An ASCB | EMBO Meeting

CELL BIO 23

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# Abstracts: Oral Presentations

# *AWARDS*

## Keith R. Porter Lecture

A1

**Dynamic interplay between force and membrane geometry during actin-mediated vesicle formation**

**D. G. Drubin**; University of California, Berkeley, CA

Clathrin-mediated endocytosis is a critical process by which cells take up nutrients, control serum cholesterol levels and regulate cell signaling. It is now appreciated that from budding yeast to human cells, a burst of actin assembly generates forces to assist plasma membrane invagination during clathrin-coated vesicle formation. The studies presented address mechanisms by which mechanical forces from actin assembly are harnessed for efficient vesicle formation. These studies use both budding yeast and human cells to investigate force production, load adaptation and feedback between membrane geometry and biochemical reaction rates. Reconstitution experiments in budding yeast demonstrate that actin assembly on the surface of a lipid bilayer is sufficient to drive vesicle formation, suggesting that membrane-associated actin assembly might represent an ancient vesicle-forming mechanism. Yeast genetics have identified the core actin associated proteins required for efficient vesicle formation, and a combination of genetics, biochemistry and mathematical modeling are revealing the biophysical mechanisms for force generation and harnessing. Other studies in budding yeast indicate that cargo controls the rate of vesicle formation, while studies in mammalian cells indicate the membrane geometry plays a key role in controlling biochemical reaction rates. Feedback from membrane geometry, cargo, lipid composition and physical load are key parameters dictating progress toward vesicle formation.

## E. B. Wilson Medal Presentation and Address

A2

**The Genome in Space and Time.**

**T. Misteli**; National Cancer Institute, Bethesda, MD

The genome is arguably the major functional component of every organism. Inside each cell, the hereditary information encoded in the DNA's one-dimensional sequence assumes complex three-dimensional architecture. Over the past two decades, the confluence of genetics, biochemistry, biophysics, and cell biology has uncovered some of the fundamental principles and molecular mechanisms that shape the organization of genomes in space and time. The most prominent cell biological features of genomes are the existence of chromatin loops and domains, the non-random positioning of genomic loci, the presence of sub-nuclear compartments, and the highly dynamic nature of genome architecture. Based on genome-wide mapping and single-cell analysis, recent findings also suggest that genomes are self-organizing systems driven by the interplay of structure and function with a high degree of inherent stochasticity. Disruption of spatial genome architecture and nuclear organization is associated with many disease states, including developmental disorders, cancer, and aging. The exploration of basic principles of genome architecture in the context of disease has given novel insight into fundamental mechanisms of genome function and has the potential to lead to novel diagnostic and therapeutic applications in a variety of diseases.

# *SYMPOSIA*

## Symposium: Communication

S1

**Programming Multicellular Pattern Formation with Synthetic Cell-cell Signaling.**

**S. Toda**; Kanazawa University, Kanazawa, JAPAN.

In developing embryos, cells communicate with each other using various molecules to control cell behaviors and organize complex tissue patterns. Tissue patterns emerge as a collaboration between cell-cell signaling and responsive cell behaviors, but the mechanisms through which signaling molecules define precise spatial patterns under biological fluctuations remain unclear. To investigate a logic of how interacting cells generate tissue patterns, we have been developing an in vitro platform to design new cell-cell communication rules there and test multicellular behaviors. Recently, we have converted a fluorescent protein GFP into an intercellular signaling molecule and designed cell-cell communication based on the secretion and reception of GFP. Using this system, we tested how secreted proteins can generate and regulate multicellular patterns and revealed the important interplay between signal diffusion and cell adhesion. In this talk, I will introduce our synthetic biology approach to engineer cell-cell communication and discuss the design principles of tissue patterning.

S2

**Dissecting cargo receptor-mediated membrane protein trafficking in health and disease.**

**A. Greka**; Harvard Medical School, Cambridge, MA.

No Abstract Submitted

S3

**Neurobiology and Evolution of Learned Vocal Communication and Spoken Language.**

**E. Jarvis**; the Rockefeller University, New York, NY.

I believe understanding the evolution of language is a window into understanding the brain's mechanisms that control highly complex behaviors. A challenge for achieving this goal is that language has been considered unique to humans, making it difficult to study. However, the last several decades have seen a surge in non-human animal studies that inform us about human language. I will present a modern synthesis of these studies, from behavioral, circuit, physiological, to molecular levels. Key new concepts include that components of language are continuous among species, that the vocal learning component is the most specialized and rarest, and the brain pathway for spoken language evolved by brain pathway duplication from an ancient motor learning pathway. These concepts have important implications for understanding brain mechanisms and disorders of language

## Symposium: Migration

S4

**Microenvironment Regulation of Metastasis.**

**K. Tanner**; Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health

In the event of metastatic disease, emergence of a lesion can occur at varying intervals from diagnosis and in some cases following successful treatment of the primary tumor. Genetic factors that drive metastatic progression have been identified, such as those involved in cell adhesion, signaling, extravasation and metabolism. However, organ specific biophysical cues may be a potent contributor to the establishment of these secondary lesions. We combine a novel preclinical model of metastasis with a suite of biophysical tools to elucidate the role of tissue biophysical properties of in the establishment of metastatic lesions in vivo. Specifically, I will discuss our efforts to determine what physical cues influence disseminated tumor cells in different organ microenvironments using in vitro and in vivo preclinical models such as 3D culture systems and zebrafish.

S5

**Making a Migratory Monarch: Defining the Behavioral and Physiological Transitions of Monarch Migration.**

**D. A. Green**; University of Michigan, Ann Arbor, MI.

Long distance migrations over continental or global scalesreveal the collective action of up to billions of individuals who completejourneys with precision and regularity. Similar to migrating cells withintissues or metastatic cancer cells, migrating organisms transition betweencertain states that are characterized by quite different behavioral,physiological, and metabolic demands. We use the monarch butterfly migration asa model system to understand how individual migrants transition between thesestates, towards an ultimate goal of detailing how this iconic collectivemigration is achieved. Physiological and gene expression analyses reveal thedependence of different individual states on early life environmental context.Using a modified flight simulator and novel analysis platform, we partitionmigratory behavior into component parts and identify critical behavioraltransitions that monarchs undergo during their migration. We arecollaboratively developing technology that will allow us to measure behavioraltransitions along the migratory flight. Altogether, this research moves uscloser to better understanding migration states, including how they aregenetically (and, ultimately, molecularly) programmed, how transitions betweenstates are accomplished, and how environmental factors control thesetransitions.

S6

**Supracellular Contractility of Cancer-associated Fibroblasts in Tumor Progression.**

**D. Matic Vignjevic**; Institut Curie, Paris, FRANCE.

The tumor microenvironment plays an important role in tumor progression. It is made of extracellular matrix (ECM), blood vessels, immune cells and cancer-associated fibroblasts (CAFs). Besides biochemical signals, mechanical forces from the microenvironment also play a role in tumor progression. CAFs have enhanced contractility and capacity to synthesize, deposit and crosslink ECM making stroma stiffer. By

accumulating around the tumor, they generate a physical barrier constraining tumor expansion. However, by exerting mechanical forces on the ECM, CAFs also enhance tumor invasion and dissemination. I will discuss these antagonistic roles of forces produced by CAFs in tumor growth, invasion and drug resistance.

## Symposium: Networks

S7

**Feeling water: Exploring the cellular basis for moisture sensing in plants.**

Y. Rui[1], P. Dahlberg[2], **J. Dinneny**[1]; [1]Stanford University, Stanford, CA, [2]SLAC National Linear Accelerator, Stanford, CA.

Water is essential for life on earth and plays a particularly important role in plant cells where the accumulation of water pressurizes cells and provides mechanical support for plant tissues. We have sought to gain insight into the processes that allow plant cells to sense water availability and the downstream cellular responses that mediate adaptations to water limiting conditions such as drought. To understand how osmotic stress affects the physicochemical properties of cells we have recently established a new FRET sensor that reports the molecular crowding status of cells. This sensor was the first designed based on a natural intrinsically disordered protein domain in plants that undergoes massive conformational changes in response to molecular crowding. Current work is using this sensor in yeast as a physiological readout of osmotic stress, which can then be correlated to the impact this stress has on the organization of cellular structures observed by Cryo-electron tomography. To identify protein complexes that mediate the acclimation of plant cells to osmotic stress, we are studying membrane localized proteins that inducibly form microdomains under stress. Proximity labeling of proteins at these foci have identified important proteins associated with membrane remodeling and mechanosensitive ion channels, which may be important for allowing cells to lose water to the environment while maintaining membrane integrity.

S8

**Mitochondrial Form and Function - Networks.**

**S. Lewis**; University of California, Berkeley.

No Abstract Submitted.

S9

**How Smart Are Cells?**

**L. Pelkmans**; Institute for Molecular Systems Biology, Zurich, SWITZERLAND.

Individual vcells make decisions that are adapted to their internal state and surroundings, but how cells can reliably do this remains unclear. To study the information processing capacity of human cells, we conducted multiplexed quantification of signaling responses and markers of the cellular state. Signaling nodes in a network displayed adaptive information processing, which led to heterogeneous growth factor responses and enabled nodes to capture partially non redundant information about the cellular state. Collectively, as a multimodal percept this gives individual cells a large information processing capacity to accurately place growth factor concentration within the context of their cellular state and

make cellular state-dependent decisions. Heterogeneity and complexity in signaling networks may have coevolved to enable specific and context-aware cellular decision-making in a multicellular setting.

## Symposium: Time

S10
**Paneth Cell Expression of Antimicrobial Proteins Is Regulated By the Circadian Clock.**
**J. Brooks**; Princeton University, Princeton, NJ.

The circadian clock directs physiological processes in coordination with the day-night cycle. However, the mechanisms by which the circadian clock regulates innate immune functions remain unclear. Paneth cells are specialized secretory cells within the intestinal epithelium that inhibit bacterial dissemination through the production of varied antimicrobial proteins, including α-Defensins and lysozyme. Here, we show that the expression of key α-Defensins and the protease Mmp7, which promotes α-Defensin antimicrobial activity, is directed by the Paneth cell-intrinsic circadian clock. The circadian clock promotes the expression of these genes independently of microbial signals, through direct binding of Clock and Bmal1 transcription factors to their respective promoters. Rhythmic production of α-defensins drives daily rhythms in mouse susceptibility to infection with the foodborne pathogen, *Listeria monocytogenes*. Our findings provide novel insight into how the cell-intrinsic circadian clock directs innate immune functions at a critical host-microbe interface.

S11
**The Stem Cell Zoo Uncovers the Mechanisms For Species-specific Developmental Time.**
**M. Ebisuya**; Physics of Life TU Dresden, Dresden, GERMANY.

While mechanisms of embryonic development are well conserved among mammals, the progression speed tends to be slower in larger species. To study differences in time between species, my group uses in vitro systems. We recapitulate the segmentation clock, the oscillatory gene expression during early development, from human and mouse pluripotent stem cells. The oscillation period of the human segmentation clock is 5-6 hours, while the mouse period is 2-3 hours. We found that this period difference between species stems from slower biochemical reactions in human cells, including slower protein degradation and longer delays in gene expression processes. We have recently extended this approach of cross-species comparison to other mammalian species, including the marmoset and rhinoceros. This ‘stem cell zoo’ approach uncovered several unexpected scaling and non-scaling laws of species-specific developmental tempo, which I will discuss in the meeting.

S12
**The Role of Molecular Heterogeneity in Circadian Cellular Synchronization.**
**J. Hurley**; Rensselaer Polytechnic Institute, Troy, NY.

Circadian rhythms are highly conserved, 24-hour, oscillations that tune physiology to the day/night cycle, enhancing fitness by ensuring that appropriate activities occur at biologically advantageous times. Circadian rhythms are generated at the cellular level via a transcription-translation based negative feedback loop, or molecular clock, which coordinates cells, tissues, and organisms to time all levels of biological function, including immunity and metabolism. Because of this extensive regulation, disruption of proper circadian timing negatively impacts organismal fitness, making understanding the mechanism underlying circadian regulation over cellular physiology critical to appreciating a fundamental rule of life

on earth. Our work has demonstrated extensive circadian regulation of immune function via metabolic coordination that, when disrupted, increases Alzheimer's Disease and Related Dementia pathophysiology. The current paradigm for how this negative affect is imparted is via the dysregulation of the transcriptional programing generated by the clock. However, our research has revealed that transcriptional programing cannot wholly account for clock physiological regulation, as we discovered only weak correlation between mRNAs and proteins that oscillate with a circadian periodicity. This suggested extensive post-transcriptional regulation through an unknown mechanism. In parallel, our data established that intrinsic protein disorder in the repressive complex of the clock enables the formation of "fuzzy-like" macromolecular complexes with proteins that regulate metabolism. We have revealed that changes in the formation of these time-of-day specific complexes enabled by this molecular heterogeneity is likely the mechanism by which the clock both post-transcriptionally controls cellular physiology and imparts negative fitness consequences upon disruption. As this fuzzy-like architecture is conserved in eukaryotic clocks, molecular heterogeneity is a probable mechanism to impart circadian timing and disruption across species.

## Symposium: Excitability

S13

**From Microbes to Living Electronics: The Choreography of Extra- and Inter-cellular Electron Transport.**
**M. El-Naggar**; University of Southern California, Los Angeles, CA.

We are used to thinking of the respiratory electron transport machinery as being confined within individual cells. However, it turns out that life invented bioelectronic solutions for interactions between microbial cells, as well as between microbes and the abiotic world. Recent studies at the interface of microbiology, electrochemistry, and physics have uncovered metalloprotein electron conduits and conductive nanowires that power bacteria by electronically linking them to extracellular surfaces ranging from environmental minerals to solid-state electrodes. Since extracellular electron transport naturally evolved to interact with external surfaces, this phenomenon has special implications for new biotic-abiotic interface technologies that study and harness the activity of living cells, including living electronics that process external signals and host synthetic genetic circuits. We will describe our recent progress in understanding extracellular and intercellular electron transport at multiple length scales, from the biophysics of individual electron conduits to the electrophysiology of whole bacteria and multicellular communities such as biofilms and cable bacteria. Using single molecule tracking, stochastic electron transport simulations, lithographic patterning of electrode attached biofilms, and metalloprotein 'doping', we describe how the interplay of cytochrome dynamics and electron hopping can give rise to long-distance electron conduction. In addition, we describe strategies to characterize and harness the electrochemical activity and quantum conduction properties of bacterial electron conduits in both synthetic structures and living biofilms.

S14

**Adaptive Cell Mechanics: Excitability in the Actin Cytoskeleton.**
**M. Gardel**; University Chicago.

Control of shape and movement is essential for cell physiology, from cell migration to control of tissue shape. Cytoskeletal assemblies both regulate how forces generated by individual mechanoenzymes are transmitted to cell and tissue scales as well as how mechanical properties evolve, or adapt, over time in

response to varied biochemical and mechanical stimuli. Such adaptation is necessary both to preserve shape (maintain homeostasis) as well as mediated smooth shape transitions. I will overview recent understanding of the origins of adaptive mechanics within the actin cytoskeleton, including the growing appreciation of the role of excitable dynamics in within the cortex, stress fibers and cell-cell adhesions. Together with a growing experimental toolset, this provides a new dynamical systems framework to to reveal design principles by which the actin cytoskeleton senses, generates, and adapts to mechanical force.

