colony formation, motility, cell cycle and reactive oxygen species (ROS) production assays in human breast cancer cells. Moreover, we also performed cellular localization assays using an EP-fluorescent probe together with organelle co-localization markers, and a target-based drug discovery approach by affinity purification using our EP-biotin probe, pulled down with streptavidin coupled with mass spectrometry analysis. Our results show that EP selectively affects cancer cell viability, and induces apoptosis, cell cycle arrest and ROS. Moreover, EP affects the signaling of cancer promoting proteins involved in cancer cell survival, and decreases mitochondria membrane potential, respiration and glycolysis. Cellular localization studies revealed that EP co-localizes in the endoplasmic reticulum. Finally, using two TNBC cell lines we showed that EP targets the Ubiquitin Protein Ligase E3 Component N-Recognin 4 (UBR4) and Rab interacting lysosomal protein like 1 (RIPL1) in both cancer cell lines. In conclusion, our studies validate EP’s therapeutic potency in TNBC cell models. In addition, we provide preliminary insights to its localization, and mode of action in aggressive breast cancer models. Target validation, as well as compound safety and efficacy studies in in vivo models are currently being conducted to further assess EP’s therapeutic potential.

SG247

Investigating the anti-cancer effects of La’au Lapa’au (Native Hawaiian plant-based medicine) and other traditional medicines

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Background Due to Native Hawaiian cultural and social determinants of health, Native Hawaiians suffer disproportionately from chronic illnesses such as cancer. Therefore, alternative and more affordable treatments that have ties to the Native Hawaiian culture may help to reduce Native Hawaiian cancer health disparities. This project investigates the anticancer effects of plant extracts and natural products from La’au Lapa’au (Native Hawaiian medicine) and other traditional medicines. Methods Cell proliferation assays were used to screen natural product and extract libraries using paired patient
derived neuroblastoma (NB) tumor cells that include tumors excised from patients at diagnosis, and tumors excised from the same patients after relapse, as well as other types of cancer such as colorectal cancer & breast cancer. Western blot analysis of the whole cell lysates determined whether the extracts and natural products altered the expression of cell cycle, apoptotic and autophagy proteins. Ratiometric Fura-2 calcium (Ca2+) measurements were performed to determine the effects on Ca2+ signaling. Fluorescent Ca2+ markers specific for the endoplasmic reticulum, mitochondria and nucleus were used to determine the effects on inter-organellar Ca2+ signaling in live cells using a laser-scanning confocal microscope. **Results** The results identified several natural products with potent anti-cancer effects, decreasing NB cell viability significantly compared to untreated controls. In addition, our results have shown that the extracts induce apoptosis in NB. Finally, we also identified aberrant Ca2+ signals that converge at the ER & mitochondria to induce apoptosis in HRNB.

SG248
*Investigating Natural Products in Cancer Research: Engaging Native Hawaiian and Pacific Islander Students*

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High-risk Neuroblastoma (HRNB) is a pediatric cancer in which up to 65% of patients experience therapy resistant relapse. While recent biomedical advances have improved treatment options for cancer patients, there are no effective treatments for relapsed HRNB. This project investigates the anticancer effects of plant extracts and natural products from La’au Lapa’au (Native Hawaiian medicine) and other traditional medicines. Cell proliferation assays were used to screen natural product and extract libraries using paired patient derived neuroblastoma (NB) tumor cells that include tumors excised from patients at diagnosis, and tumors excised from the same patients after relapse. Western blot analysis of the whole cell lysates determined whether the extracts and natural products altered the expression of cell cycle, apoptotic and autophagy proteins. Ratiometric Fura-2 calcium (Ca2+) measurements were performed to determine the effects on Ca2+ signaling. Fluorescent Ca2+ markers specific for the endoplasmic reticulum, mitochondria and nucleus were used to determine the effects on inter-organellar Ca2+ signaling in live cells using a laser-scanning confocal microscope. P450-glo and UGT-glo assays from Promega were used to determine whether the natural products and extracts were substrates, inhibitors or inducers of P450 cytochrome and UDP glucuronosyltransferase enzymes that are commonly used to metabolize drugs. The results identified several natural products with potent anti-cancer effects, we identified aberrant Ca2+ signals that converge at the mitochondria to induce apoptosis in relapsed HRNB. Determining the dynamic interactions between Ca2+ influx and ER-mitochondrial Ca2+ will open a path for new approaches to treat relapsed HRNB, as well as a broad spectrum of diseases. The results also identified some Native Hawaiian medicines that alter the activity of some P450 enzymes. This knowledge will provide critical information that could lead to the safe practice of La’au Lapa’au in patients who use prescription medications. Due to the inclusion of over 60 Native Hawaiian and Pacific Islander high school, undergraduate and graduate students, this work is training the next generation of researchers, is effective at engaging underrepresented minority students, and will increase diversity in the STEM workforce.
Reimagining Cell Biology: Emerging Tools for Imaging, Modeling, and Systems Integration

SG249
Quantifying the dynamics of gene expression at multiple spatial and temporal scales in developing embryos
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During early development gene expression patterns progressively emerge as cell fates are determined and the embryo takes form. The regulation of patterning occurs across a broad range of spatial and temporal scales. These scales range from the molecular scale dynamics of regulatory proteins binding to genomic loci to activate or repress expression, to the organization of chromatin and distribution of proteins within nuclei at sub-micron to micron scales, to the emergence of domains of gene expression at the scales of hundreds of microns to millimeters. Here I will discuss the application of high-resolution lattice light-sheet microscopy, single molecule tracking, and new analysis approaches to functionally bridge data acquired across this wide range of spatial and temporal scales. I will present data on how transient assemblies of transcriptional regulators, called hubs, shape the nucleoplasm and regulate gene expression during embryogenesis in *Drosophila melanogaster*. By combining our imaging data with systematic mutagenesis of key transcription factors we can now dissect how incorporation of proteins into hubs influences their ability to efficiently find and bind their genomic targets and regulate transcriptional activity.