## Symposium: Rejuvenation and Regeneration

S15

**Mechanisms of Axon Growth and Regeneration.**

**F. Bradke**; the German Center for Neurodegenerative Diseases (DZNE), Bonn, GERMANY.

Almost everybody who has seen neurons under a microscope for the first time is fascinated by their beauty and their complex shape. Early on during development, however, neurons look round and simple without signs of their future complexity. How do neurons develop their sophisticated structure? How do they initially generate domains that later have distinct functions within neuronal circuits, such as the axon? And, can a better understanding of the underlying developmental mechanisms help us in pathological conditions, such as a spinal cord injury, to induce axons to regenerate? Here, I will talk about the cytoskeleton as a driving force for initial neuronal polarization and axon growth. I will then explore how cytoskeletal changes help to reactivate the growth program of injured CNS axons to elicit axon regeneration after a spinal cord injury. Finally, I will discuss whether axon growth and synapse formation could represent mutually excluding processes. Following this developmental hypothesis helps us to generate a novel perspective on regeneration failure in the adult CNS and to envisage new paths to overcome it. Thus, this talk will describe how we can exploit developmental mechanisms to induce axon regeneration in the adult after a spinal cord injury.

S16

**Rejuvenation of Eggs.**

**M. Schuh**; Max Planck Institute for Multidisciplinary Sciences, Göttingen, GERMANY.

The Schuh lab studies meiosis in mammalian oocytes. In particular, we are interested in how oocytes segregate their chromosomes, and how aneuploidy arises in eggs, which is a leading cause of miscarriages and infertility. We found previously that human oocytes are prone to assemble meiotic spindles with unstable poles, which can favor aneuploidy in human eggs. In the first part of my presentation, I will talk about the origin of spindle instability in human oocytes, and about a method to stabilize the spindle and reduce chromosome segregation errors. In the second part of my presentation, I will present a new method to reduce age-related aneuploidy in eggs.

## Symposium: Perception and Sensation

S17

**No Abstract Submitted**

**I. Abdus-Saboor**; Zuckerman Mind Brain Behavior Institute, New York City, NY.

No Abstract Submitted.

S18

**The Missing Link of Surface Mechanics: How Membrane Fluidity and Its Attachment to the Cortex Synergistically Regulate Cortical Dynamics.**

S. Dar[1], A. Gopalan[1], Z. Sun[2], R. Sprenger[3], I. Palaia[4], M. Murrell[5], A. Šarić[4], J. Belmonte[6], C. Ejsing[3], M. Leptin[1], **A. Diz-Muñoz**[1]; [1]EMBL, Heidelberg, GERMANY, [2]Yale Systems Biology Institute and Physics Department, New Haven, CT, [3]SDU, Odense, DENMARK, [4]ISTA, Viena, AUSTRIA, [5]Yale Systems Biology Institute, New Haven, CT, [6]NC State University, Raleigh, NC.

Animal cell shape changes are primarily driven by its surface, a composite interface comprising a thin cortical actin network physically tethered to the plasma membrane by specialized membrane-to-cortex attachment (MCA) proteins. Previous studies have focused on how gradients in myosin motors or actin network architecture drive cell deformations, overlooking any mechanical contribution of the membrane *per se*. Here, we identify membrane fluidity and the length of MCA proteins as additional forces that synergistically regulate cortical dynamics. By combining *in silico, in vitro and in cellula* approaches we show that contractility in actin networks scales linearly with membrane fluidity when MCA proteins are short (< 8nm). However, this effect becomes significantly amplified by several orders of magnitude with intermediate (10-15nm) or long (>24nm) linker proteins due to their caging, ultimately blocking actin network remodelling by myosin motors. On the basis of our findings, we propose that local changes in lipid composition or the choice of MCA protein allow cells to confine cortical tension changes leading to cellular morphogenesis.

# *SPECIAL INTEREST SUBGROUPS*

## Organelle Dynamics and Quality Control (SPONSORED Joint Session by CSCB)

SS1

**Segregation of the stemness program from the proliferation program in intestinal stem cells.**

**Y. Chen**[1,2]; [1]Biological Sciences and Biotechnology, Tsinghua University, Beijing, CHINA, [2]Nanchang University, Nanchang, CHINA.

Stem cells can undergo continuous self-renewal and meanwhile retain the stemness capability to differentiate to mature functional cells. However, it is unclear whether the proliferation property can be segregated from the stemness in stem cells. The intestinal epithelium undergoes fast renewal, and the $Lgr5^{+}$ intestinal stem cells (ISCs) are essential to maintain homeostasis. Here, we report that methyltransferase-like 3 (Mettl3), a critical enzyme for N6-methyladenosine (m6A) methylation, is required for ISCs maintenance as its deletion results in fast loss of stemness markers but has no effect on cell proliferation. We further identify four m6A-modified transcriptional factors, whose ectopic expression can restore stemness gene expression in $Mettl3^{-/-}$ organoids, while their silencing leads to stemness loss. In addition, transcriptomic profiling analysis discerns 23 genes that can be segregated from the genes responsible for cell proliferation. Together, these data reveal that m6A modification sustains ISC stemness, which can be uncoupled from cell proliferation.

SS2

**Vimentin filament entanglement patterns ER shape and subcellular distribution.**

**A. Moore**, J. Lippincott-Schwartz; Howard Hughes Medical Institute, Janelia Research Campus, VA.

The endoplasmic reticulum (ER) is a membrane-enclosed organelle that plays central roles in the maintenance of eukaryotic cell health and survival. The ER forms an elaborate, contiguous network that extends from the nuclear envelope to the cell edge. Despite this global continuity, the ER manifests an astonishing diversity of nanoscale membrane structures, including flat sheets, curved tubules, and a nuanced continuum of shapes therebetween. These complex geometries are maintained through the coordination of ER shaping proteins, which fine-tune nanoscale membrane curvature, and cytoskeletal interactions that stabilize and position them within the cytoplasm. Here, using advanced light and electron microscopy techniques, we describe a novel, cytoskeleton-stabilized ER subdomain, which we term ER tubule clusters. These clusters represent dense 3D enclaves of confined, aggregated ER tubules nestled amid otherwise sparse peripheral ER. Tubule clusters, distinct from matrices or fenestrated ER sheets, form at the cell edge and migrate inwards, ensnaring and incorporating increasing ER tubule mass as they travel. Dense, mature clusters exhibit an increased surface area-to-volume ratio, partially excluding luminal content while acting as sinks for curvature stabilizing membrane proteins. Critically, we find that ER-tubule clusters are entangled with and scaffolded by dense vimentin filament knots. Using cryogenic structured illumination microscopy correlated with focused ion beam scanning EM, we mapped and computationally reconstructed ER tubule clusters, identifying close apposition of vimentin filament bundles with the ER surface. We further show that vimentin filaments are scaffolded on the ER surface through plectin-mediated tethering by the LINC complex protein nesprin-3. Prolonged expression of nesprin-3 enhances ER-vimentin coupling, while disruption of the interaction or depletion

of vimentin by siRNA eliminates the ER tubule clusters and promotes expansion of planar ER geometry. Together, our results provide the first evidence for vimentin-ER entanglement in the maintenance of ER structure and affirm the important function of vimentin intermediate filaments in organizing subcellular membrane positioning.

SS3

**Molecular regulation of mitochondrial homeostasis and its contribution to aging associated diseases.**

**Q. Chen**; State Key Laboratory of Medicinal Chemical Biology, College of Life Sciences, Nankai University, Tianjin, CHINA.

Mitochondria are highly dynamic and undergo constant turnover through two opposing processes: mitophagy which selectively removes superfluous and damaged mitochondria by the autolysosomal pathway versus mitochondrial biogenesis, which generates fresh, functional mitochondria from existing ones. We have previously characterized that FUNDC1 functions as a mitophagy receptor to mediate hypoxia-induced mitophagy. Interestingly, we found that PGC-1α and NRF1, master regulators of mitochondrial biogenesis and metabolic adaptation, also transcriptionally up-regulate *Fundc1* in response to cold stress in brown adipose tissue (BAT). Specific knockout of *Fundc1* in BAT results in reduced mitochondrial turnover and accumulation of functionally compromised mitochondria, leading to impaired adaptive thermogenesis. Recently, we showed that inducible loss of endothelial *Fundc1* in postnatal mice was sufficient to spontaneously cause pulmonary arterial hypertension (PAH), a currently non-curable disease. *Fundc1* deficiency in endothelial cells, but not in smooth muscle cells, leads to metabolic reprogramming, pseudohypoxia, and senescence. We further characterize the molecular pathways driving pulmonary arterial remodeling and suggest new strategies to ameliorate PAH. Collectively, our results demonstrate that FUNDC1 dictates mitochondrial quantity, quality and turnover and contributes to adaptive thermogenesis and aging related diseases.

SS4

**Lipid Composition Defines Endoplasmic Reticulum Morphology and Function.**

**U. Eggert**; King's College London, London, UNITED KINGDOM.

The complex network of the endoplasmic reticulum (ER) is primarily composed of sheet-like cisternae and dynamic tubules. While sheets are involved in synthesis, folding and secretion of proteins, tubules are recognized as the main sites of calcium storage, lipid synthesis and the ER's contact with other organelles. The fine balance that regulates the ratio of ER sheets to tubules is not fully understood. We show that the cellular and organelle lipid compositions are crucial to maintain ER morphology and function, including the ratio of ER sheets to tubules. We conducted a phenotypic siRNA screen in which HeLa cells were treated with 258 different siRNAs targeting lipid-metabolizing enzymes. Removal of several enzymes, and therefore perturbation of the lipids they metabolize, resulted in dramatic changes to the ER. We developed a protocol to rapidly isolate ER lipids and identified which lipids change in organelles and cells with morphological alterations. Double knock down of enzymes with opposing phenotypes restored near wild type morphology and lipid composition, providing evidence for lipid involvement. In parallel, we analyzed the impact of these lipidomic changes in functional assays, systematically linking lipid composition to ER structure and function.

SS5

**Modulation of Phase Condensates Regulates Organelle Quality Control and Proinflammatory Signaling On Damaged Organelles.**

E. R. Gallagher[1], O. Harding[1], J. F. Riley[1], **E. Holzbaur**[2]; [1]University of Pennsylvania, Philadelphia, PA, [2]University of Pennsylvania.

Organelle quality control pathways are central to the maintenance of cellular homeostasis. Acute damage to organelles can induce cellular response mechanisms leading to organelle repair or to organelle turnover, with selective autophagy as the major pathway driving removal. Phase condensate dynamics have been implicated in the regulation of multiple aspects of the selective autophagy of lysosomes and mitochondria. We will discuss recent progress in understanding the formation of phase condensates following organelle damage, how the fluidity of these phase condensates is modulated by upstream signaling pathways and downstream effectors, and the cellular effects of alterations in phase condensate behavior during selective autophagy. Specifically, we find that the fluidity of p62/SQSTM1 phase condensates is modulated by the small heat shock protein HSP27 and promotes the clearance of damaged lysosomes. We also see the formation of phase condensates of p62 on damaged mitochondria during mitophagy. In this context, however, we find that these condensates mediate the assembly of NEMO into a signaling platform that activates proinflammatory NF-kB signaling. Thus, phase condensates regulate both organelle turnover and cellular responses to organelle damage.

SS7

**No Abstract Submitted.**

**D. Li**; Institute of Biophysics Chinese Academy of Science.

SS8

**EB1 condensation dynamics guides microtubule dynamics in mitosis.**

**X. Liu**; University of Science and Technology of China, Hefei, CHINA.

Liquid-liquid phase separation (LLPS) of biomolecules drives the formation of subcellular compartments with distinct physicochemical properties. These compartments, free of lipid bilayers and therefore called membraneless organelles, include nucleoli, centrosomes, heterochromatin, and centromeres. These have emerged as a new paradigm to account for subcellular organization and cell fate decisions. Using chromosome segregation in mitosis as model system, we aim to delineate the mechanisms of action underlying LLPS-driven process in centromere assembly and mitotic spindle plasticity, highlighting a functional role for phase behavior and material properties of proteins assembled onto heterochromatin, centromeres, and central spindles via LLPS. Our current interest is focused on the visualization of specific biomolecules condensation in real space and time as perturbation of cellular plasticity and dynamics is detrimental to health.

## Subgroup: Bioelectricity in Development, Regeneration, and Cancers

SG1

**Neural cell primary cilia calcium dynamics regulate Hedgehog signaling-dependent neurogenesis in the embryonic neural tube.**

**L. N. Borodinsky**, S. Shim, R. Goyal, A. A. Panoutsopoulos, O. A. Balashova, D. Lee; University of California, Davis, Sacramento, CA.

The balance between neural stem cell proliferation and neuronal differentiation is paramount for the appropriate development of the nervous system. Sonic hedgehog (Shh) is known to sequentially promote cell proliferation and specification of neuronal phenotypes, but the signaling mechanisms responsible for the developmental switch from mitogenic to neurogenic have remained unclear. By using multiple loss and gain of function approaches and live imaging of ciliary calcium dynamics, here we show that Shh enhances calcium activity at the neural cell primary cilium of developing *Xenopus laevis* embryos through calcium influx via TRPC3 and release from intracellular stores in a developmental stage-dependent manner. This ciliary calcium activity in turn antagonizes canonical, proliferative Shh signaling in neural stem cells by downregulating Sox2 expression and upregulating expression of neurogenic genes, enabling neuronal differentiation. These discoveries indicate that the Shh-calcium-dependent neural cell ciliary signaling triggers the switch in Shh action from canonical-mitogenic to neurogenic. The molecular mechanisms identified in this neurogenic signaling axis are potential targets for the treatment of brain tumors and neurodevelopmental disorders.

SG2

**Cellular herding: bioelectric programming of collective cell behaviors.**

**D. J. Cohen**; Princeton University, Princeton, NJ.

Naturally occurring, direct-current (DC) electric fields arise from ion gradients within tissues and can trigger numerous ancient signaling processes conserved across many multicellular systems—imagine the potential if we could harness and control these interactions! I will discuss new work from my group using engineered electrical signals to manipulate cell migration (‘electrotaxis’) and actuate water transport as mechanisms to effectively regulate the behavior of thousands of cells, inspired from human and sheepdog herding strategies.In our previous work, we have demonstrated how electrotaxis can be used to literally program large-scale collective cell migration in 2D skin-cell monolayers, which we have since extended to explore how to apply concepts from swarm control theory to better electrically control tissues of different sizes, shapes, and types. In particular, we will discuss how natural cellular collective behaviors can ‘compete’ with our external, bioelectric commands, and the cell-cell adhesion protein E-cadherin is the key to this tug-of-war.We have also recently expanded into electrically programming 3D developmental and organoid models using renal epithelial cells, intestinal stem cell organoids, and human induced pluripotent stem cells. In all cases, we observe that the interplay between the applied electric field and the 3D tissue geometry drive unexpected emergent behaviors. In particular, our new data demonstrate how external electrical stimulation of 3D, lumenized (hollow) organoids can program both the size and shape of the organoids, which we can now relate directly to specific ion transporter activation and 3D cell migration around the organoid. We even observe symmetry breaking in circular hiPSC colonies, where colony morphology and dynamics split straight down the middle of the colony during electrical stimulation.In addition to studying the biophysical bases for these behaviors, we are also trying to understand the ramifications, such as effects on cell fate and

general physiology. The outlook of these data is that bioelectric regulation of cell dynamics offers a powerful and emerging strategy to directly program complex developmental, engineering, and disease processes.

SG3

**Probing the Relationship between Bioelectric Signaling and Reactive Oxygen Species (ROS) Accumulation during Adult Tissue Repair.**

**S. J. Hack**, J. Vuckovic, E. Quereshi, W. S. Beane; Biological Sciences, Western Michigan University, Kalamazoo, MI.

Bioelectric signaling, specifically membrane depolarization and subsequent calcium ($Ca^{2+}$) flux, has been shown to regulate regenerative tissue repair in many species—including planarian flatworms. However, physiological responses to trauma are complex, and how ion flux mechanistically links bioelectric signaling to growth processes remains unclear. Recent work has shown that signaling regulated by reactive oxygen species (ROS) mediates injury responses to both wound healing and new tissue growth. In planarians, our own data reveal that precise control of trauma-induced ROS accumulation is critical, as differential ROS threshold levels determine whether wound healing alone, wound healing plus regenerative growth, or chronic wounds occur. Biochemically, ROS signaling is required for gene expression changes and stem cell proliferation following injury. In non-regenerative contexts, ROS are known to induce depolarization of the mitochondrial inner membrane, while conversely depolarization of the plasma membrane (as well as activation of voltage-gated $Ca^{2+}$ channels) can lead to the production of ROS. Therefore, we hypothesized that changes in membrane voltage ($V_{mem}$) and $Ca^{2+}$ flux may regulate ROS accumulation during regenerative growth. To investigate the epistatic relationship between injury-induced bioelectric signaling and ROS production, we used *in vivo* fluorescent reporter dyes to visualize changes in $V_{mem}$, $Ca^{2+}$ flux, and general ROS within the first 3 hours after injury. We found that all three were upregulated at the wound site within 30 minutes, but with varied temporal patterns. Furthermore, we used species-specific reporter dyes to individually visualize $H_2O_2$ and superoxide ($O_2^-$) accumulation during the same three hours. Our preliminary results suggest that bioelectric signaling, in the form of $V_{mem}$ changes and $Ca^{2+}$ flux, may be upstream of injury-induced ROS accumulation and ROS-mediated gene expression required for stem cell proliferation. Together, the data suggest that complex feedforward and feedback mechanisms between ROS and bioelectric signaling could be involved in adult tissue repair. Our current investigations will reveal epistatic relationships between bioelectric signaling and ROS accumulation during wound closure and stem cell proliferation, where our future studies will identify potential genetic regulators of injury-induced bioelectric signaling.

SG4

**Mechano-electrical regulation of glioblastoma stem cell confined migration.**

**C. Junqueira Alves**[1], A. Dixon[2], T. Hannah[1], R. Wiener[3], H. Nguyen[1], M. Tipping[4], P. Slesinger[5], K. Costa[1], H. Zou[1], R. Friedel[1]; [1]Department of Neuroscience, Icahn School of Medicine At Mount Sinai, New York City, NY, [2]Department of Biology, Case Western Reserve University, Cleveland, OH, [3]Department of Medicine, Icahn School of Medicine At Mount Sinai, New York City, NY, [4]Molecular Cytology Core Facility, Memorial Sloan Kettering Cancer Center, New York City, NY, [5]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY.

Glioblastoma is a malignant brain tumor of uncontrolled proliferation and infiltration. GBM stem cells (GSCs) are constantly challenged by confined spaces during their migration through the brain

parenchyma. Understanding the factors that drive GSC confined migration (CM) is thus critical for effective drug development. Using microchannel devices, we showed that GSCs are adept at squeezing through narrow constrictions at faster speed than through non-constricted spaces.During CM, cytoskeletal components and plasma membrane self-organize to establish polarity and propel the cell through the constrictions. Among the mechanical plasticity observed for meeting the challenges of CM the cells remodel their shape and plasma membrane through increased endocytosis. Rapid endocytosis of inner leaflet anionic phospholipids-such as PI(4,5)P2 and phosphatidylserine at the front of the cell collectively contribute to drive actin filaments and positive-charged calcium ions to the rear end of cells, abruptly leading to localized changes in membrane potential and propelling the cell. Correspondingly, inhibiting endocytosis abrogates polarity and results in failure to CM. These effects were recapitulated by deletion of the cell surface receptor Plexin-B2. Plexin-B2-CRISPR knockout (KO) GSCs displayed reduced capability to squeeze through constrictions in microchannel devices. Mechanistically, Plexin-B2 KO in GSCs compromised membrane dynamics and reduced endocytosis as consequence of low cell stiffness and membrane tension. Computational simulations demonstrated that membrane tension couples with cell stiffness in feedback loops and support the model that Plexin-B2 controls membrane tension through actomyosin contractility. On a molecular level, the locked extracellular ring domain of Plexin-B2 prevents GSCs to migrate through constrictions and recapitulates the defects associated with Plexin-B2 KO phenotype, indicating that ring bending is required for membrane tension. Thus, our results describe critical mechano-electrical events necessary to establish polarity and prompt GSCs migration under confinement and provide a mechanism by which a single mechanosensory molecule exerts regulation at the cytoskeletal and membrane levels.

SG5

**The interplay of membrane potential and cytoskeleton directs cell migration.**

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Mammalian cells uphold osmolarity balance through constant ion exchanges, leading to highly dynamic bioelectric activities. Membrane potential is the outcome of specific ion channels and transporters exhibiting unique ion selectivity and permeability. The significance of membrane potential has been well studied in excitable cells, emphasizing its role in developmental biology and cancer. However, its role in non-excitable cells remains to be elucidated. Recent studies have highlighted abnormal membrane potential is associated with cancer metastasis, correlating with enhanced cell migration. Cell migration is predominantly governed by cytoskeletal activities, with emerging evidence indicating a key role for ion channel polarization and activities, known as Osmotic Engine Model, in directing this process. Thus, we hypothesize a substantial interplay between the cytoskeleton and membrane bioelectric activities during this process. In this study, we reveal that cell membrane potential is dynamically tuned by actomyosin activities at the leading and trailing edges of migrating cells. Such interplay is achieved by polarized ion transporter distribution and cytoskeletal activation ion transporters such as Sodium-Hydrogen exchanger 1(NHE1). Our work elucidates a novel mechanism for cell migration, which advances our understanding of the role of membrane potential in non-excitable cells.

SG6

**Calcium transients during cell division requires a a mechanosensitive polycystin channel Pkd2.**

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Calcium is an essential secondary messanger in eukaryotic cells. Neverthless, its role in cell division remains poorly understood. We recently discovered the novel calcium transients accompanying cytokinesis, the last stage of cell division. However, it is unknown what gives rise to these so-called cytokinetic calcium spikes. Here we showed that an essntial ion channel Pkd2 promotes calcium influx that contributes to the calcium spikes in the model organism fission yeast. Pkd2 is a homologue of the human polycystin channel, mutations of which lead to a common genetic disorder Autosomal Polycystic Kidney Disorder. This plasma membrane localized ion channel enriches at the expanding microdomains in the membrane, including the equatorial division plane during cytokinesis. In vitro reconsititution found that elevated membrane tension activates this channel to allow calcium passage. In vivo, Pkd2 directs calcium influx during cytokinesis, leading to sharp calcium spikes. Depletion of Pkd2 leads to failed separation of the daughter cells during cytokinesis. Combined, we discovered a novel force-sensitive channel that promotes calcium transient during cell division. Such mechanism is likely evolutionally conserved, revealing a potentially novel function of human polycystins in cell division.

SG7

**Calcium channel Trpv6 regulates epithelial cell renewal by reprogramming growth factor signaling and mitochondria metabolism.**

**C. Duan**; University of Michigan, Ann Arbor, MI.

Tissue homeostasis and regeneration require tight control of cell renewal. Although adult stem cells were long considered the source of new cells, recent studies suggest that many differentiated cells can reenter the cell cycle to replace the lost cells upon injury. While it is evident that cell plasticity plays a key role in tissue homeostasis and regeneration, the underlying mechanisms are poorly understood. Whether a fully differentiated cell can dedifferentiate and reenter the cell cycle in a physiological context is not clear. We have recently developed a zebrafish model, in which a population of differentiated epithelial cells (known as ionocytes or NaR cells) can be induced to reenter the active cell cycle under a physiologic context. NaR cells are structurally and functionally similar to human renal and colon epithelial cells. NaR cells are located on the yolk sac epidermis during the larval stage, making them easily accessible for observation and manipulation. A hallmark of NaR cells is the expression of the calcium channel Trpv6. Our studies show that Trpv6 is constitutively open in vivo. Trpv6 mediates $Ca^{2+}$ influx and maintains high cytoplasmic $[Ca^{2+}]_i$. The high $[Ca^{2+}]_i$ activates the phosphatase PP2A, which suppresses Akt-Tor signaling and promotes cell cycle exit. When Trpv6 is deleted or $Ca^{2+}$ is depleted from the medium, NaR cells rapidly reenter the cell cycle and divide. Using this whole animal platform, we performed RNA-seq, chemical biology screens, and genetic studies. The results suggest that the (re)activation of the insulin-like growth factor (Igf)-Igfbp5a-Igf-PI3 kinase-Akt-Tor signaling pathway is critical for cell renewal. Acting further downstream is mitochondria. NaR cell reactivation is accompanied by a robust and sustained increase in mitochondrial membrane potential and ATP production. Elevated mitochondrial metabolism is required and sufficient for cell renewal. Mechanistically, elevated mitochondrial metabolism increases mitochondrial ROS levels, which induces the expression and mitochondrial localization of Sgk1, a conserved Akt-like kinase. Sgk coordinates mitochondrial activity with ATP synthesis by phosphorylating F1Fo-ATP synthase. Similarly, ROS-

dependent mitochondrial expression of SGK1 promotes S phase entry in human breast cancer cells. These findings suggest that calcium channel-mediated $Ca^{2+}$ influx regulates cell fate by reprogramming growth factor signaling and mitochondrial metabolism.

SG8

**Unraveling the interplay of calcium signaling and SNARE proteins in morphogenesis: implications for *Drosophila* wing development and BMP/Dpp signal transduction.**

**M. Follmer**, E. Bates; Developmental Biology, University of Colorado - Anschutz Medical Campus, Denver, CO.

Morphogenesis requires a strict control of cell-cell signaling. How cells release signals at the right time and place in developing tissues remains an open question. Evidence from our lab shows that developmental signaling in the developing *Drosophila* wing shares distinct parallels with neuronal signaling, including excitability, ion channel regulation, and SNARE complex proteins. We have shown that the larval wing disc and pupal wing both display spontaneous calcium oscillations during *Drosophila* wing development. We found that a group of ion channels that are responsible for regulating cytoplasmic and ER calcium stores (Stim, Orai, SERCA, SK, and Best2) are necessary for wing morphogenesis. Interestingly, wing specific knockdown of these channels phenocopies loss of bone morphogenetic protein (BMP, Dpp in flies) signaling. Both the SERCA inhibitor thapsigargin and RNAi knockdown of SERCA and Stim disrupt endogenous calcium oscillations in the larval wing disc and pupal wings. Disruption of SERCA and Stim decreased Dpp-GFP release from larval wing disc cells. Taken together, these results suggest a vital role for ER calcium in regulating BMP/Dpp release during wing morphogenesis. The dependency of BMP/Dpp release on cytoplasmic calcium suggested that BMP signaling may be reliant on the same calcium-dependent protein machinery as neurotransmitter release. We are investigating the role SNARE proteins Syntaxin1A (Syx1A) and SNAP24 play in Dpp signal transduction for *Drosophila* wing development. Our data shows defects in wing size and venation with RNAi knockdown of Syx1A, along with an increase in phosphorylated Mad (pMad) signal in the larval wing disc, indicative of Dpp gain of function. Additionally, SNAP24 knockdown causes missing anterior and posterior cross veins which are Dpp loss of function phenotypes. Our evidence suggests that Syx1A and Snap24 are both required for wing development, and they may work in opposing ways to regulate Dpp signaling.

SG9

**Depolarization regulates calcium dependent BMP release in the developing palate.**

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Palatal defects are prevalent yet remain challenging to treat. Ion channels have emerged as key regulators of palatal development because their genetic and pharmacological inhibition causes cleft palate in humans and animal models. However, the mechanisms underlying how bioelectrical properties impact palate formation remains an open question because ion channels have primarily been studied in neurons or in animals with few tools for probing this question. We use a mouse knockout of *Kcnj2 (Kcnj2$^{ko/ko}$)*, a potassium ($K^+$) channel that sets resting membrane potential, as a tool to study how bioelectrical properties contribute to craniofacial morphogenesis. Disruption of *Kcnj2* causes cleft palate and other craniofacial defects in humans and mice. We found that loss of *Kcnj2* disrupts Bone Morphogenetic Protein (BMP) signaling within the developing palate. BMP signaling is essential for the morphogenesis of several craniofacial structures, including the palate. We introduce a tool to image

release of BMP4 from mammalian cells. Using this tool, we show that depolarization induces BMP4 release from mouse embryonic palate mesenchyme cells in a calcium-dependent manner. We discovered native transient changes in intracellular calcium occur in cranial neural crest cells, the cells from which embryonic palate mesenchyme derives. Waves of transient changes in intracellular calcium suggest that these cells are electrically coupled and may temporally coordinate BMP release. These transient changes in intracellular calcium persist in palate mesenchyme cells from embryonic day (E) 9.5 to 13.5 mice. Disruption of *Kcnj2* significantly decreases the amplitude of calcium transients, but does not abolish them suggesting that Kcnj2 works in conjunction with other ion channels to regulate electrical activity. Our single cell RNA sequencing of the anterior palate reveals that potassium, sodium, and calcium channels are expressed in the E13.5 palate along with gap junctions revealing potential candidates for regulating bioelectric properties of the palate. Together, these data suggest that temporal control of developmental cues can be bioelectrically regulated by ion channels, depolarization events, and changes in intracellular calcium for mammalian palate formation.

SG10

**Electrifying secrets of directed cell migration: identifying the genes that support neutrophil galvanotaxis.**

**N. M. Belliveau**, T. E. Eustis, M. J. Footer, J. A. Theriot; University of Washington & Howard Hughes Medical Institute, Seattle, WA.

Migratory cells must constantly integrate physical and chemical cues within their environment to move effectively. Galvanotaxis, also known as electrotaxis, involves directed cell movement in response to electric fields. Experiments in vitro have demonstrated that many motile cell types will rapidly respond to changes in applied electric fields. Physiologically, disruptions to epithelial barriers such as a cut in skin will disrupt the transepithelial potential that is normally present, resulting in a wound current and the generation of an endogenous electric field with the cathode at the site of injury. This study aims to identify the molecular players involved in galvanotaxis in human neutrophils through an unbiased functional genomics approach. We used pooled CRISPR interference (CRISPRi) in the HL-60 human neutrophil-like cell line for a genome-wide knockdown screen. To identify gene perturbations that alter galvanotaxis, we have engineered a device that allows us to expose millions of cells to a direct current (DC) electric field in the physiologically relevant range of a few V/cm, directing the cells' movement through track-etch membranes containing 3 m pores. Genes are identified by measuring sgRNA gene-target enrichment or depletion in the cell population that successfully migrates through the membrane in response to the electric field. We have identified several hundred genes whose knockdown affects cell migration in both the presence and absence of electric fields, including expected components involved in regulation of the actin cytoskeleton and phosphoinositide signaling. Excitingly, we have also identified genes whose knockdowns affect galvanotaxis but have little or no effect on cell migration in response to serum stimulation. These include poorly characterized membrane proteins, TMEM154 and TM2D2, as well as a variety of genes involved in the synthesis of precursors necessary for protein glycosylation. We confirmed their specific effects on the directional response during galvanotaxis using individual knockdown cell lines. Glycosylation often imparts additional negative charges to cell surface proteins, and our results lend new support to the hypothesis that electrophoresis of charged proteins on the cell surface plays a key role in the sensory mechanism of galvanotaxis.