SG250
Seeing in vivo forces - development of light-producing intracellular nanosensors to quantify mechanical signaling in living cell chromosomes
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Mechanobiology describes living systems by cycles of mechanosensation, mechanotransduction and mechanoresponse. The cycles of mechanical signaling between the extracellular environment and the nucleus, alongside biochemical cascades, are increasingly recognized to be a major driving mechanism in genome regulation. We hope to extend the mechanobiological approach to ask how forces arise, accumulate, and spread in chromosomes, and how such mechanical patterning contributes to the genome function, with special interest in accurate segregation during cell division [Chu et al., PNAS 2020; Mol. Cell 2020]. To visualize mechanical patterning within and among chromosomes, in its normal physiological context, we are developing two new tools that will allow luminescence imaging of mechanical effects \textit{in situ}. Our first approach builds on our recent discovery of the nanoscale mechanism for elastic and repeatable mechanoluminescence (ML) in ZnS doped with Mn (ZnS:Mn) [Mukhina et al., ACS Nano 2021]. We found that, \textit{in absence of photoexcitation}, single microcrystals of ZnS:Mn emit repeatable pulses of light in response to the application of mechanical pressure, i.e. directly convert mechanical energy to optical emission, i.e. produce mechanoluminescence. The threshold pressure for ML appearance registered in our experiments varied between 0.2 and 47 pN/nm\textsuperscript{2} which is in the range of intracellular forces. Furthermore, we provided evidence that ML in ZnS:Mn occurs at stacking faults, a special type of structural defect. Given this insight, we have now synthesized ZnS:Mn nanorods with
stacking faults and have shown that these nanostructures produce ML. These nanorods will be fully suitable for use as a force nanosensor after introduction and targeting to objects of interest in living cells. In our second approach, we are creating a dynamic DNA origami nanostructure which senses local depletion forces by switching between open and closed states separated by an energy barrier of 4.1 pN nm, which is on the scale of thermal energy. These nanostructures can be targeted to the chromosomes of proliferating cells using a bio-orthogonal Cu(I)-catalyzed cycloaddition reaction (“click” chemistry). We envision that both of these sensors, when attached to the chromosomes, will report a five-dimensional (f,x,y,z,t) map of bending, compressive, and tensile intracellular forces, thereby facilitating quantitative understanding of the mechanical basis of diverse chromosomal functions.

SG251
Using image-based reconstructions of cellular geometries for computational modeling
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That cell shape influences many cellular processes is well-known and well-accepted by now. Many studies have used a combination of computational models and experiments to demonstrate specific relationships between cell shape and readouts that are specific to a biological process. A vast majority of these studies often used idealized geometries in simulations because of lack of access to detailed cellular reconstructions. The explosion of imaging data and 3D reconstruction methods now offers us a mechanism to potentially use these reconstructed geometries for simulations. More important, this effort will allow us to qualitatively define the morphological features of cells and their organelles from these reconstructions. In this presentation, I will discuss the efforts of my group to render the 3D reconstructions of cells from FIB-SEM and other modalities suitable for computational simulations for both biochemical signal transduction and for biophysical problems. Our simulations reveal that the relationships between cell shape and signaling are more complex that previously thought because of the complexities involved in cell shape itself. Our efforts are focused on problems that are inspired by neurobiology but the methods are broadly applicable to any cell type.

SG252
Targeting intracellular organization via microscopy-based high-content phenotypic screening and generative neural networks
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Disruption in cell organization determined by the cell’s organelles composition in space and proper organelle-organelle organization leads to impaired cell function in many diseases. Thus, discovery of drugs that revert the cell structure and organization to its “healthy” state is an initial step in some drug discovery pipelines. High-content image-based screening is emerging as a powerful technology to identify phenotypic differences in cell populations with several applications including drug screening. While current computational approaches pool image-based features from different modalities, each of a distinct organelle, we develop new methodology to measure alterations in the spatial dependencies between different organelles, and apply it to identify new treatments that interfere with specific spatial dependencies between organelles. This will enable discovery and mechanistic interpretability of the effects each treatment has on specific aspects of cell organization in terms of “breaking” existing
relations between multiple cell structures, which are currently inaccessible. Our preliminary results indicate that this approach is feasible, and that spatial organelle dependencies are much more sensitive, specific and interpretable readouts for phenotypic cell screening. Specifically, we show that (1) known phenotypes are dramatically amplified making it easier to identify subtle phenotypes, (2) new phenotypes that are missed by traditional analyses can be discovered, and (3) spatial dependencies are differentially determined based on organelles composition and perturbation, implying a more specific and interpretable readout. Together, the proposed technology will be the first to target defects in inter-organelle spatial dependencies, and holds the promise for broad translational applicability in drug discovery, repurposing existing drugs, and combinatorial drug therapy.

SG253
Using Biochemical Reconstitution to Understand the Emergent Behavior of Signaling Pathways
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Cells are immensely complex, containing thousands of molecular components that synergistically control cellular functions. Biochemical reconstitution can be a powerful tool to dissect the underlying molecular interactions that give rise to the emergent behaviors of complex cellular systems. We have use biochemical reconstitution to study the formation and function of signaling clusters that form at the plasma membrane, including nephrin clusters and integrin clusters. Using purified lipids and recombinant proteins, we can carefully control the identity and concentrations of lipids and proteins in the system. To mimic the plasma membrane in vitro, we attach his-tagged receptors to Nickel-NTA lipids within supported phospholipid bilayers. With reconstitution we can determine the minimum set of components necessary and sufficient to observe cluster formation or downstream signaling. When paired with live-cell imaging and computational modeling approaches, biochemical reconstitution can be a powerful tool for understanding the molecular parameters that control signaling. For example, we used biochemical reconstitution to study actin polymerization downstream of nephrin receptor clustering. In a simplified in vitro reconstitution, we observed that the membrane dwell-time of proteins positively correlated with the amount of actin polymerization. With computational modeling, we predicted that membrane-dwell time could be controlled by changing the stoichiometry of components within clusters, and experiments in the reconstituted system confirmed this prediction. Finally, we designed cell-based experiments to compare the in vitro observations with cellular behavior. Iterating between in vitro reconstitution, computational modeling, and cell-based experiments can be a powerful experimental work flow for understanding complex cellular systems. Future technical innovations in biochemical reconstructions of signaling pathways, such as incorporating more complex membrane compositions or transmembrane proteins, will enable scientists to address new biological questions with this approach.