SG11

**T-cells in zebrafish epidermis migrate away from wounds due to endogenous electric fields.**

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Skin wound healing is a complex process that requires precise spatio-temporal coordination between multiple cell types including epithelial and immune cells. Intact skin maintains a trans-epithelial potential (TEP) due to asymmetric ion flow. Wounding disrupts the TEP and induces a lateral electric field (EF). This motivated us to develop an adult zebrafish scale explant system as a model skin tissue ('skin-on-glass') to explore the role of the wound-induced EF in leukocyte and epithelial cell behaviour. The explant retains several key features of animal skin, including the co-existence of multiple cell types (epithelial cells, mechanosensory cells, T-cells, neutrophils and macrophages) in a near-native organisation. In 'skin-on-glass' exposed to EFs, we observed the epithelial cells and neutrophils rapidly migrating towards the cathode (i.e. toward the wound), corroborating previous reports. However, surprisingly, T-cells migrated towards the anode (i.e. away from the wound). Isolated adult primary zebrafish T-cells also displayed directed migration toward the anode, indicating that this unusual directional response is a cell-intrinsic behaviour. These observations led us to predict that in vivo, T-cells will migrate away from the wound while neutrophils will go towards it. Using transgenic zebrafish lines expressing fluorescent proteins in specific leukocyte cell types, we standardised a laceration wounding protocol in larvae aged 11-14 days post-fertilization, when both neutrophils and T-cells are abundant throughout the epidermis. This robust and reproducible wounding assay showed that neutrophils exhibit rapid migration toward the wound site, as expected, while T-cells moved away from the wound with a strong bias migrating against the flow of the epidermal tissue. Additionally, disruption of the endogenous wound-generated EF led to failure of both neutrophils and T-cells to migrate with their usual directional biases. After about one hour post-wounding, T-cells resume their normal surveillance behaviour in the vicinity of the closed wound margin, indicating that their EF-dependent avoidance of the wound site is an acute behaviour as part of the wound response. We are currently exploring the biological implications of T-cell wound avoidance, distinct from the neutrophil migration toward the wound site.

SG12

**Bioelectric regulation in zebrafish embryos and fin patterning.**

**G. Zhang**; Purdue University, West Lafayette, IN.

**Title:** Bioelectric regulation in zebrafish embryos and fin patterning.

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**Abstract:** Embryonic development is a self-autonomous and robust process that requires coordinated and complex cellular events such as proliferation and differentiation. The morphogen gradient and transcription network are currently the mainstay patterning mechanisms verified in various organisms. Recently, mounting evidence revealed that bioelectricity is an emerging patterning signaling during embryonic development. To further investigate the roles of bioelectricity in zebrafish, we first validated

the bioelectricity in zebrafish early embryos using a genetically encoded voltage indicator, ASAP1, and found that the zebrafish embryos display stage-specific characteristic depolarization events. Furthermore, we explored the role of bioelectricity in zebrafish fin development, which has been an extensively studied system for organ patterning. We found that ectopically expressing two inwardly rectifying potassium channel (*kir*) genes can cause long fins in adult zebrafish. Moreover, we showed that ectopic expression *kir* genes in the somite of transgenic zebrafish is essential for long-fin formation. Our results support that bioelectricity can function as a patterning signal in zebrafish embryogenesis and fin patterning.

## Subgroup: Biological Timing and Patterning: From Fundamental Principles to the Development of Novel Approaches

SG13

**How P-bodies Influence Our Biological Clocks.**

**S. Yadlapalli**, Y. Xiao, Y. Yuan; University of Michigan, Ann Arbor, MI.

**How P-bodies Influence Our Biological Clocks**

Circadian clocks are cell-autonomous timekeepers that orchestrate ~24-hour rhythms in the expression of a large number of genes, thereby controlling much of our physiology and behavior, including sleep-wake cycles, and metabolism. These clocks are based on negative transcription translation delayed feedback loops. Specifically, in Drosophila, this clock regulation involves four core clock proteins: activators CLOCK (CLK) and CYCLE (CYC), and repressors PERIOD (PER) and TIMELESS (TIM). CLK/CYC homodimers activate transcription by binding to E-boxes of genes, including per and timeless. After a time delay, PER and TIM enter the nucleus where they inhibit CLK/CYC, suppressing their own transcription as well as that of other clock-regulated genes.

While past studies used traditional techniques to study PER nuclear complexes in mammalian cells, we have adopted a novel BioID-based proximity labeling method to identify in vivo PER partners in Drosophila clock neurons across the circadian cycle. By creating a unique fly line with endogenous PER tagged using the miniTurbo enzyme (biotin ligase) and employing 10-plex tandem mass tag (TMT) isobaric labeling, we successfully mapped PER's interactions throughout the circadian rhythm. Surprisingly, we found that several components of Processing bodies (P-bodies) such as Pcm/Xrn1 (5'-3' exonuclease), Edc3 (Enhancer of decapping-3), and Me31B/DDX6 (DEAD-box family RNA helicase) interact with PER protein during the activation phase. P-bodies, which are dynamic, membrane-less ribonucleoprotein granules in the cytoplasm, function by selectively sequestering RNAs for either decay or delayed translation. Yet, the potential role of P-bodies in post-transcriptionally regulating key clock mRNAs has not been explored.

Here, using imaging and sequencing based methods we show that Period and Timeless mRNAs are localized to P-bodies specifically during the activation phase, and this localization is required for translational repression of PER and TIM proteins during the activation phase. Loss of me31B or pcm causes PER and TIM to continuously accumulate in the nucleus, disrupting rhythms in gene expresssion and extending the circadian cycle to 27 hours. Taken together, P-bodies play a crucial role in repressing the translation of core clock proteins, ensuring their timely nuclear entry and maintaining the regular ~24-hour circadian rhythms. We are currently investigating the molecular mechanisms that govern localization of Period mRNAs to P-bodies.

SG14

**A topological mechanism for robust and efficient global oscillations in biological networks.**

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Long and stable timescales are often observed in complex biochemical networks, such as in emergent oscillations. How these robust dynamics persist remains unclear, given the many stochastic reactions and shorter time scales demonstrated by underlying components. We propose a topological model with just a couple of parameters that produces long oscillations around the network boundary, effectively reducing the system dynamics to a lower-dimensional current. Using this to model KaiC, which regulates the circadian rhythm in cyanobacteria, we compare the coherence of oscillations to that in other KaiC models. Our model localizes currents on the network edge for an efficient regime with simultaneously increased precision and decreased cost. Our work presents a new mechanism for emergent oscillations utilizing dissipative cycles to achieve robustness and efficient performance.

SG15

**Identifying *in vivo* substrates of CDK-opposing phosphatases using a global phosphoproteomic approach.**

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Temporal ordering of cell cycle events is ensured by the reversible phosphorylation of hundreds of proteins. This is an interplay between Cyclin-dependent kinases (CDKs) and counteracting phosphatases (PPases). To understand phosphorylation signalling networks, kinase and PPase substrates must be unambiguously identified. Phosphoproteomic studies have revealed CDK substrates and their phosphorylation timing during the cell cycle. However, our understanding of which phosphatases oppose these CDK sites and what role they play in ordering CDK substrate phosphorylation timing is still incomplete. We set out to identify which PPases oppose CDK substrate phosphorylation *in vivo* using a global mass spectrometry-based phosphoproteomics approach in fission yeast. We focused on the PPases PP2A and Clp1, which have a negative regulatory impact on the G2/M transition. Since PP2A is a holoenzyme, we degraded its B55 and B56 regulatory subunits separately, to discern differences in substrate specificity of the different PP2A holoenzymes. We inhibited CDK activity in the presence and absence of each of these phosphatases and compared the dephosphorylation kinetics of known CDK substrates. We defined PPase substrates as phosphosites with a substantially decreased dephosphorylation rate in the absence of the PPase. Interestingly, the majority of CDK sites were dephosphorylated by a specific PPase, and only a minority of phosphosites were jointly targeted by multiple PPases. Comparing the groups of PPase substrates revealed differences in amino acid preferences, with $PP2A^{B56}$ substrates showing a strong depletion of acidic residues surrounding the phosphorylated residue, $PP2A^{B55}$ substrates being enriched for P at the +1 position and Clp1 substrates enriched for K at the +3 position. We hypothesised, that the division of labour between these phosphatases in opposing CDK substrates could play a crucial role in ordering the phosphorylation of CDK substrates throughout the cell cycle. We show that CDK substrates opposed by $PP2A^{B55}$ are on average phosphorylated later during the cell cycle compared to CDK substrates opposed by Clp1 or $PP2A^{B56}$. This suggests, that $PP2A^{B55}$ restricts some CDK substrates from being phosphorylated early during the cell cycle, thereby imposing order on the timing of CDK substrate phosphorylation. Consistent with this, we show that phosphorylation of a putative $PP2A^{B55}$ substrate happens earlier in the absence of $PP2A^{B55}$, using a single cell phospho-sensor. Together, these data show that there is a division of

labour between different cell cycle PPases and highlight the importance of these PPases in regulating timing of CDK substrate phosphorylation during the cell cycle.

SG16

**Quantifying the role of nuclei in mitotic wave propagation in *Xenopus laevis* extracts.**

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The mitotic timing is regulated by the network of molecular oscillators centered on the cyclin-dependent kinase 1 (Cdk1) that can serve as a bistable medium for trigger wave propagation. Such waves of Cdk1 activity are considered to be the mechanism for mitotic events to achieve spatial synchrony beyond the molecular length scale, highlighting their pivotal role in the patterning and coordination of cell divisions, for example, across an embryo during early development. Literature shows they emanate from foci with higher local oscillator frequencies, or pacemakers, which are, in fact, nuclei that localize and concentrate cell cycle regulators. Here, as a further clarification of the role of nuclei, we present a comparative study of 1-dimensional (1D) mitotic waves propagating in media either with or without nuclei. We use *Xenopus laevis* egg extracts to implement a quasi-1D system where mitotic waves propagate up to 1 cm and persist longer than a day (>10 cycles), with the mitotic activity being directly quantified with a Cdk1 FRET sensor. Based on a novel approach exploiting metaphase-arrested cytostatic factor extracts as a drive, we produce directed waves that do not necessitate reconstituted nuclei as wave sources. Also, we investigate the undirected waves arising in cycling extracts with and without nuclei. Our comparative study reveals the role of nuclei in the speed and entrainment dynamics of mitotic waves: while nuclei slow down the wave propagation, a system entrains more quickly in the presence of nuclei. We also present computational studies in parallel for a more systematic understanding of the time-dependent behavior of mitotic waves.

SG17

**Cytoplasmic division cycles without the nucleus and mitotic oscillator.**

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Cytoplasmic divisions are thought to rely on nuclear divisions and mitotic cues. We demonstrate in Drosophila embryos that cytoplasm can divide repeatedly without nuclei and the Cdk/Cyclin oscillator. We find that the Cdk/Cyclin oscillator normally slows an otherwise faster cytoplasmic division cycle, coupling it with nuclear divisions. When uncoupled, cytoplasm starts dividing before mitosis. In cycling embryos, we show that cytoplasm can divide without centrosomes; whereas in arrested embryos lacking nuclei and mitotic microtubules, cytoplasmic divisions do require centrosomes. Thus, at least one microtubule pool, but not the mitotic spindle or a specific pool per se, helps transmit the cues for cytoplasmic divisions. Strikingly, autonomous cytoplasmic divisions occur even in normal development, where they appear to help extrude mitotically stalled nuclei from the blastoderm. We postulate that cytoplasmic divisions occur in cycles governed by a clock mechanism autonomous from the Cdk/Cyclin oscillator, but their tight coordination helps achieve successful mitoses.

SG18

**A metabolic clock driving the eukaryotic cell cycle.**

**M. Heinemann**; University of Groningen, Groningen, NETHERLANDS.

The eukaryotic cell division is thought to be controlled by periodic activity of the cyclin dependent kinase (CDK) machinery. However, the fact that CDKs came late in the evolution of eukaryotes, and the fact that oscillations in global transcription and the anaphase promoting complex activity were also found in during cell cycle arrest, suggest that cell cycle regulators external to the cyclin/CDK machinery could exist. We hypothesized that an autonomous metabolic oscillator could represent such global cell cycle regulator. Using microfluidics, in combination with single-cell metabolite and cell cycle reporters, we found that yeast metabolism is a CDK-independent oscillator, which orbits across nutrients and at different metabolic modes, in synchrony with the cell cycle, but also in non-dividing cells. Using environmental perturbations and conditional protein depletion experiments, we found that the metabolic oscillator and the cyclin/CDK machinery form a system of coupled oscillators. Through analysis of our experimental data with a Kuramoto model, we unraveled the high-level topology of this coupled oscillator system. In this system, the metabolic oscillator robustly gates the phase of the early and the late cell cycle, whereas a minimal metabolic frequency threshold must be reached for the cell cycle to START. This work suggests that cell cycle control is not just the result of the cyclin/CDK machinery, but emerges as a higher order function from coupled and mutually entrained oscillators, including the oscillating metabolism. Given the evolutionary conservation of metabolic pathways across life kingdoms, the metabolic oscillator may constitute an ancestral regulator of cell division.

SG19

**Dissipation and energy propagation across scales in an active cytoskeletal material.**

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Cytoskeletal machines use energy from metabolic processes to generate mechanical forces that pattern living systems. Driving these cellular-scale machines requires a constant influx of energy, which at the protein-scale comes in part from the chemical energy converted by molecular motor proteins into mechanical work. While this energy is required for the proper functioning of cytoskeletal machines, what fraction of energy injected at the protein-scale propagates up to the cellular-scale activity of these machines is unknown. Here, we begin addressing this question by characterizing the energetics of a well-studied *in vitro* system composed of purified microtubules and artificial Kinesin motor clusters, which generates emergent flows on length scales far greater than the size of the constituent components. Using a recently developed picowatt calorimeter, we experimentally measure the system's total rate of energy consumption. By characterizing these flows through quantitative light microscopy, we estimate the rate of viscous energy dissipation by the emergent flows. By combining these, we find that the energetic efficiency of this system is surprisingly low - only approximately one-billionth of the system's total energy consumption propagates to the scale of the emergent flows. We develop a chemical kinetics model which accurately predicts how the rate of energy use scales with ATP and microtubule concentration, but breaks down at high motor concentrations, suggesting an interaction between motors. Finally, we estimate how energetic losses accumulate across scales. Taken together,

these results raise the question of the energetic efficiency of cytoskeletal machines *in vivo* and are a step towards developing a nonequilibrium thermodynamics of living systems.

SG20

**Regulation of nuclear morphology under conditions of cellular stress.**

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Normal cells have a nuclear size within a defined range, while aberrations in nuclear size and shape are often characteristic of diseased conditions, for instance in cancers and laminopathies. Certain factors have been identified to play a role in the regulation of nuclear size, however mechanisms that regulate nuclear size, shape, and structure under conditions of stress are largely unknown. We are investigating the impact of osmotic (mannitol), oxidative (hydrogen peroxide), and proteotoxic (proteasome inhibitor MG132) stress on nuclear morphology, studying nuclei assembled de novo in *Xenopus* egg extracts as well as in *Xenopus* A6 and HeLa cells. The extract system allows us to investigate transcription-independent stress responses, and the extract's biochemical composition can be easily manipulated. We found that MG132-treatment reduced the stability of nuclei during assembly in extracts, as observed by frequent ruptures, while osmotic and oxidative stress had little effect on the nuclear assembly process. All stress conditions induced changes in nuclear morphology in extract-assembled nuclei as well as in cells, typically a reduction in nuclear size and wrinkling of the nuclear envelope. Delocalization of Lamin B3 and the appearance of ring-like intranuclear structures that stained for Aquaporin 2 were characteristic of stress-treated nuclei assembled in extracts. We also observed the presence of nuclear Aquaporins in *Xenopus* A6 cells and in HeLa cells. To investigate the mechanistic basis for stress-induced changes in nuclear morphology, we performed mass spectrometry to compare the proteomes of unstressed and stressed egg extracts. of the dozens of proteins whose levels were affected by stress, regulators of microtubule dynamics were a prominent hit. Consistent with microtubules contributing to stress-induced changes in nuclear morphology, we observed that microtubule dynamics were altered in stress-treated extracts and that microtubule depolymerization could partially rescue stress-induced changes in nuclear morphology. Our future work will elucidate microtubule-based and other mechanisms that regulate nuclear morphology in response to cellular stress.

SG21

**ERK signaling regulates nuclear divisions in fly embryos.**

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The extracellular signal regulated kinase (ERK) cascade governs animal development and is implicated in neurodegenerative diseases and cancers when dysregulated. In the 2-3 hour *Drosophila* embryo, ERK phosphorylates the transcriptional repressor CIC and releases it from the regulatory regions of genes that promote head and tail development of the future larva. Contrary to this simple circuit, the list of ERK substrates in other organisms is long and functionally diverse. Whether or not ERK's role in the early fly embryo extends beyond gene de-repression remains unknown. Here, we sought to uncover novel ERK functions using acute optogenetic perturbations and phosphoproteomics, and found that upregulated phosphoproteins are enriched for cell cycle machinery. This effect is consistent with an observed decrease in inhibitory phosphosites on cyclin-dependent kinase 1 (CDK1), a master regulator of mitotic entry. Based on this, we show with loss-of-function genetic tools and ectopic optogenetic

stimuli that cell cycle speed and synchrony in the embryo require endogenous ERK signaling at the poles. Together, these results reveal that the processes controlled by ERK in the early embryo are more extensive than previously thought.

SG22

**Changing nuclear pore numbers regulates nuclear composition and early embryonic development.**

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The precise temporal coordination of cellular processes is critical for cellular homeostasis and cell state transitions. For example, the maternal-to-zygotic transition (MZT) is an early milestone in animal development, where activation of the zygotic genome, the emergence of ordered DNA replication domains, and cell cycle remodeling all occur in parallel. However, it remains poorly understood how these pathways are coordinated to achieve a robust cell state transition. One possible mechanism involves modulation of cellular machinery that is common to these multiple pathways. Here, we show that the nuclear pore complex (NPC), the gateway for macromolecular trafficking across the nuclear membrane, regulates the MZT in *Drosophila*. Using an endogenously-tagged nuclear pore marker, we find a progressive reduction in NPC number during early embryogenesis without a change in the composition of individual NPCs. FRAP (fluorescence recovery after photobleaching) experiments demonstrate a corresponding slowing of the rate of passive transport. Notably, we find that changes in NPC number do not uniformly affect the nuclear transport of all cargoes. We observe that reduced numbers of NPCs results in increased nuclear accumulation of NLS-tagged RFP. This somewhat counterintuitive result suggests that the number of NPCs modulates the balance of passive and active nuclear transport processes, resulting in cargo-specific changes in nuclear composition. Importantly, given that many of the previously identified MZT regulators are nuclear proteins, the developmentally changing number of NPCs may serve as a global mechanism that coordinates the activities of multiple pathways through the regulation of their nuclear transport. Indeed, reduction of NPC number by RNAi leads to an overall slowing of cell cycle progression and delays the MZT. Together, our data suggest a model in which the changing number of NPCs sets the pace of early embryonic development by modulating nuclear composition.

SG23

**Epigenetic Mechanisms Underlying Spatiotemporal Activation of Germ Layer-Specific Programs in Early Embryogenesis.**

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In early embryo development, zygotic genome activation (ZGA) is essential for initiating gene expression to specify diverse cell types, including forming the three germ layers ectoderm, mesoderm, and endoderm. The order of the germ layer onset is debated, and little is known about the mechanisms underlying hierarchical onset of germ layers during ZGA. Using *Xenopus* early embryos as a model, we previously discovered that the ectoderm genes are activated earlier than the endoderm genes, consistent with a gradual spatiotemporal pattern of ZGA in the blastula, but whether the epigenetic mechanisms are involved in its regulation remain to be studied. To test whether chromatin accessibility is correlated with the spatiotemporal ordering of germ layer specific onset, we developed an ATAC-seq protocol that is friendly for yolk-rich early embryos. By profiling the chromatin accessibility in early-stage embryos before, during and after ZGA, we discovered that while the chromatin accessibility is gradually

gained for the promoter regions of both ectoderm and endoderm genes, it is much higher in ectoderm genes than endoderm genes once ZGA starts. This finding supports the notion that chromatin accessibility primes ZGA and that sequential germ layer-specific onset is correlated with the chromatin accessibility. To further test whether it holds true in space - the animal pole (AP) is the presumptive ectoderm and the vegetal pole (VP) is the presumptive endoderm, we performed ATAC-seq on the AP and VP regions individually dissected from early embryos. Surprisingly, we found that the chromatin accessibility of endoderm genes is higher than that of ectoderm genes when ZGA starts. These data suggest that distinct spatial mechanisms exist to coordinate the sequential germ layer onset. In supporting this, ChIP-seq analysis for histone marks in different regions of early embryos show spatial bias. Overall, our study provides new insights into epigenetic mechanisms underlying sequential spatial onset of cell fate specification in early embryo development.

SG24

**Optogenetic control of YAP reveals a dynamic communication code for gene activation and cell fate.**

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YAP is a transcriptional regulator that transmits extracellular information to gene expression programs driving developmental decisions such as pluripotency, proliferation and differentiation. How cells ensure selective gene activation to choose between these programs has remained unclear. Leveraging optogenetics, live-imaging of transcription, and cell fate analysis we reveal that embryonic stem cells decode the concentrations and timing of YAP activation to control proliferation, cell fate, and expression of the pluripotency regulators Oct4 and Nanog. We reveal that oscillatory YAP inputs mimicking endogenous YAP dynamics induce Oct4 expression and proliferation. In contrast, cellular differentiation acts as a persistent detector decoding low YAP levels. Using live imaging of transcription, we demonstrate that the dynamic decoding capacity of Oct4 is based on an adaptive change sensor. Together, we reveal how YAP concentrations and dynamics enable multiplexing of information transmission for the control of developmental gene activation and cellular decision-making.

SG25

**Reconstruction and deconstruction of human somitogenesis with stem cells.**

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The body of vertebrates displays a segmental organization which is most conspicuous in the periodic organization of the vertebral column and peripheral nerves. This metameric organization is first implemented when somites, which contain the precursors of skeletal muscles and vertebrae, are rhythmically generated from the presomitic mesoderm (PSM). Somites then become subdivided into anterior and posterior compartments essential for vertebral formation and segmental patterning of the peripheral nervous system. How this key somitic subdivision is established remains poorly understood. Here we introduce novel tridimensional culture systems of human pluripotent stem cells (PSCs), called Somitoids and Segmentoids, which can recapitulate the formation of epithelial somite-like structures with antero-posterior (AP) identity. Using these systems, we identified a key organizing function of the

segmentation clock in converting temporal rhythmicity into the spatial regularity of anterior and posterior somitic compartments. We show that an initial salt-and-pepper expression pattern of the segmentation gene MESP2 in the newly formed segment is transformed into defined compartments of anterior and posterior identity via an active cell sorting mechanism. Our work demonstrates that the major patterning modules involved in somitogenesis including the clock and wavefront, AP polarity patterning and somite epithelialization which are normally integrated during development, can be dissociated and operate independently in our in vitro systems. Together we put forward a novel framework accounting for the symmetry breaking process initiating somite polarity patterning. Our work provides a valuable platform to decode general principles of somitogenesis and advance knowledge of human development.

## Subgroup: Building the Cell

SG26

**Chiral and nematic phases of flexible active filaments.**

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The emergence of large-scale order in self-organized systems relies on local interactions between individual components. During bacterial cell division, the tubulin-homolog FtsZ polymerizes into treadmilling filaments that further organize into a cytoskeletal ring. In *in vitro* assays FtsZ filaments demonstrate a striking capacity to form dynamic chiral assemblies. However, how the active and passive properties of individual filaments relate to these large-scale self-organized structure remains poorly understood. Here, we combined minimal active matter simulations, biochemical reconstitution experiments with fluorescence microscopy and high-speed AFM, to bridge the gap between single filament properties and the mesoscopic scale. Our experiments and simulations reveal that density and flexibility of active chiral filaments define their global order: At intermediate densities, curved, flexible filaments organize into chiral rings and polar bands. An effectively nematic organization dominates for high densities and for straight, mutant filaments with increased rigidity. Our predicted phase diagram captured these features quantitatively, demonstrating how the flexibility, density and chirality of active filaments affect their collective behavior. Our findings shed light on the fundamental properties of active chiral matter and explain how treadmilling FtsZ filaments organize during bacterial cell division.

SG27

**Length control is an emergent property of actin cable bundles.**

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The sizes of many subcellular structures are coordinated with cell size to ensure that these structures meet the functional demands of the cell. Until now, our understanding of how cells control the size of their subcellular structures has relied upon mechanisms that confer size-dependent feedback on the rate at which molecular building blocks are either added or removed from that structure. Here, we

present and experimentally test a new mathematical model of actin cable length control that explores how the specific geometry and architecture of a cable can encode length control. This model is a significant departure from previous length control models, because there is no length-dependent molecular feedback mechanism that tunes the rates of assembly or disassembly. Instead, the control over actin cable length naturally emerges from the geometric arrangement of the filaments within this higher order cytoskeletal network. We use this model to further dissect the mechanisms that allow actin cables to grow longer in larger cells, and identify specific assembly factors that enable cells to scale cable length with cell length. This work reveals a new strategy that cells use to coordinate the size of their internal parts with their linear dimensions. We suspect that similar design principles may control the size and scaling of other subcellular structures whose physiologically important dimension is their length.

SG28

**Friction patterns guide actomyosin network contraction on micropatterned surfaces.**

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Actomyosin network self-organization and contraction play essential roles within the cell. Through molecular-scale interactions of myosin with actin filaments, global cell-scale contractile behaviors emerge, harnessed for cellular processes such as cell organization, motility, and intracellular transport. However, the relative importance of these active, local stresses compared to anchoring/friction forces and cell geometry in directing actin network deformation is still not well understood. Here we tackle this question using a combined experimental and modeling approach.

Our experimental efforts employed reconstituted actomyosin networks on micropatterned surfaces of various cell-sized shapes, where we modulate friction by patterning on glass or lipid bilayers. We found that on both surfaces, the system self-organizes with myosin rapidly condensing into a few randomly positioned aggregates, triggering a contraction process. Interestingly, regardless of myosin distribution, the whole network would contract to the pattern's geometric center, with faster deformation on lipids pointing to the role of friction in resisting network contraction. We then designed heterogeneous micropatterns comprised of both glass and lipids and found, notably, that the compaction point was biased to more adhesive regions.

To uncover the mechanisms underlying this robust, friction-dependent contraction, we developed a mathematical model of the actomyosin network as a 2D deformable viscoelastic cable-network material with active contractile stresses generated by advected myosin. The internal network forces were balanced by drag due to friction with the underlying surface.

Analysis and numerical simulation demonstrated that our model successfully reproduces key experimental results. Most notably, the model predicts robust, friction-dependent contraction and explains why the friction, not myosin, pattern determines the compaction point through a center of drag argument.

In conclusion, our findings show how robust, cell-scale contractile behaviors can arise from patterning of resistive forces, independent of spatial patterning of active forces driving the contraction. This work explains how homogeneous networks could contract asymmetrically in cells and tissues and has broad implications of how contractile actin networks might behave in cells which can generate or harness gradients of resistive forces.

SG29

**Studying Motor-Free Ultrafast Motion in Synthetic Cells.**

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Ciliated protists are single celled eukaryotic organisms with a striking particularity - some of them display the fastest motions in the biological world! the mechanism that enables such phenomenon is a motor-free, calcium-based cytoskeletal system. Unlike the actin-myosin based cytoskeletons, ATP is not directly consumed; a local increase in cytosolic $Ca^{2+}$ concentration is what triggers the contraction. In this project, we aim to study the mechanical effects of calcium-based motor-free contraction in the cellular membrane, and how the interplay between these two elements allows ultrafast cell motility. We study this system by using a bottom-up approach: we are going to build a synthetic contractile system inside of a cell-sized liposome, which mimics the structure and composition of a cellular membrane. The main component of our is tetrahymena calcium-binding protein 2 (tcb2), the main component of *Tetrahymena termophila* cytoskeleton that shows calcium-dependent contractile behavior. In order to create a controlled artificial calcium increase within the liposome, we incorporate a compound that chelates calcium and releases it upon irradiation with UV light. The method of choice for assembling the synthetic cells is cDICE (Continuous Interface Crossing Encapsulation). The results display that we are able to trigger the contraction of the protein network inside the liposomes by using light, from which we quantify the speed of contraction and the membrane deformations that arise from it. The study of motor-free cytoskeletons is important in the context of bioengineering, since it opens new venues for the design of more energetically efficient systems and devices. The ability to optically control calcium signaling pathways is also a rising field in biomedicine, which has already offered solutions for diagnosis and treatment of various diseases, including cancer, and several muscular and neurological disorders. The convergence of these two disciplines in the presented work will contribute to building a solid ground for the development of the next generation of optically-controlled bioactuators and biosensing devices.

SG30

**Digital Organization of sheet and rim Golgins at Golgi Apparatus.**

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The protein organization of the Golgi apparatus remains incompletely understood despite its well-established compartmentalized membrane by electron microscopy. Here we used multicolor super-resolution microscopy 4Pi-SMS to map the relative positions of golgins at 10+ nm resolution. We found that golgins are organized into hierarchical sheet-like and rim structures with minimal overlap. Different rim Golgins localize to the rims of cis (GMAP-210), medial (Giantin, Golgin84, TMF1), trans (GCC185) cisternae. The localization of Golgins strongly correlates with their propensity of forming oligomers and long filaments *in vitro*. Our precise mapping of golgins enables future investigation of the structure and functions of Golgi apparatus at molecular level.

SG31

**A tubule-sheet continuum model for the mechanism of nuclear envelope assembly.**

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Nuclear envelope (NE) assembly defects cause chromosome fragmentation, cancer, and aging. However, major questions about the mechanism of NE assembly and its relationship to nuclear pathology are unresolved. In particular, how cells efficiently assemble the NE starting from vastly different, cell type-specific endoplasmic reticulum (ER) morphologies is unclear. Here, we identify a NE assembly mechanism, ‘‘membrane infiltration,’’ that defines one end of a continuum with another NE assembly mechanism, ‘‘lateral sheet expansion,’’ in human cells. Membrane infiltration involves the recruitment of ER tubules or small sheets to the chromatin surface by mitotic actin filaments. Lateral sheet expansion involves actin-independent envelopment of peripheral chromatin by large ER sheets that then extend over chromatin within the spindle. We propose a ‘‘tubule-sheet continuum’’ model that explains the efficient NE assembly from any starting ER morphology, the cell type-specific patterns of nuclear pore complex (NPC) assembly, and the obligatory NPC assembly defect of micronuclei.