SG254
Polaris: accurate spot detection for single-molecule FISH images with deep learning and weak supervision

Recent advances in imaging and machine learning have increased our ability to capture information about biological systems in the form of images. Live-cell and spatialomics assays allow us to capture both the “parts list” and spatial and temporal variation in living systems. Therefore, images have the
potential to be a universal data type for biology. A common and challenging computational task required for the analysis of images from a number of live-cell and end-point imaging assays is fluorescent spot detection. This problem is challenging to solve with supervised learning methods because the notion of ground truth is ambiguous - most images contain too many spots for humans to manually curate. Moreover, expert human annotators disagree significantly on the number and location of spots in images. In this talk, we present Polaris, a weakly supervised approach to spot detection. Rather than manually annotating each spot, humans fine tune a collection of classical spot detection algorithms on a set of images to create a set of annotations. We then perform generative modeling to create a consensus annotation set which is then used to train a deep learning model for spot detection. We show that when trained in this fashion, our deep learning model has spot detection capabilities that generalize to image sets from a wide range of assays. When paired with our deep learning-based methods for cell segmentation and tracking, this spot detection method can be applied to the analysis of a number of live cell reporters and end-point spatial-omics assays. To improve accessibility, we have developed an image analysis pipeline for singleplex and multiplex RNA FISH image sets. Furthermore, we demonstrate the ability of our spot detection method to quantify dynamics of phase separating live cell reporters and lipid droplet formation. These advancements improve our ability to capture biological information with increased speed and accuracy from images produced by a number of different assays.

SG255

Mapping cell structure across scales by fusing protein images and interactions

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The cell is a multi-scale structure with modular organization across at least four orders of magnitude. Two central approaches for mapping this structure - protein fluorescent imaging and protein biophysical association - each generate extensive datasets, but of distinct qualities and resolutions that are typically treated separately. Here, we integrate immunofluorescence images in the Human Protein Atlas with affinity purification experiments from the BioPlex resource to create a unified hierarchical map of eukaryotic cell architecture. Integration is achieved by configuring each approach to produce a general measure of protein distance, then calibrating the two measures using machine learning. The map, called the Multi-Scale Integrated Cell (MuSIC 1.0), currently resolves 69 subcellular systems of which approximately half are undocumented. Based on these findings we perform 134 additional affinity purifications, validating close subunit associations for the majority of systems. The map reveals ribosome biogenesis components, including a novel pre-ribosomal RNA processing assembly and numerous accessory factors which we show govern rRNA maturation. The map also elucidates roles for SRRM1 and FAM120C in chromatin and for RPS3A in splicing. By integration across scales, MuSIC substantially increases the mapping resolution obtained from imaging while giving protein interactions a spatial dimension, paving the way to incorporate diverse types of molecular data to create proteome-wide cell maps.

SG256

Organelle topology is a new breast cancer cell classifier in 2D and 3D cultured systems: classification of 3D rendered organelle objects using high resolution imaging & machine learning

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Breast cancer is a highly heterogenous disease, both phenotypically and genetically, and a correlation between intra-tumoral heterogeneity, drug resistance and negative clinical outcome has been established. Quantity or subcellular location of protein biomarkers have been compared to identify tumor tissue types, and cell morphology has been indicated as a direct readout of the functional phenotypic state of an individual cancer cell. Here, the hypothesis was proposed that the spatial context of organelles, specifically their subcellular location and inter-organelle relationships (topology), can be used to inform breast cancer cell classification. Since numerous correlations between biologic behaviors and pathologic findings have been well-established, organelle topological heterogeneity reveals the long-term adaptation of organelles and cytoskeletal networks to match breast cancer cell type status. Thus, Organelle Topology-based Cell Classification Pipeline was introduced as a novel approach that quantifies, for the first time, the topological features of subcellular organelles, removing the bias of visual interpretation, to classify different breast cancer cell lines using a machine learning method, which aims to investigate the heterogeneity of organelle topology and morphology in breast cancer cells to increase the understanding of cancer biology on a subcellular level. It was tested on three different organelle datasets in 2D and 3D culture systems: mitochondria, early endosomes and recycling endosomes in a panel of human breast cancer cells, including T47D (estrogen receptor-positive), MDAMB231, MDAMB436 and MDAMB468 (triple negative), AU565 (HER2 positive), and non-cancerous MCF10A cells. High resolution Airyscan microscopy was used to collect z-stacks across cells labeled with fluorescently labeled-transferrin uptake (ERC) and immunostained with anti-Tom20 (Mito) and anti-EEA1 (EEC) to label the recycling and early endosomal and mitochondria networks, respectively. Subsequently, 3D rendering of organelle objects was performed using IMARIs software. A morphometric evaluation of organelles resulted in 34 topology and morphology parameters. We systematically evaluated how different parameter combinations affected the Random Forest machine learning-based cancer cell classification and discovered that topology parameters were crucial to achieve the highest classification accuracy such as 97.1% in 2D cells and 93.8% in 3D spheroids, indicating that organelle topology is a novel, highly precise classifier to differentiate cell lines of differing subtype. These findings lay the groundwork for using quantitative topological organelle features as an effective method to analyze and classify breast cancer cell phenotypes.

SG257
Characterizing the subnuclear micro-environments of genes by multi-modal data integration
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Recent advances in microscopy and sequencing-based genomics technologies have provided an opportunity to produce quantitative models of whole genome structures to advance our understanding of the causal connection between chromatin structure and function. However, it remains a major challenge to systematically integrate multi-modal data from different technologies. Here, we introduce a population-based modeling method for data integration to produce highly predictive genome structures from a variety of data sources. The resulting models accurately predict the subnuclear positions of genes, nuclear bodies, local chromatin compaction, and preferences in chromatin compartmentalization on a genome-wide scale. Therefore, these models reveal a gene’s subnuclear microenvironment, as well as its variability between cells, which in turn correlates with its functional potential for gene expression and DNA replication timing. Moreover, quantitative assessment of genome structures generated from different data combinations shows that multi-modal data integration can compensate for systematic
errors in some of the data sources and thus, greatly increases accuracy and coverage of genome structure models.