SG32

**Evaluating rules of intracellular organization in human induced pluripotent stem cells using generative computational models via cellPACK.**

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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 hiPSCs transition between states during differentiation and disease. To achieve this, we develop a variety of assays and computational tools that are openly available at allencell.org. Here we describe an iterative model development pipeline to test hypotheses for rules underlying intracellular organization in hiPSCs using peroxisomes and endosomes as initial structures of interest. cellPACK is an open-source software that builds 3D spatial models of the biological mesoscale, integrating entities ranging from ~10 to 10,000 nm in size. We leveraged the generative modeling capabilities of cellPACK to simulate hypothetical distributions of subcellular structures within experimentally measured cellular/nuclear shapes of hiPSCs. We applied our previously developed analysis framework for integrated intracellular organization to create parameterized intracellular location representations (PILRs) for peroxisomes and endosomes in hiPSCs as well as their simulated distributions generated by cellPACK. The PILR establishes a shape-independent intracellular coordinate system and enables interpretable quantitative comparisons between observed and simulated distributions of subcellular structures. Starting with peroxisomes, we evaluated a null hypothesis of random distribution due to a lack of any previously established organizational pattern. We compared the average PILRs for peroxisome distribution in hiPSCs with cellPACK simulations and found that a bias towards the nuclear membrane correlated best with observations. In contrast to peroxisomes, observations suggest endosomes may prefer localizing at the apical surface of cells. We used our analysis pipeline to investigate the directionality and strength of a potential apical-basal polarization following a similar approach for peroxisomes. Our unique multimodal data-driven approach lets us go from quantitative

descriptors to generative rules of intracellular organization. Ongoing developments of the cellPACK toolkit will extend these analyses to subcellular structures with more complex geometries or interactions to uncover an expansive library of organizational rules.

SG33

**Measuring and modeling the dynamics of mitotic error correction.**

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When functioning properly, the mitotic spindle segregates an equal number of chromosomes to daughter cells with high fidelity. Over the course of spindle assembly, many initially erroneous attachments between kinetochores and microtubules are fixed through a process called error correction. Despite the importance of chromosome segregation errors in cancer and other diseases, we lack methods to characterize the dynamics of error correction and how it can go wrong. Here, we present an experimental method and analysis framework to quantify chromosome segregation error correction in unsynchronized human tissue culture cells with live cell confocal imaging of spindle assembly, timed premature anaphase, and automated counting of kinetochores after cell division. Our results are consistent with an exponential decrease of errors over time. A coarse-grained model, in which errors are corrected in a chromosome autonomous manner at a constant rate, can quantitatively explain both the measured error correction dynamics and the distribution of anaphase onset times. We further validated our model by measuring error correction rates under perturbations that destabilize microtubules and change the initial configuration of chromosomal attachments. Taken together, this work provides a quantitative framework for understanding the dynamics of mitotic error correction. Ongoing work is determining how these error correction dynamics are perturbed in cancer.

SG34

**Advected Percolation: A Unifying Principle for Amoeboid Cell Locomotion in Complex 3D Environments.**

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Spontaneous locomotion is a fundamental characteristic of animal cells, typically attributed to the actomyosin network's essential properties. While the details of this force-producing machinery have been extensively studied, particularly in lamellipodium-driven migration, a unifying principle for understanding actomyosin network behavior inside contraction-driven amoeboid cells remains elusive. In this study, we performed live imaging on stable motile blebs to examine the actin cortex dynamics at the single filament level. Surprisingly, we uncover the existence of three distinct phases of the actin network, each exhibiting unique rheological properties (gas, passive solid, and contractile solid), respectively distributed at the front, middle, and rear regions of cells.Through physical modeling, we unveil that these three phases spontaneously organize due to a rigidity percolation transition, coupled with an active advection of the percolated network. We term this phenomenon "advected percolation." This novel mechanism provides a minimal and generic explanation for amoeboid cell locomotion and sheds light on how these cells efficiently propel themselves through complex 3D environments, a trait shared by immune and cancer cells alike. Furthermore, our model elucidates the formation and shape of

stable blebs, the diverse front dynamics observed in amoeboid cells, and the intriguing phenomenon of migrating cytoplasts originating from blebbing cells, as recently observed through intravital microscopy.Our findings present a new understanding of the mechanical properties of the actin network from the scale of single actin filaments to the entire cell and offer valuable insights into amoeboid cell locomotion. Given its significance in both physiological and pathological contexts, the concept of advected percolation opens new avenues for therapeutic interventions aimed at modulating cell migration in immune response regulation and cancer metastasis inhibition.

## Subgroup: Cell Death 2.0: Novel and Diverse Killing Modalities across Systems

SG35
**Programmed Cell Death in Microorganisms.**
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Programmed cell death is intrinsic to cellular life, and likely arose before multi-cellularity. However, cell death research has focused on animal models to understand cancer, degenerative disorders and developmental processes. Although once a rejected concept, recent advances have accelerated our understanding of self-inflicted cell death in microorganisms. Elegantly delineated pathways in bacteria reveal ancient origins of animal cell death intertwined with immunity, and are likely to be samplings of the true diversity of death pathways. Much less is known about fungal cell death, and this topic has not yet been explored in most actively studied human pathogens. We identified a vesicle trafficking fungal cell death pathway using the model organism *Saccharomyces cerevisiae* that appears to be conserved in the human pathogen *Cryptococcus neoformans*. A genome-wide screen identified five death-promoting genes from two vesicle trafficking pathways. One of these requires the AP-3 vesicle trafficking complex, which transports newly made membrane-associated proteins to the lysosome/vacuole membrane. Deletion of any AP-3 subunits or one of its cargo proteins, the Yck3 kinase, results in resistance to several cell death stimuli and inhibits permeabilization of the lysosome/vacuole membrane. Vesicle trafficking pathways also appear to promote cell death in the human opportunistic pathogen *Cryptococcus neoformans*, a free-living yeast found in the environment and the cause of significant mortality worldwide. Death-resistance in vitro appears to correlate with virulence in mouse, further suggesting that cell death-resistance may be an important previously unrecognized feature of fungal disease pathogenesis. This shift in current thinking raises the possibility of alternative therapeutic strategies for treating fungal infections.

SG36
**Ribosome collisions is targetable vulnerability in glioma during ferroptosis exposure.**
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Accumulating evidence has revealed the particular characteristics of ferroptosis in glioma cells, demonstrating the potential therapeutic effect of ferroptosis inducers on gliomas. Despite advances, ferroptosis resistance in glioma due to high tumor heterogeneity and suppressive tumor microenvironment is still a challenge. Here, we used genome-wide CRISPR screening to identify that Endothelial Differentiation Factor 1 (EDF1) confers resistance to ferroptosis in glioma cells. Previous research has implicated EDF1 in ribosome quality control (RQC) by protecting against ribosome collisions. Indeed, during ferroptosis, ribosome collision were increased, evident by the activation of the

ribotoxic stress. Public database search revealed that EDF1 was elevated in glioma and its high expression was associated with reduced patient survival. We show that EDF1 regulates glioma proliferation, stemness, and response to ferroptosis. Mechanistically, we show that EDF1 maintains proteostasis by preventing global translational reduction and regulating Ribotoxic Stress Response (RSR) pathway during ferroptotic stress. By integrating multi-omics analysis, we found translation, translation efficiency, and mRNA stability of certain ferroptosis-related genes (FRGs) were modulated by EDF1. Our findings established EDF1 as a regulator of ferroptosis at the level of mRNA translation and a potential therapeutic target for glioma.

SG37

**Phagocytosis Machinery Is Co-Transcriptionally Regulated to Promote Non-Apoptotic Developmental Germline Death.**

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Vacuolar-ATPases (V-ATPases) are ATP-driven mechanoenzymes that transport protons across membranes to maintain organelle acidity. During oogenesis in *Drosophila melanogaster*, these complexes are harnessed by terminally differentiated somatic cells called Stretch Follicle Cells (SFCs) to cull the germline. At the close of oogenesis, SFCs express V-ATPases on their plasma membrane, which allows SFCs to acidify the germline externally, thereby promoting its death non-apoptotically. To understand how SFCs exploit V-ATPase function at the appropriate spatiotemporal juncture, we profiled V-ATPase activity in the egg chamber using multiple modalities, under various conditions. We leveraged two publicly available single cell RNA-seq atlases of the fly ovaries by integrating them and performing pseudotime analysis to place SFC sub-populations along the developmental trajectory and uncovered expression dynamics of V-ATPase subunits along this trajectory. Next, we performed TRAP-seq to identify differentially translated mRNAs in SFCs. Using HITindex, we identified that the second exon of the V-ATPase 'a' subunit, was enriched in SFCs. To observe the localization and assembly of the complex, we performed multiplexed immunohistochemistry (mIHC) with antibodies against subunits in both the V0 and V1 domains, which revealed that the complex is basally polarized during mid oogenesis, whereas uniformly punctate in younger SFCs, indicating vesicular expression, ultimately becoming diffuse due to plasma-membrane enrichment at the interface of SFCs and the germline. We previously determined that RNAi against certain subunits impaired acidification. We further characterized the components of the complex required for proper localization, assembly, and acidification in these mutants using mIHC and Lysotracker staining. Finally, to determine the mechanism by which the complex is trafficked to the plasma membrane of SFCs, we perturbed genes in the microtubule-mediated transport and SNARE-mediated membrane fusion pathways, as well as candidates identified from the scRNA-seq and TRAP-seq datasets using *in vivo* RNAi and CRISPR-Cas9 techniques. Collectively, these experiments demonstrate how evolutionarily conserved proteins with hyper specialized functions can be coopted in a context-dependent manner, facilitated by multiple-levels of regulation.

SG38

**Metabolic control of inflammatory cell death.**

**C. L. Evavold**; Ragon Institute of Mass General, MIT, and Harvard, Massachusetts General Hospital, Boston, MA.

Reactive oxygen species (ROS) regulate the activities of inflammasomes, which are innate immune signaling organelles that induce pyroptosis. The mechanisms by which ROS control inflammasome activities are unclear and may be multifaceted. Herein, we report that the protein gasdermin D (GSDMD), which forms membrane pores upon cleavage by inflammasome-associated caspases, is a direct target of ROS. Exogenous and endogenous sources of ROS, and ROS-inducing stimuli that prime cells for pyroptosis induction, promote oligomerization of cleaved GSDMD, leading to membrane rupture and cell death. We find that ROS enhance GSDMD activities through oxidative modification of cysteine 192 (C192). Within macrophages, GSDMD mutants lacking C192 show impaired ability to form membrane pores and induce pyroptosis. Reciprocal mutagenesis studies reveal that C192 is the only cysteine within GSDMD that mediates ROS responsiveness. Cellular redox state is therefore a key determinant of GSDMD activities.

SG39

**Mitochondrial transport directed by CED-3/caspase and opposing kinesins regulates Compartmentalized Cell Elimination.**

**P. Ghose**; the University of Texas At Arlington, Arlington, TX.

Polarized cells with elaborate architecture such as neurons are a common feature amongst metazoans, and, like many other cells, may be eliminated during normal development as well as pathological or injury contexts. Nevertheless, precise molecular mechanisms demise of morphologically complex cells remains to be fully understood. In addition, highly intricate cells have different compartments that may be subject to different degeneration modalities and such cells can be removed in full or in part (as in region-specific neurite pruning). We discovered a novel 'tripartite' cell death program in the embryo of the nematode *C. elegans* whereby different compartments of two sets of morphologically complex cells, an epithelial cell and a set of sensory neurons, die is disparate ways, which we term Compartmentalized Cell Elimination (CCE). CCE bears striking hallmarks to developmental pruning with the proximal segment of the cell's long process/dendrite undergoing beading and fragmentation and the distal segment retracting. While cell body elimination resembles apoptotic death of simpler cells, CCE is genetically distinct from apoptosis, though it does require the main caspase protease of *C. elegans*, CED-3. We have previously shown that partial loss of CED-3 results in compartment-specific cell death, with mutants often having only the soma or only the process persist. We subsequently asked how these fragments are salvaged and noted that mitochondria are reported to protect against injury-induced axon degeneration as well as assist in regeneration, and that a role for these organelles in developmental pruning has not been defined. As such, we examined whether mitochondria are present in the persisting process fragments. We found this to be so and also that, in wild type embryos, mitochondria travel retrogradely prior to cell death. A forward genetic screen and several other data reveals the microtubule motor UNC-116/Kinesin 1 is required for this retrograde transport of mitochondria and a candidate gene screen and supporting data reveals that CED-3/Caspase targets another motor UNC-104/Kinesin 3 to prevent anterograde traffic, thus keeping the cell process free of protective mitochondria in preparation for cell death. Our findings illuminate a novel regulation for complex cell elimination, a molecular mechanism for the novel phenomenon of CCE, presents a new caspase target and a second non-killing function of

CED-3/caspase and reveals mitochondria as a previously unknown cargo of UNC-104/Kinesin 3. We also provide insight on the regulation of developmental neurite pruning, and by extension neurodegeneration, by calling to attention a role for mitochondria and their transport.

SG40

***Clearing the dead:*** **Hair follicle stem cells orchestrate apoptotic cell phagocytosis to maintain tissue homeostasis.**

**K. Stewart**, E. Fuchs; the Rockefeller University, New York, NY.

Apoptotic cell clearance occurs in most tissues throughout the adult body and is absolutely critical to maintain immune homeostasis. Although it has been long recognized that epithelial cells contribute to the clearance of dying cells, remarkably little is known about how phagocytic ability is regulated in an epithelial tissue, nor about the functional impact and fitness consequences to these “non-professional” phagocytes. The murine hair follicle is well-suited for tackling this problem, as it undergoes cyclical bouts of tissue (hair) growth. As part of this process, the mature hair-generating follicle suddenly enters a period of massive apoptosis (“catagen”), where only some stem cells survive to regenerate a new follicle upon the launching of a new cycle. Interestingly, during this process, the epithelial cells, including the stem/progenitor cells, clear their neighboring corpses by phagocytosis. Through single cell RNA sequencing and accessible chromatin profiling of the hair follicle stem/progenitor cells we identify a heterogenous upregulation of a phagocytic program during catagen, concomitant with an expansion of the lysosomal machinery. Using genetic loss and gain of function approaches, we further link the expression of the phagocytic program in hair follicle stem/progenitor cells to activation of retinoid X receptor (RXR) signaling. Finally, we demonstrate that activation of RXR signaling occurs in response to secreted signals from dying hair follicle cells, offering an elegant mechanism to specify non-professional phagocytes near to where they are required within the constraints of an epithelial tissue. This novel paradigm is likely to be broadly applicable to adult tissues, which must constantly eliminate dying cells to maintain immune and epithelial tissue homeostasis.

SG41

**Cholesterol controls location and pore forming activity of mammalian gasdermins.**

**Y. Liang**, V. De Andrade, C. L. Evavold; Ragon Institute of Mass General, MIT, and Harvard, Massachusetts General Hospital, Boston, MA.

Pyroptosis is a lytic form of cell death that requires pore formation by members of the gasdermin family of proteins. Upon proteolytic cleavage, gasdermin family proteins release N terminal pore forming fragments that execute unconventional cytokine secretion and lytic cell death. A number of host- and pathogen-derived proteases are known to positively mediate this conversion during pyroptosis. Moreover, positive associations between acidic phospholipids including phosphatidylserine, phosphoinositide, or cardiolipin are thought to encourage gasdermin targeting of specific membranes, such as the plasma membrane and mitochondrial membrane, during cell death. Herein, we report an inverse relationship between host cholesterol content and gasdermin pore forming activity. Through survivor enrichment analysis after expression of the N terminal fragment of gasdermin D (NT-GSDMD), we discovered specific endosome adaptors control NT-GSDMD pore formation at the plasma membrane. These endosome adaptors include Rabgef1 and Vps family proteins involved in the HOPS/CORVET complexes. Mechanistically, deficiency in these endosome adaptors cause cholesterol accumulation in membranes within macrophages that result in defects in NT-GSDMD pore formation.

Depletion of cholesterol through several orthogonal means rescues pyroptosis in macrophages. Prior studies have illustrated that cells adapt the cholesterol content of the plasma membrane to prevent bacterial intoxication and viral entry. Our results suggest that these same cholesterol adaptations that endow a cell with resistance to initial pathogen encounter also poise the host to commit to pyroptosis.

SG42

**Non-apoptotic developmental cell death in development and neurodegeneration.**

**O. Yarychkivska**, A. Milocevic, S. Shaham; the Rockefeller University, New York, NY.

Cell death is a major developmental cell fate. While apoptotic cell death has been extensively explored, it does not account for all developmental cellular destruction. Studies in *C. elegans* uncovered a developmental cell death program, linker cell-type death (LCD), which is morphologically and molecularly distinct from apoptosis. LCD, which eliminates the male-specific linker cell, is accompanied by nuclear envelope crenellation, cytoplasmic organelle swelling, and cell splitting into unequal fragments. Cell death with common features is often observed during vertebrate development and in neurodegenerative diseases, suggesting that LCD may play an important role in these settings. Consist with this idea, previously-identified LCD genes have been implicated in neurodegeneration. To identify additional LCD genes, we performed a linker-cell-specific RNAi screen, seeking genes whose inactivation results in inappropriate linker cell survival. Among these genes, several encode cell-cycle and cytokinesis regulators. Loss of one of these, NMY-2/Myosin, a component of the cell-division contractile ring, blocks LCD. An NMY-2::GFP fusion protein progressively aggregates as LCD proceeds, and ~80% of the protein is segregated into the anuclear cellular fragment. Concomitantly, TBA-1/tubulin, another cytoskeletal protein, also aggregates. Protein aggregation is common in neurodegenerative disorders, such as Alzheimer's (AD), Parkinson's (PD), and Huntington's (HD) diseases. To further probe the connection between LCD and neurodegeneration, we are performing a transcriptome analysis of *C. elegans* linker cells at different stages of the death process using FACS purified cells and 10x single-cell RNA sequencing. We aim to assess whether LCD and neurodegeneration have common gene expression signatures. A preliminary comparison of our transcript list with a compendium assembled from neurodegenerative disease models and patients using a network-guided differential expression analysis (NetDiff) and network-guided shortest path method (NetPath) developed by the Troyanskaya lab at Princeton University, identified *nmy-2*/MYH9 as a highly ranked gene in AD, PD, and HD. Our findings uncover commonalities between LCD and neurodegenerative diseases, and raise the possibility that some neurodegenerative conditions may result from inappropriate activation of a common cell death program.

## Subgroup: Emerging Cell Biology Concepts in Neural Function and Disease

SG43

**TRIM9 regulates netrin dependent morphogenesis through the regulation of DCC and UNC5C on the plasma membrane.**

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Netrin-1 is a guidance cue that generates both attractive and repulsive axon responses. One potential mechanism by which netrin-1 elicits multiple guidance responses is through the engagement with distinct receptors on the membrane. Using microfluidic-based and bulk netrin treatment assays, we

have previously shown that netrin-1 induces a bifunctional response, attraction at lower concentrations, and repulsion at higher concentrations. Here we show both attractive and repulsive turning and growth cone responses are disrupted by the loss of TRIM9. TRIM9 is a neuronally enriched E3 ubiquitin ligase that interacts with the attractive netrin receptor DCC and is required for the appropriate development of the corpus callosum. We demonstrate that TRIM9 also interacts with the repulsive netrin receptor, UNC5C using a mutant of TRIM9 lacking its ligase domain (TRIM9ΔRING) and coimmunoprecipitation assays. Further, using TIRF-based live imaging, we find partial colocalization of UNC5C receptor with TRIM9ΔRING at the membrane, suggesting TRIM9 may organize UNC5C. We also observe increased DCC at the plasma membrane of *Trim9*$^{-/-}$ cortical neurons. We hypothesize that the netrin-unresponsiveness of growth cones in *Trim9*$^{-/-}$ cortical neurons at least partially stems from the deregulation of DCC and UNC5C on the membrane. Consistent with this hypothesis, a function-blocking antibody that binds the DCC extracellular domain rescues excessive morphogenesis in *Trim9*$^{-/-}$ cortical neurons, such as aberrant growth cone size and exuberant axon branching. Interestingly, we did observe DCC-UNC5C interaction in a coimmunoprecipitation assay, but the interaction was independent of netrin and TRIM9. Thus, TRIM9 is likely to regulate DCC and UNC5C distinctly. Currently, we are investigating mechanisms of deregulation of DCC and UNC5C receptors in *Trim9*$^{-/-}$ cortical neurons. Ongoing work using FRAP analysis and TIRF-based receptor clustering experiments will highlight the role of TRIM9 in organizing netrin receptors on the membrane. Organized receptors on the membrane provide localized scaffolds to regulate signaling and downstream morphogenesis. Current work will address unknown mechanisms of the formation of the netrin receptor complex and their functions in neuronal morphogenesis.

SG44

**Laminin and BDNF synergistically promote local translation in neuronal growth cones.**

**N. Kirkise**, K. Welshhans; Department of Biological Sciences, University of South Carolina, Columbia, SC.

Accurate neural wiring in the developing brain is mediated by growth cones, which are the pathfinding tips of growing axons that respond to environmental cues to find their synaptic partners. Environmental cues stimulate the local translation of mRNAs, such as *β-actin*, within growth cones to regulate this process of axon guidance. Although it is well-established that neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), stimulate local translation, the contribution of extracellular matrix (ECM) proteins, such as laminins, to this process is poorly understood. Our lab has previously shown that point contacts, which are growth cone adhesions that link the ECM to the intracellular cytoskeleton, are a preferred site for local translation of *β-actin* mRNA. Thus, we hypothesized that laminin may stimulate local translation in axonal growth cones. Embryonic day 17 mouse cortical neurons were acutely stimulated with laminin 111 ± BDNF by bath application and used in (1) an O-propargyl puromycin (OPP) assay to label nascent protein synthesis and (2) a puromycin-proximity ligation assay (Puro-PLA) to label only newly translated *β-actin*. Stimulation with laminin 111 and BDNF together significantly increases local protein synthesis, as compared to either laminin 111 or BDNF alone. Stimulation with laminin 111 and BDNF together also increases axon length and point contact density. Interestingly, stimulation with BDNF alone significantly increases *β-actin* local translation, suggesting that laminin 111 signaling may not be needed for *β-actin* local translation. However, there are multiple isoforms of laminin, each with unique temporal and spatial distributions. Thus, we repeated the puromycin assay using BDNF and either Laminin 111, 511, 211, or 221. Neurons cultured on laminins 211 and 221 in the presence of BDNF show a remarkable decrease in local protein synthesis and axon length compared to the other isoforms, suggesting that laminin isoforms have differential functions during development. Thus, these results

indicate that laminin and BDNF synergistically affect local translation, thereby impacting the speed of growth during axon guidance.

SG45

**Axonal ER tubules regulate local translation via P180/RRBP1-mediated ribosome interactions.**

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Local mRNA translation in axons is critical for the spatial and temporal regulation of the axonal proteome and neuron function. A wide variety of mRNAs are localized and translated in axons, however how protein synthesis is regulated at specific subcellular sites in axons remains unclear. Here, we establish that the axonal endoplasmic reticulum (ER) supports axonal translation in developing neurons. Axonal ER tubule disruption impairs local translation and ribosome distribution. Using nanoscale resolution imaging, we find that ribosomes make frequent contacts with ER tubules in the axon in a translation-dependent manner and are influenced by specific extrinsic cues. We identify P180/RRBP1 as an axonally distributed ribosome receptor that binds specific mRNAs and regulates local translation in an mRNA-dependent manner. Finally, we find that impairment of axonal ER - ribosome interactions causes defects in axon morphology. Our results establish an important role for the axonal ER in dynamically localizing mRNA translation which is important for proper neuron development.

SG46

**Muscleblind-like RNA binding protein trafficking dynamics are regulated by molecular interactions with early and recycling endosomes.**

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Transport of mRNAs to distal parts of the cell and subsequent localized translation are considered crucial for neuronal development and function. Recent findings revealed a role of endosomes in RNA granule transport and as platforms for localized translation in conjunction with RNA binding proteins. Muscleblind-like (MBNL) proteins are RNA binding proteins that regulate alternative splicing and mRNA localization in muscle and brain. MBNLs are depleted from the cytoplasm in myotonic dystrophy type 1 (DM1) by sequestration on the expanded CTG repeats in the 3' UTR of dystrophia myotonica protein kinase (DMPK) transcripts. We have recently demonstrated a role for MBNL-kinesin interactions in mRNA localization although the role of membrane association in this process is not understood. We hypothesize that the unstructured carboxy terminus of MBNL1 regulates endomembrane attachment necessary for mRNA localization. Using immunofluorescence, colocalization of MBNL1 with early (Rab5, Neep21), late (Rab7) and recycling (Rab11) endosomes was observed in n2a cells. Using live imaging in primary neurons, EGFP-MBNL1 colocalization and/or co-transport with early endosomes (Rab5, Neep21), recycling endosomes (Rab11), late endosomes (Rab7) and lysosomes (Lamp1) in neuronal processes was observed. Expression of full-length EGFP-MBNL1-41, but not a carboxy terminus deletion mutant of MBNL1, revealed decreased movement of Rab5 early endosomes and Rab11 recycling endosomes. Conversely, EGFP-MBNL1-41 particle movement was decreased upon co-expression of mCherry-Rab5, and not when co-expressed with Rab5 dominant negative (S43N) mutant. Lastly, we used the MS2 system to image mRNA tracking in live cells, and observed co-trafficking of a MBNL1 target RNA encoding NR1 subunit with EGFP-MBNL1-41 particles and Rab5 early endosomes. We

conclude that MBNL1 mRNA granules are transported with early and late endosomes via piggybacking and that the unstructured C-terminus of MBNL1 acts as an anchor that bi-directionally regulates its mobility with endosomes. Work in progress is to evaluate the role of the C-terminus of MBNL1 in endosome coupled local translation of synaptic proteins. Taken together our findings suggest that one of the mechanisms for MBNL1 to regulate mRNA cargo distribution and localized translation in neurons is endomembrane anchoring.

SG47

**Guidance receptor recycling is required for dendrite growth.**

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During neuronal morphogenesis, adhesion receptors play essential roles in guiding axonal growth cone navigation and mediating dendritic branching. However, it is unclear how guidance receptors are regulated in newly formed neurites to support their growth. Using *C. elegans* dendrite branching receptor DMA-1 as a model, we show that the lateral diffusion of DMA-1 into nascent filopodia is restricted by its binding to its transmembrane ligand SAX-7/L1CAM. Endocytosis of DMA-1 and its subsequent reinsertion onto the plasma membrane via RAB-10 recycling endosomes generate a pool of diffusible DMA-1, allowing for its rapid recruitment to nascent filopodia. The Furin-like proprotein convertase, KPC-1 cleaves the DMA-1 coreceptor, HPO-30/Claudin to enable DMA-1 endocytosis. Inhibition of KPC-1 prevents DMA-1 endocytosis, which leads to severe loss of dendritic arbor. These data reveal a mechanism by which guidance receptor endocytosis and recycling strips transmembrane ligands and regenerates a diffusible pool of receptors on the plasma membrane to promote dendrite outgrowth and branching.

SG48

**Brain-derived neurotrophic factor downstream pathways regulate signaling endosomes transport and RabGTPasas activity in axons and dendrites of cortical neurons controlling neuronal plasticity.**

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The brain-derived neurotrophic factor (BDNF) and its receptor TrkB (tropomyosin kinase receptor B) control the connectivity of different neuronal networks in the central nervous system (CNS) by regulating the activation of signaling pathways leading to transcriptional regulation and translation of proteins. After being bound by BDNF, TrkB is endocytosed into endosomes and continues signaling within the cell soma, dendrites, and axons in signaling endosomes. Although the dynein-mediated transport of BDNF and TrkB in axons has been reported, the functional role of BDNF/TrkB axonal signaling is unknown. Recent lab findings demonstrated that dynein-dependent BDNF-TrkB-containing endosome transport is required for long-distance induction of dendritic growth. On the one hand, axonal signaling endosomes increase PLC-gamma in axons, allowing signaling endosome transport. On the other hand, CREB and mTOR kinase activity in the cell body, and the activity of both last proteins

was required for general protein translation and the expression of Arc, a plasticity-associated gene, indicating a role for BDNF-TrkB axonal signaling endosomes in coordinating the transcription and translation of genes whose products contribute to increase neuronal plasticity.The exact molecular signature of BDNF/TrkB signaling endosomes along the endocytic routes in different parts of the neurons (somatodendritic and axons) and the mechanism that allows them to mobilize in proximity to the nucleus to trigger CREB-dependent transcriptional regulation is not clear. The family of monomeric Rab GTPases is the principal regulator of membrane trafficking and governs the intracellular transport of different organelles. Membrane-bound Rabs bind varying effectors, including microtubule-based molecular motors, participating in membrane recognition and fusion steps. Our lab has shown a bidirectional relation between BDNF signaling and RabGTPas controlling the early-recycling endocytic pathways (Rab5 and Rab11, respectively) to allow membrane transport and neuronal plasticity. This talk will present an integrated view of the cellular mechanism controlling BDNF neuronal plasticity in SNC neurons.

SG49

**Probing Transport Selectivity at Branch Junctions Using Optogenetic Tools.**

**S. Tymanskyj**, B. Curran, A. Escorce, L. Ma; Thomas Jefferson University, Philadelphia, PA.

For complex neuronal function, a neuron generates axonal branches to connect to multiple regions of the brain and spinal cord that are far away from the cell body. This poses two challenging problems of transporting protein, membrane, and RNA that are synthesized in the cell body to nerve terminals: 1) how do cargo travel long distance along axons, and 2) how can they navigate through highly branched axonal networks. Although microtubule-based transport has been well established as responsible for moving cargos via kinesin motors for long distance delivery, it is not clear how transport is regulated at branch junctions along the axon. Recently, using cultured embryonic sensory neurons from the DRG, we showed that transport of some membrane vesicles, such as lysosomes and synaptic vesicles through branch junctions is not random. Rather, transport is selective and influenced by branch length and growth cone dynamics. To probe the mechanism underlying this type of transport regulation, we developed two optogenetic tools. First, to demonstrate the role of growth cone signaling, we generated a bicistronic expression system that uses the CRY2 protein to locally activate receptor activity by light, altering growth cone motility. In combination with lysosome markers, we found that changes in growth cone signaling could rapidly alter transport selectivity at branch junctions, further supporting the notion that transport is not random. Second, to identify the molecular mechanism mediating transport selectivity, we developed an ‘artificial cargo’ system to probe motor functions axons. The artificial cargo is based on protein clusters that are induced by light, or small chemical molecules, or via spontaneous assembly. Combined with an optogenetic approach (based on LOV-PDZ interaction) to recruit motors to these clusters, this approach provides a sensitive way to analyze motor-specific transport in cells. Using this method, we demonstrated the role of MAP7 in regulate motor specific transport as well as the effect on transport by pathogenic mutations found in human motor proteins. We are currently using the approach to determine the role of specific kinesin motor in mediating transport selectivity. Overall, we have developed two optogenetic approaches to understand the mechanisms underlying transport regulation in axons.