**Staging of the Centrosome-Cilium Complex for Inside-Outside Cellular Signaling**

SG258

**Centriolar satellites act as transit sites for regulators cilium biogenesis and ciliary signaling**

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Centriolar satellites are membrane-less granules that localize and move around centrosomes. Their assembly is scaffolded by PCM1, which interacts with a diverse array of proteins implicated in the biogenesis and function of centrosomes/cilia and linked to developmental disorders. To uncover their disease mechanisms and relationship to centrosomes and cilia, we have combined proximity proteomics, loss-of-function experiments and high resolution imaging and defined their functions and mechanisms in mammalian cell lines. Phenotypic characterization of cells chronically depleted for centriolar satellites by genome editing or acutely inhibited for satellite activity by inducible trafficking assay revealed satellites as regulators of ciliogenesis and ciliary signaling. So far, we identified two mechanisms by which centriolar satellites regulate cilium assembly. First, they are required for targeting IFT-B complex components to the basal body and the cilium. Second, they inhibit Aurora Kinase A (AURKA)-induced cilium disassembly in quiescent cells by regulating cellular and basal body abundance and activation of AURKA. These results suggest that centriolar satellites function as transit sites where centrosome proteins are assembled, modified, stored and/or trafficked. In line with their trafficking and sequestration functions, systematic quantitation of satellite dynamics showed that they display both microtubule-based bimodal and diffusive motility. Moreover, photoactivation experiments showed exchange of proteins between centrosomes and centriolar satellite granules. Together, these results unveiled the mechanisms by which centriolar satellites spatiotemporally regulate cilium biogenesis and functions. Future studies are required to determine how they sense extracellular stimuli and respond.

SG259

**Using Cryo-ET to take an In Situ look at the Ciliary Base and Transition Zone, revealing native TZ structure and IFT train assembly in Chlamydomonas reinhardtii**

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Thanks to its superb native contrast and textbook organelle architecture, the unicellular green alga *Chlamydomonas reinhardtii* is an ideal specimen for use in cryo-electron tomography (cryo-ET). We use focused ion beam (FIB) milling of frozen cells followed by state-of-the-art cryo-ET in order to image macromolecules with nanometer-precision localization, and perform high-resolution structural analysis, all within the native cellular environment. In this presentation, I will show the results of a selection of these studies. I will talk about cilia-related structures, charting the macromolecular landscape of the ciliary base and transition zone in *Chlamydomonas*. With the help of the labs of Prof. Dr. Gaia Pigino and Prof. Dr. Paul Guichard, we were able to investigate IFT train assembly, and create averages of assembling intraflagellar transport train particles, as well as of structural components of the transition
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zone. We used these to create a comprehensive model of the ciliary base and transition zone, shedding some light on how the recruitment and transport of ciliary components to- and from cilia works.

SG260

Revealing membrane architecture directing the assembly of the primary cilium

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Ciliogenesis requires a highly complex yet poorly understood sequence of membrane and cytoskeletal axoneme assembly processes. In cells that build the cilium partially in the cytoplasm, the earliest steps of ciliogenesis involves the docking of preciliary membrane vesicles (PCV) to distal appendages on the mother centriole (MC). PCVs are thought to fuse to form a larger ciliary vesicle (CV) that covers the distal end of the MC. The CV subsequently seeds the ciliary membrane, that surrounds the axoneme, and the ciliary pocket membrane, which connects the cilium to the plasma membrane. The formation of the CV is associated with removal of CP110/CEP97/MPP9 (MC cap) from the MC distal end required before the axoneme can develop, and also with the recruitment of transition zone (TZ) proteins that function to control ciliary transport at the base of the cilium. Here, using correlative light-electron microscopy (CLEM) approaches with super-resolution fluorescence and volume-based focused ion beam scanning electron microscopy (FIB-SEM), we investigated the 3D structures of membranes from the PCV to CV stages. We observed PCVs docked preferentially to one side of the MC and discovered two uncharacterized tubulovesicle membrane structures that we concluded are intermediates of the PCV and CV stages. We predicted that traditional 2D ultrastructure imaging could misidentify these newly discovered stages as PCVs and/or the CV. Consistent with this idea, FIB-SEM analysis of cells depleted of the membrane trafficking regulators Rab8a and Rab8b revealed a pre-CV ciliogenesis dysfunction which is an earlier requirement than was previously reported from 2D ultrastructures. We also investigated ciliogenesis in EHD1 depleted cells by FIB-SEM and confirmed this membrane shaping protein functions at the PCV stage, supporting a role in assembling tubulovesicle membranes at the MC distal end. Because EHD1 is also required to uncap the MC we next investigated the effects of membrane structure on the removal of CP110 and CEP97. We demonstrated that loss of the MC cap correlates with pre-CV membrane structures and identify a role for EHD1 membrane organizing function in this process. Finally, we demonstrate the TZ proteins B9D2, RPRGIP, and the predicted integral transmembrane protein TMEM67 also accumulates asymmetrically on the MC during ciliogenesis suggesting the TZ participates in establishing pre-CV membrane structures. Together our findings demonstrate requirements for membrane architecture in directing early stages of ciliogenesis, and provides insight into how pre-CV membrane organization could help establish the ciliary membrane and ciliary pocket membrane.

SG261

Regulation of Primary Ciliogenesis by C-terminal Eps15 Homology Domain Proteins

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Primary ciliogenesis is a closely regulated process that is key for signal transduction processes, including that of sonic hedgehog signaling, and a variety of factors influence the generation and maintenance of primary cilia. In recent years, many proteins involved in the regulation of endocytic trafficking have also been implicated in primary ciliogenesis. In particular, the small GTP-binding proteins Rab8 and Rab11
regulate ciliogenesis, and mutation, depletion, or loss of function of these Rab proteins disrupts the generation of primary cilia. More recently, the endocytic protein Eps15 Homology Domain Protein 1 (EHD1) was identified as a key regulator of primary ciliogenesis, in addition to its well-defined roles in the regulation of endocytic recycling and endosomal fission. EHD1 belongs to a family of C-terminal EHD proteins which has four human orthologs: EHD1, EHD2, EHD3 and EHD4. Thus far, EHD1 and EHD3 have been shown to play important roles in primary ciliogenesis. In the process of primary cilia generation, EHD1 coordinates with SNARE protein SNAP29 to remove centriolar capping protein CP110 in a poorly understood manner, thus facilitating the fusion of distal appendage vesicles (DAVs) into a larger ciliary vesicle (CV). Formation of the CV is required for basal body docking at the plasma membrane, which is followed by fusion of the CV with the membrane and subsequent axoneme extension, thus generating a functional primary cilium. Without CP110 removal and the resulting fusion of DAVs into a CV, the primary cilium fails to assemble, impairing signal transduction and leading to a variety of disease states known as ciliopathies. As such, with both EHD1 and EHD3 characterized as key regulators of primary ciliogenesis, we hypothesized that the EHD2 and EHD4 orthologs may also regulate ciliogenesis. Herein, we demonstrate that EHD4, but not EHD2, regulates ciliogenesis by facilitating the removal of CP110. Moreover, we determine that EHD4 is recruited to primary cilia independently of EHD1, as siRNA knock-down of EHD1 does not impede EHD4 recruitment. Furthermore, reintroduction of wild-type EHD1 in CRISPR/Cas9 gene-edited EHD1 knock-out cells rescues ciliogenesis to normal levels, while reintroduction of EHD1 mutants that are unable to bind ATP or interact with binding partners through its EH-domain fail to rescue ciliogenesis. Overall, these data support a novel role for EHD4 in ciliogenesis, and further define the mechanistic role of EHD1 in the process by demonstrating a requirement for ATPase function and ligand binding.

SG262
Understanding Transducer Immobilization in Signaling Through the Primary Cilium
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In living organisms information flows through cells by molecular relay. The Hedgehog (Hh) signaling pathway is a molecular relay that orchestrates the formation of organs in vertebrate embryonic development. In response to the Hh morphogen, a GPCR-related protein, Smoothened (Smo) localizes to the primary cilium, an organelle specialized for cellular signaling. Several molecular relay steps proceed through the cilium ending with transcription of Hh target genes in the nucleus. The activation of the key transducer Smo and its translocation to the ciliary membrane are essential to downstream molecular relay events. The accumulation of Smo in the ciliary membrane depends on the transition zone, a super-assembly of multiple proteins at the ciliary base that functions as a molecular gateway to the ciliary membrane. The mode of movement of Smo and its dependence on the transition zone remain unclear. To understand the dynamics of Smo movement in the ciliary membrane we have performed live imaging and fluorescence recovery experiments. We have measured the mobility of Smo in the ciliary membrane in wild-type mouse embryonic fibroblasts (MEFs), as well as in cilia where the transition zone is disrupted by genetic deletion of Tmem231. In transition zone mutants Smo accumulates to low levels and downstream transcription of Hh target genes is reduced compared to wild-type cells. We predicted that the disruption to signal transduction in transition zone mutants would be associated with a change in the dynamic movement of Smo in the ciliary membrane. In wild-type cilia we have observed that Smo becomes immobilized when it accumulates to a high concentration in the ciliary membrane. We have
found that immobilization of Smo depends on an intact transition zone; in Tmem231<sup>−/−</sup> cells Smo does not reach a high concentration in the primary cilium and does not become immobilized. These findings suggest that Smo immobilization enhances Hh signal transduction, and that the transition zone controls accumulation of Smo at the cilium. We are currently investigating how Smo becomes immobilized in the ciliary membrane, the role of the transition zone in this process and the implication of Smo immobilization on downstream Hh signal transduction.

SG263

**Interactions Between Tulp3 Tubby Domain Cargo Site and Arl13b Amphipathic Helix Promote Lipidated Protein Transport to Cilia**

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The membrane of the primary cilium has a distinct protein and lipid composition, despite being contiguous with the rest of the plasma membrane. To understand the role of cilium-generated signaling in homeostasis, it is necessary to decipher the mechanisms that drive such unique compartmentalization. We previously showed that the tubby family protein, TULP3, transports integral membrane proteins into cilia through binding with the intraflagellar transport complex-A (IFT-A) and phosphoinositides. These transmembrane cargoes have short motifs that are necessary and sufficient for TULP3-mediated trafficking. However, the mechanisms underlying ciliary compartmentalization of non-integral, membrane-associated proteins are poorly understood. Here, using Tulp3 knockout mouse embryonic fibroblasts and cell lines, we show that TULP3 is required for ciliary transport of ARL13B, a palmitoylated atypical GTPase, that is highly enriched in cilia. By promoting ARL13B trafficking, TULP3 also determines ciliary enrichment of ARL13B-dependent lipidated cargoes, including farnesylated INPP5E, and myristoylated NPHP3 and CYS1. Trafficking of transmembrane cargoes, such as G-protein coupled receptors, by TULP3 requires both IFT-A and phosphoinositide binding. In contrast, we found that lipidated protein trafficking to cilia required TULP3 binding to IFT-A but not to phosphoinositides, reflective of increased affinity of interactions between TULP3 and ARL13B, compared to transmembrane cargoes. Furthermore, we used proximity biotinylation followed by mass spectrometry, and domain mapping studies to determine precise TULP3-ARL13B interaction sites. We identified a conserved lysine in TULP3’s tubby domain that mediated direct ARL13B binding and promoted ciliary trafficking of both lipidated and transmembrane cargoes. Correspondingly, an N-terminal amphipathic helix preceding the GTPase domain and flanking the palmitoylation site mediated direct ARL13B binding to TULP3. This helix was required for ciliary trafficking of ARL13B irrespective of palmitoylation and RVxF sorting motifs, both of which were previously implicated in ciliary trafficking. These findings indicate an expanded role of a shared tubby domain site in TULP3 that captures short sequences of diverse cargoes to directly mediate their transport into cilia.
SG264

Deciphering the Ciliary Extracellular Vesicle (EV) Proteome

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The cilium acts as an antenna receiving and sending signals, the latter via ciliary EVs that bud from the ciliary membrane. Ciliary EV shedding is evolutionarily conserved. Disrupted ciliary EV signaling is likely an important and yet to be recognized driver of the pathophysiology of many ciliopathies, such as polycystic kidney disease, retinal degeneration, obesity, skeletal malformations, defects in organ placement, and related disorders. While cilia and EVs are of profound medical importance, the field lacks a basic understanding of how EVs form, what cargo is packaged in different types of EVs originating from different cell types, and how different cargoes influence the range of EV bioactivities. Methods: Here we used evolutionarily conserved polycystin-2 PKD-2::GFP EV cargo of Caenorhabditis elegans as a marker of ciliary EVs in fractions resolved by the buoyant density centrifugation. Most enriched fractions were analyzed by transmission electron microscopy to confirm vesicle presence and by mass spectrometry to identify animal EV proteome. Top candidates were experimentally confirmed using genetically-encoded fluorescent protein reporters and visualizing using Airyscan super-resolution imaging in living animals. Results: We discovered that ciliary EVs carried nucleic acid binding proteins (dsRNA transporter SID-2, replication licensing factor MCM-3, ectonucleotide pyrophosphate phosphodiesterase ENPP-1) and other conserved proteins of EV biogenesis, such as TSP-6 (homolog of human tetraspanin CD9) and KLP-6 (homolog of human KIF13B). Conclusion: Our findings indicate that cilia produce multiple EVs with distinct protein content and that EVs from different ciliated neuronal types carry different EV cargo. Discovery of the RNA transporter as a cargo of ciliary EVs suggests that cilia possess molecular machinery for extracellular transport of RNA and thus might participate in horizontal transfer of genetic information and epigenetic regulation of gene expression. Overall, our work establishes C. elegans as a discovery platform for animal EV biology. We envision that our dataset can be exploited and used as an experimental springboard in three different ways: (i) identification of conserved and species-specific drivers of ciliary EV biogenesis, (ii) discovery of novel bioactivities that have not been associated with extracellular vesicles, (iii) in combination with other identified EV proteomes, understanding of evolutional specialization of EV-based communication.

SG265

The ciliary necklace is involved in localization of mating signals in Chlamydomonas

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The ciliary necklace is a collection of highly organized intramembranous proteins (IMPs) in the proximal ciliary membrane that surrounds the transition zone. Numerous groups have suggested a role in establishing the ciliary diffusion barrier or in retention of various proteins. We used quick-freeze, deep-etch electron microscopy to visualize the Chlamydomonas IMPs. Several ift mutants (ift52, ift81, ift121, ift139, ift144) exhibit reduced and disorganized IMPs. A cytoplasmic dynein mutant (fla24-1) has reduced but organized IMPs. Four Chlamydomonas transition zone mutants (nph4, cep290, tmem67, and rpg1) assemble disorganized ciliary necklaces with fewer IMPs. Connections between the microtubule axoneme and the ciliary membrane known as the Y-linkers remain partially intact in all of the mutants, which support the idea that Y-linkers are complicated structures that are not lost in any of
the single mutants. *Chlamydomonas* has a second feature in the ciliary membrane that is called the ciliary bracelet, which is present in all of the mutants tested. However, in each of the four independent *rpg1* alleles (*RPGRIP1L* homolog), the ciliary bracelet is duplicated and appears as two rings rather than the one ring observed in wild-type cells or other mutants. SAG1 is a membrane protein that is needed for successful recognition during mating in *Chlamydomonas*. The membrane-bound C-terminal fragment of SAG1 fails to localize to cilia of gametic *rpg1* cells. This suggests that ciliary necklace may be needed for its localization in the cilia. The other transition zone mutants are being tested for SAG localization. We will ask if the localization defect is involves its transport, retention, or placement in ectosomes, since SAG1 is released from the cilia in ectosomes.

SG266

**Ciliary Signaling Mechanisms directing mesenchymal stem cell fate**

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Adult stem and progenitor cells need to respond to a broad range of tissue-specific ligands to enable tissue regeneration and expansion. Failure of adult stem cells to appropriately respond can lead to metabolic and aging-related diseases. For most adult tissues, the identity of these ligands and the mechanisms through which they direct cell fate are poorly understood. All mesenchymal stem and progenitor cells are uniformly ciliated, and the clinical features of some ciliopathies include defects in mesenchymal tissues, such as obesity, diabetes, hypotonia, and skeletal dysplasia. We showed that genetic ablation of ciliation in pre-adipocytes impairs adipogenesis *in vitro* and *in vivo*, and results in a failure of white adipose tissue to expand. We further showed that pre-adipocyte primary cilia express the omega-3 fatty acid receptor FFAR4, and activation of ciliary FFAR4 by omega-3 fatty acids such as DHA (docosahexaenoic acid) triggers adipogenesis *in vitro* and activates pre-adipocytes *in vivo* to proliferate and differentiate. Non-FFAR4 ligands, including saturated fatty acids such as palm oil, fail to trigger adipogenesis *in vitro* and *in vivo*. DHA promotes adipogenesis by causing a localized increase in ciliary cAMP levels, activating the cAMP effector protein EPAC, and promoting the looping of chromatin enhancers to promoter sites of adipogenic genes in a CTCF-dependent manner to initiate the adipogenic transcriptional cascade. Thus, we have linked a physiological ligand, the nutritional flux of a subset of dietary fatty acids, to the activation of the ciliary second messenger cAMP to direct pre-adipocyte cell fate by promoting the expression of adipogenic genes. Similarly, we showed that genetic ablation of ciliation in muscle stem cells impairs muscle stem cell proliferation *in vitro* and *in vivo*, and results in a failure of muscle to regenerate in response to injury. We further showed that Hedgehog signaling is required for muscle stem cell proliferation and injury-induced muscle repair. Intriguingly, in aged muscle about half of all muscle stem cells lost ciliation, correlating with the known decrease in regenerative capacity of aged muscle stem cells that contributes to sarcopenia. Exogenous activation of Hedgehog signaling promoted muscle stem cell proliferation *in vitro* and *in vivo*, and restored the regenerative capacity of muscle in aged mice. We propose that the primary cilium is a powerful tool to discover physiologically-relevant signaling pathways that direct adult stem cell fate, and that the study of ciliary signaling pathways in these contexts may provide key insights into a myriad of human diseases.
SG267

Shedding light on the spatial and temporal organization and function of ciliary signaling in health and disease

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The primary cilium constitutes an organelle specialized for signal transduction. It senses and locally transduces environmental information, spatially separated from the cell body. Dysregulation of ciliary signaling results in ciliopathies like polycystic kidney disease. However, how dysregulated ciliary signaling triggers disease development is not well understood. We investigate the role of ciliary cAMP signaling in the development of cyst formation in kidney epithelial cells using a combination of optogenetics and RNA-sequencing. Our data reveal that an increase in ciliary but not somatic cAMP signaling is sufficient to induce cyst formation. We delineate the molecular identity and sequence of events that transduce a change in ciliary cAMP levels into a cellular response and identify a novel concept how the primary cilium controls cellular functions in a specific and spatially distinct manner.

Wnt Signaling in Development and Disease

SG203

Targeting FZD7 signaling in development and disease

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The WNT-FZD signaling pathway is critically involved in a myriad of developmental processes and is frequently de-regulated in a variety of pathologies, including birth defects, neurodegenerative disorders and cancer. With 19 WNT genes and a similar number of WNT receptors, including 10 FZDs, encoded in the mammalian genome, an unresolved question has been how signaling specificity is achieved. Furthermore, this level of complexity provides ample opportunities for specific and selective targeting of this important developmental signaling pathway. We have identified the WNT receptor FZD7 to be highly upregulated in human pluripotent stem (hPS) cells, as well as in a large number of cancers. Using a highly selective antibody to human FZD7 as a starting point, we have developed tools to selectively target and activate WNT/beta-catenin signaling in hPS cells and thereby demonstrated that signaling through FZD7 promotes mesendodermal differentiation. Furthermore, since FZD7 is often overexpressed in cancer cells, we have engineered a FZD7-specific antibody drug conjugate (ADC). This ADC selectively targets and kills FZD7-positive ovarian cancer cells in vitro and in vivo. These experimental strategies demonstrate that selective targeting of WNT receptors can be leveraged in both regenerative medicine to promote differentiation and development of specific cell types and tissues and in oncology to target and kill tumor cells.
SG204

The ubiquitin ligase HUWE1 regulates WNT signaling through a new mechanism
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HUWE1 is a HECT-domain ubiquitin ligase involved in dozens of cellular processes through the ubiquitination of diverse substrates. Both oncogenic and tumor suppressive functions have been ascribed to HUWE1. HUWE1 has been postulated as a negative regulator of WNT signaling through at least two distinct mechanisms: inhibition of DVL activity and degradation of CTNNB1. However, in an unbiased, genome-wide, forward genetic screen in haploid human cells for mediators of hyperactive WNT signaling induced by loss of the CTNNB1 destruction complex kinase CSNK1A1, we previously identified HUWE1 as a positive regulator of the WNT pathway. We also demonstrated that HUWE1 potentiates WNT signaling in cells and Xenopus laevis embryos. Here we show that HUWE1 promotes WNT/CTNNB1 signaling through a mechanism independent of the control of CTNNB1 protein stability. This mechanism requires the ubiquitin ligase activity of HUWE1. Furthermore, a subset of CTNNB1 destruction complex components, including APC, AXIN1 and GSK3 but excluding CSNK1A1 and AXIN2, are required for HUWE1 to potentiate WNT signaling. These results reveal a new role for some destruction complex components in mediating WNT signaling through HUWE1, distinct from their established, concerted activity in controlling CTNNB1 stability.

SG205

The dynamics of the APC-Axin complex determines Wnt/β-catenin pathway’s activity
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Wnt/β-catenin pathway controls crucial cellular functions such as cell proliferation and cell differentiation. The dysregulation of Wnt/β-catenin pathway often results in severe diseases like cancer. A hypothetical “destruction complex” is at the core of the Wnt pathway, composed of several proteins, such as scaffold protein APC, Axin, and kinase GSK3. However, the “destruction complex’s” activity control remains an open question. In this study, we provide direct observation of the dynamics of the “destruction complex” during Wnt pathway activation, down to the single-molecule level. We found that a fraction of Axin1 is on the plasma membrane without Wnt ligands, which dissociates from the plasma membrane after Wnt pathway activation. This observation is surprising because it contradicts the widely accepted view that the Axin1-APC complex is recruited to the plasma membrane after Wnt activation. Additionally, we found Axin1 membrane localization is primarily dependent on APC and reflects the binding to membrane-bound APC proteins. We believe the dissociation of the APC-Axin1 complex is the control step of the Wnt pathway activation, which in turn determines the GSK3’s activity on β-catenin.

SG206

Bcl-2 up-regulation mediates taxane resistance downstream of APC loss
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Patients with triple negative breast cancer (TNBC) are treated with traditional chemotherapy, such as paclitaxel (PTX). Despite the efficacy of taxanes, many tumors will recur due to drug resistance.
Therefore, the need to understand the mechanism behind drug resistance is critical to improve patient outcome and survival. Our lab was the first to show that loss of the Adenomatous Polyposis Coli (APC) tumor suppressor caused resistance to PTX in the MDA-MB-157 human TNBC cell line. In the absence of APC, apoptosis induction was decreased, as measured through cleaved caspase 3 and annexin/PI staining. To understand the molecular mechanisms behind APC-mediated PTX response, we analyzed the BCL-2 family of proteins and found a robust increase of the pro-survival family member, Bcl-2. The BH3 mimetic, ABT-199 (Venetoclax), which specifically targets Bcl-2, has been used as a single or combination therapy in multiple hematologic malignancies. In addition, ABT-199 has shown promise in multiple subtypes of breast cancer. Therefore, we used ABT-199 in combination with PTX to address the hypothesis that APC-induced Bcl-2 increase is responsible for PTX resistance. Combination treatment in three CRISPR-mediated APC knockout TNBC cell lines (MDA-MB-157, MDA-MB-231, and SUM159) caused changes in cell proliferation and apoptosis. Combined, these data suggest restored sensitivity to PTX using ABT-199. Our studies are the first to show that Bcl-2 inhibition can restore PTX-sensitivity in APC mutant breast cancer cells. These studies are critical to advance better treatment regimens in patients with TNBC.

SG207
Cross-talk between Non-canonical Wnt and Transforming growth factor beta pathways control the epithelial-mesenchymal transition and cell migration
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The epithelial-mesenchymal transition (EMT) is a central mechanism for diversifying the cells found in complex tissues. During EMT, epithelial cells adopt mesenchymal properties by altering their morphology, cellular architecture, adhesion, and migratory capacity. This dynamic process helps organize the formation of the body plan, and EMT is well studied in the context of embryonic development including mesoderm and neural crest formation. Previously, we discovered a new non-canonical Wnt5-Frizzled 2 (Fzd2) pathway that drives EMT and mediates cell migration. Our follow-up work uncovered that EMT depends on co-operation between two developmentally important pathways - Wnt5/Fzd2 and Transforming growth factor beta (TGFβ). We found that Fzd2-driven EMT is associated with upregulation of TGFβ receptor levels and subsequent activation of TGFβ pathway. Similarly, TGFβ-driven EMT is accompanied by increased expression of Wnt5a and Fzd2. Together with our observations that expression of Fzd2 turns mesenchymal cells hyper-sensitive to TGFβ stimulation, whereas Fzd2 inhibition activates a novel compensatory response that also ensures sustained TGFβ signaling, this suggests a complex co-regulatory interaction between these two evolutionary conserved pathways. Importantly, inhibition of both Fzd2 and TGFβ pathway completely abrogates the key feature of EMT, the mesenchymal cell migration. Thus, we propose that EMT is driven by a robust co-regulatory mechanism that depends on both Fzd2 and TGFβ signaling to ensure a complete transdifferentiation.

SG208
Wnt5a-ror regulates cell migration and contractility via rhoa-myosin-actin (rma) axis
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The Wnt5a-Ror pathway is a highly conserved developmental signaling pathway that regulates morphogenetic processes in organisms from cnidarians to vertebrates. The pathway functions independently of the canonical Wnt/β-catenin pathway to control cell migration and other cytoskeleton-
dependent processes during embryonic development. Despite this, little is known about how Wnt5a-Ror signaling functions at the cellular level to exert its biological effects. Through a combination of pharmacological and genetic approaches, we experimentally elevated or diminished Wnt5a-Ror activity in immortalized mouse embryonic fibroblasts (iMEFs). Interestingly, we observed that in a 2D environment, elevated Wnt5a-Ror signaling suppresses migration, whereas, in a 3D environment, it promotes migration. Based on previous literature on the role of contractility in cell migration (Liu et al, Br J Pharmacol 2010, Even-Ram et al, Nat Cell Biol, 2007), we hypothesized that the contrasting effect of Wnt5a-Ror signaling in 2D and 3D migration might be explained by changes in cellular contractility. Indeed, we observed that increased Wnt5a-Ror activity not only increased cellular contractility in iMEFs, but also resulted in enhanced stress fiber formation and mature focal adhesions. Importantly, we further established that Wnt5a-Ror signaling induces changes in several key regulators of the cell contractility, including altered subcellular localization of RhoA which elevated activity of its downstream target Rho-associated protein kinase (ROCK) and subsequently increased phosphorylation of non-muscle myosin light chain (MLC) at Thr18/Ser19, a major molecular switch that controls contractility. Collectively, these data suggest that a major cellular function of the Wnt5a-Ror pathway is to activate the “RhoA-Myosin-Actin” (RMA) axis, which in turn controls cell contractility and focal adhesion formation to modulate migratory cell behavior.

SG209

**Wnt11 family dependent morphogenesis during frog gastrulation is marked by the cleavage furrow protein anillin**

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Wnt11 family proteins are activating ligands for dishevelled-mediated non-canonical Wnt signaling. Loss of their function causes defects in gastrulation and/or anterior-posterior axis extension in all vertebrates. Non-mammalian vertebrate genomes encode two Wnt11 family proteins whose distinct functions have been unclear. We knocked down zygotic Wnt11b and Wnt11, separately and together, in *Xenopus laevis*. Wnt11b and Wnt11 single morphants exhibited similar delayed blastopore closure but different phenotypes during the tailbud period. We further characterized the dual morphant by time-lapse imaging and whole embryo immunofluorescence. Wnt11 family dual morphant embryos exhibit slowed blastopore closure, failure to internalize the endoderm at the dorsal blastopore lip, and disrupted archenteron extension. The cleavage furrow protein anillin provided an excellent cytological marker for maturation of the blastopore, revealing multiple steps. The membrane proximal localization of anillin during blastopore groove formation and archenteron extension suggests a function in regulating contractility and/or stiffness in epithelial cells undergoing dramatic shape changes. By using intact embryos, rather than Keller or animal cap explants, we revealed a likely role of anillin in morphogenesis of specific organizational states of the embryo epithelium while filling a knowledge gap in Wnt11 family biology.
Aa`aSG210

**Wnt/planar cell polarity signaling contributes to glioblastoma multiforme invasiveness**

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Glioblastoma multiforme (GBM) is the most common form of malignant brain cancer, and is highly aggressive, recurrent, and difficult to treat. The highly infiltrative nature of GBM cells diminishes the clinical efficacy of surgery, and while the prospect of more effective patient-tailored therapies has been explored, such strategies have thus far failed because these tumors are inherently highly resistant. Wnt/Planar Cell Polarity (Wnt/PCP) is a non-canonical Wnt signaling pathway that interprets global directional cues to produce locally polarized cell behavior, leading to increased cell motility and invasiveness. Wnt/PCP is critical for embryonic developmental processes, where it modulates cell adhesion and migration. The emerging role for Wnt/PCP signaling in tumor malignancy highlights the recurring theme that tumors reactivate developmental programs to promote their aggressive behaviors. Based on previous studies and preliminary observations, we hypothesize that GBM tumors engage Wnt/PCP pathway components to promote invasiveness and therapeutic resistance, underscoring the notion that a deeper understanding of Wnt/PCP in GBM could uncover novel therapeutic approaches.

To characterize Wnt/PCP components in cell motility, we used _in vitro, ex vivo, and in vivo_ assays to elucidate the effect of Wnt/PCP perturbation on GBM cell migration and invasion. Because it is unclear mechanistically how cell motility is regulated by Wnt/PCP components, pathway activation and signaling was assessed by western blot and live imaging techniques utilizing LifeAct-GFP to examine filamentous actin, and Rac1 and RhoA biosensors to assess downstream GTPase activation. Our studies show that pathway components Wnt5a, Fzd7 and Vangl1 significantly contribute to GBM motility and invasiveness, and suggest a model whereby Wnt/PCP signals through Rho family GTPases to direct the formation of actin-rich protrusions at the leading edge of migratory GBM cells. Collectively, our observations highlight the importance of Wnt/PCP in promoting GBM malignancy, and suggest that interference with pathway signaling may offer an avenue toward the development of novel and more effective GBM therapeutic strategies and agents.