SG50

**Non-canonical roles for cell adhesion molecules in presynaptic assembly.**

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The proper formation of synapses is integral to the function of neural circuits. Disruptions in synaptogenesis during early development can lead to neurodevelopmental disorders, such as autism or intellectual disability. Yet despite decades of study, precisely how synapses form, mature, and are maintained has remained elusive. Cell adhesion molecules (CAMs) have been implicated in synaptogenesis, as their extracellular transsynaptic interactions are capable of inducing synapse assembly and conferring synaptic partner specificity. However, the intracellular signaling pathways that mediate their synaptogenic functions are less understood. Using the nematode *C. elegans*, I have identified non-canonical roles in synapse assembly for a pair of CAMs, SYG-1 and SYG-2. I have found that both SYG-1 and SYG-2 are expressed and function in the same presynaptic cell, suggesting they may interact in cis, contrary to their previously described role as transsynaptic binding partners. Furthermore, using over expression assays along with CRISPR transgenesis we have found that the intracellular domain of SYG-2 alone is sufficient for inducing presynaptic assembly, in contrast to the accepted idea that the extracellular domains of CAMs are required for their function. Using CRISPR transgenesis, I am investigating whether SYG-2 and SYG-1 are required together or separately to organize presynaptic specializations. I am also mutating motifs within their intracellular domains to elucidate the downstream intracellular pathways required for their function, as well as residues homologous to SYG-1/SYG-2 human ortholog KIRREL3 that have been implicated in autism. These experiments will provide insight into the possible function and underlying mechanisms of these proteins in regulating synapse development, and how their dysfunction leads to neurodevelopmental disorders.

SG51

**Axonal mitochondria of cortical pyramidal neurons lack mitochondrial DNA and consume ATP.**

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In the mammalian CNS, mitochondria-dependent ATP synthesis is classically thought to play an important role in supporting energy-consuming cellular functions such as neurotransmitter release along axons. However, only 50% of presynaptic boutons along axons of mammalian cortical glutamatergic pyramidal neurons (CPNs) contain mitochondria making the contribution of presynaptic mitochondria to ATP supply in the axon unclear. Axonal mitochondria are also much smaller (~1 micron) than somatodendritic mitochondria in CPNs. As small mitochondria are associated with low oxidative phosphorylation (OXPHOS) capacity, we used multiple independent approaches to determine if axonal

mitochondria contain a nucleoid and mitochondrial DNA (mtDNA). Using (1) immunofluorescence detection of mtDNA and mtDNA associated proteins TFAM or Twinkle, (2) single molecule DNA FISH for mtDNA, as well as (3) quantitative PCR from single axonal mitochondria isolated using fluorescent-based scanning ion conductance microscopy, our results reveal that mtDNA is undetectable in ~80% of axonal mitochondria. The mechanism leading to axons being populated by mitochondria lacking mtDNA appears to involve sorting as timelapse imaging experiments show the vast majority of axonal mitochondria passing through the axon initial segment, from the cell body, lack mtDNA.These results imply most axonal mitochondria in CPNs may be defective in their capacity to mediate OXPHOS. To test this, we performed live imaging using a genetically-encoded, mitochondrial matrix ATP sensor (mt-iATPSnFR1.0), as well as a matrix-targeted pH sensor (mt-SypHer), to visualize mitochondrial properties in the axons of CPNs. Our results show that axonal mitochondria do not generate significant ATP as blocking ATP export from the matrix using BKA does not result in accumulation of mt-iATPSnFR1.0 fluorescence in axonal mitochondria but does in dendritic mitochondria. Furthermore, complex V (ATP synthase) mostly functions in reverse in axonal mitochondria, consuming ATP to extrude $H^+$ out of the matrix to maintain a polarized mitochondrial membrane potential. Thus, our results call for a major revision of the role of axonal mitochondria in CPNs, as they do not play a prominent role in ATP supply despite playing other critical functions, such as presynaptic $Ca^{2+}$ buffering, that require a polarized mitochondrial membrane potential.

SG52

**Dysfunctional autophagy induces compensatory secretion in neurons.**

**S. D. Palumbos**, E. L. F. Holzbaur; Physiology, University of Pennsylvania, Philadelphia, PA.

Neurons rely on autophagy to constitutively degrade damaged proteins and organelles to maintain cellular homeostasis. When neuronal autophagy is perturbed or degradation capacity is overwhelmed, aggregated proteins and damaged organelles accumulate, contributing to neuronal dysfunction or cell death. Indeed, autophagy dysfunction is consistently associated with neurodegenerative diseases such as Parkinson's Disease. We queried the mechanisms by which mouse cortical neurons and human iNeurons respond to either acute or chronic autophagosomal dysfunction. We treated cortical neurons with Bafilomycin A1 to block lysosomal fusion which prompted the upregulation of secretion of extracellular vesicles (EVs) as assessed by nanoparticle tracking analysis (NTA). Similarly, we examined increased secretion of EVs from neurons expressing the Parkinson's Disease-causing mutation, LRRK2 G2019S, which exhibit chronic defects in basal autophagy. These neurons also exhibited a shunt of autophagy cargo toward secretion. Live-cell imaging studies showed that LRRK2 mutants have increased CD63-dependent secretion. This upregulation of CD63 secretion is rescued in the presence of the LRRK2 kinase inhibitor, MLI-2, suggesting that LRRK2 hyperactivity promotes secretion. Finally, analysis of plasma from LRRK2 G2019S animals suggest that upregulation of secretion is also likely occurring in vivo. Together, these observations highlight the induction of EV secretion as an alternative quality control mechanism to dispel cellular waste from neurons when degradation is chronically or acutely strained.

## Subgroup: Evolutionary Cell Biology

SG53

**Conservation and divergence in the uncoupled microtubule networks of *Naegleria*.**

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The microtubule cytoskeleton is remarkable both for its deep conservation as well as its versatility. For example, in all eukaryotic lineages, dynamic microtubules drive mitosis while stable microtubules form the foundation of flagella. The biochemical mechanisms that promote assembly of specialized microtubule networks have been extensively investigated, yet we still lack an understanding of the cellular constraints that guide the diversification of microtubule networks on evolutionary timescales. To explore these constraints, we study the organization and specialization of microtubule networks in the unicellular eukaryote Naegleria gruberi, which last shared an ancestor with animals and fungi over a billion years ago. Naegleria has two genetically and temporally separated microtubule cytoskeletons, one used for mitosis in its amoeba form and the second to rapidly differentiate into a transient flagellate state. Remarkably, the Naegleria tubulins expressed during intranuclear mitosis are significantly divergent relative to those of other species. *Naegleria* mitotic tubulins are also divergent compared to the *Naegleria* tubulins used during differentiation. To explore how these two distinct microtubule networks are connected, we identified homologs of known microtubule regulators—including motor proteins, nucleators, cross-linkers, and regulators of microtubule dynamics—and determined which ones are expressed during mitosis versus differentiation using RNA-Seq. Despite the distinct, divergent tubulins associated with each process, we find that both mitosis and differentiation share tubulin modifying enzymes, microtubule nucleators, plus tip regulators, and subsets of both dynein and kinesin motor families. These findings suggest that, although the core subunits of Naegleria mitotic and cytoplasmic microtubule networks have diverged, their regulators remain shared. We have also identified subsets of regulators unique to each network. We are now mapping interaction sites of shared and unique regulators onto the mitotic and cytoplasmic tubulins to test whether tubulin regions that interact with regulators tend to be more highly conserved. We find that microtubule binding proteins appear to restrict tubulin diversification at their binding sites, maintaining a highly conserved substrate with which to build diverse cellular structures.

SG54

**Dissecting β-tubulin toxicity to understand tubulin proteostasis.**

**L. C. Wethekam**, J. K. Moore; University of Colorado Anschutz Medical Campus, Aurora, CO.

Eukaryotes express families of genes for the α- and β-tubulin monomers that comprise the tubulin heterodimer. Correct balance of α- and β-tubulin expression is needed to maintain stoichiometry for heterodimer production. Our previous works shows that this balance is essential in *S. cerevisiae*, and excess β-tubulin is toxic to cells. In this study we use *S. cerevisiae* to investigate the molecular consequences of excess β-tubulin. We use a structure-guided mutagenesis approach to show that β-tubulin toxicity arises from the catalytically inactive GTP-binding pocket that is normally positioned between α- and β-tubulin in the heterodimer, and the ability of super-stoichiometric β-tubulin to recruit +TIPs away from bona fide microtubules. Our study reveals a previously unappreciated binding mode between the carboxy-terminal tail of β-tubulin and XMAP215/Stu2. We further demonstrate that excess β-tubulin may be involved in the pathology of tubulinopathy disease in humans. A subset of *TUBA1A*-tubulinopathy mutations affect the intradimer interface and we find that budding yeast cells that mimic

these mutations exhibit fitness defects that are ameliorated by decreasing the expression levels of β-tubulin. Based on these results, we propose a novel model for the evolutionary relationship between α- and β-tubulin in which α-tubulin must be maintained in excess to promote GTP hydrolysis and to prevent the accumulation of non-catalytic assemblies of β-tubulin.

SG55

**Septin evolution and positional orthology.**

**M. Momany**, B. Shuman; Fungal Biology Group and Plant Biology Department, University of Georgia, Athens, GA.

The septin cytoskeleton plays critical roles in processes including cell division, polarity establishment, and vesicle trafficking. These roles require septin monomers from four distinct groups to polymerize in a specific order across two unique interfaces designated “G” and “NC.” the heteropolymers in turn form nonpolar filaments and higher order structures that interact with membranes and cellular effectors. Septin monomers contain highly conserved GTPase domains, polybasic and polyacidic regions, and SUE and S1-4 motifs. We used phylogenetic analysis, ancestral reconstruction, and structural modeling to investigate septin evolution focusing on polymerization. The relative positions of orthologous monomers appear to be conserved across evolution leading to positional orthology within heteropolymers. The highly conserved SUE and S1-4 motifs appear to drive septin monomer-monomer interactions. We postulate that the ancestral proto septin was a GTPase and that interactions across the G interface drove the earliest polymerization events in ancestral septins. Subsequent duplication and diversification enabled NC interface interactions and eventual filament formation.

SG56

**Septins throughout phylogeny are predicted to have a transmembrane domain, which in C. elegans is sufficient to drive membrane recruitment and necessary for septin localization.**

**J. A. Perry**, M. E. Werner, B. W. Heck, A. S. Maddox; UNC Chapel Hill, Chapel Hill, NC.

The septins, a conserved family of filament-forming proteins, carry out a variety of functions in eukaryotes, including roles in cell division, motility, and polarity. Septins are thought to act in these processes by scaffolding proteins to the plasma membrane and associating with the plasma membrane via the polybasic domains or an amphipathic helix that some septins possess. However, when either the amphipathic helix or polybasic domains are removed, septins can still associate with lipid bilayers in vitro (Cannon *et al.*, 2019; Omrane *et al.*, 2019). In the nematode *Caenorhabditis elegans*, there are only two septins, which form a palindromic tetramer in vitro, whereas other animals and fungi contain 5 or more septin genes to form palindromic hexamers or octamers. One of the *C. elegans* septin genes, *unc-61*, has multiple isoforms. The isoform *unc-61a* is expressed at a lower level from an alternative start codon and encodes a larger protein product than the other two isoforms (UNC-61b and c; Finger et al., 2003). UNC-61a has an N-terminal extension, predicted to include a transmembrane helix, a feature heretofore unappreciated in septins. Knockout of the entire *unc-61a* gene or just the sequence encoding the transmembrane helix via CRISPR/Cas9 genome editing perturbed the cortex localization of the other C. elegans septin UNC-59 in the pharynx and germline, yet recruitment to stable cytokinetic rings in the syncytial germline and to blastomere cytokinetic rings was normal. The transmembrane domain of UNC-61a was sufficient to drive membrane association of a StayGold GFP fusion protein expressed in mammalian cells. Finally, a phylogenomics survey to explore the prevalence of transmembrane domain-containing septins revealed the presence of a transmembrane domain in septins from many eukaryotic

organisms including some fungi, most bony fishes, and some mammals including the fin whale and a tiny primate, the tarsier. In sum, we hypothesize that association with the plasma membrane via a transmembrane domain contributes to septin function.

SG57

**Actin-dependent Mitochondrial Transport in Germlings of a Myosin-lacking Lineage of the Red Algae.**

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Red algae (Rhodophyta) are excellent subjects for evolutionary cell biology because: 1) they are an early-diverging primary photosynthetic lineage; 2) the oldest taxonomically resolved fossil of a multicellular eukaryote is the bangiophyte red alga *Bangiomorpha* (~1 billion years old; Gibson *et al. Geology* 2018); and 3) genomic studies demonstrate significant reductions in the typical eukaryotic cytoskeleton across red algal lineages (Brawley *et al.* 2017. *PNAS*). The sequenced bangiophytes have a small set of kinesins, a divergent dynein, and, surprisingly, lack the motor protein myosin entirely (Goodson *et al. BioEssays* 2021). We are studying young stages of the *Bangiomorpha* relative *Porphyra umbilicalis* ("laver") to address the effects of these cytoskeletal losses on growth and development. We previously followed germinating spores of *P. umbilicali*s to study mitochondrial movement in long rhizoids of young germlings; energetically demanding secretion occurs at rhizoid tips (Musor *et al. Mol Biol Cell* 2021, abstract P878). Despite the absence of myosin, we found that the actin inhibitors latrunculin B (latB) or cytochalasin D (CD) caused reversible inhibition of bidirectional transport of labeled mitochondria in rhizoids. The effect of the microtubule inhibitor nocodazole was inconsistent. Here we present results of studies with the microtubule inhibitor oryzalin. In 1-to-6-celled germlings (n=11) with long rhizoids, oryzalin treatment produced nearly equal proportions of: 1) reversible inhibition of bidirectional "fast" mitochondrial movement (27%), 2) continued bidirectional movement of mitochondria during the oryzalin treatment (27%), 3) inhibition and no recovery after washout (27%), and 4) bulk cytoplasmic movement (18%). We conclude that mitochondrial motility is actin-dependent, but the inconsistent effects of microtubule inhibitors suggest a secondary, structural interaction of microfilaments and microtubules. In addition, we localized actin with Alexa 488 phalloidin following cell wall permeabilization with digestive enzymes from snails (*L. littorea*) feeding in *Porphyra* beds. We observed cortical actin and strong localization of actin in the rhizoid. Together, these observations suggest the existence of actin-dependent mitochondrial transport in myosin-lacking *P. umbilicalis.* Funded by NSF #2027389.

SG58

**Resurrection of Ancestral Mitochondrial Mic60 Reveals Residue Signatures for Supporting Respiratory Function.**

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Cristae are characteristic inner membrane (IM) invaginations in mitochondria that serve as dynamic bioreactors and house the respiratory machinery. Crista stability is essential for cellular energy production, metabolism, and programmed cell death. Cristae junctions (CJs) are neck-like membrane structures that compartmentalize and connect cristae to the boundary membrane region of the IM. The evolutionarily conserved mitochondrial contact site and cristae organizing system (MICOS), a heterooligomeric membrane protein complex, stabilizes CJs through its core protein Mic60, which induces membrane bending of the IM and scaffolds higher-order mitochondrial protein interactions. Mic60 is conserved in α-proteobacteria, the proposed extant mitochondrial progenitors, where it forms intracytoplasmic membranes (ICMs) that are morphologically similar to cristae. Mitochondrial cristae are hypothesized to have evolved from α-proteobacterial ICMs, but the evolution of Mic60 and its functional involvement in the evolution of cristae from ICMs is unknown. Here, we present a functional and phylogenetic dissection of Mic60's most conserved domain, the C-terminal mitofilin domain, from α-proteobacterial Mic60 to human and yeast Mic60 to investigate its role in CJ stability. Using reconstructed and resurrected ancestral sequences dating back ~1.2 billion years in evolutionary time, we demonstrate that Mic60's function in mitochondrial respiration and crista scaffolding diverged between the last opisthokont common ancestor and modern humans. This suggests that yeast Mic60 reflects a more ancestral respiratory function, whereas animal Mic60 has diverged and diversified from the common ancestor state. We identify residue signatures, which support Mic60's role in respiration. Our results provide insight into the evolution of different MICOS complexes in mitochondria and push ancestral sequence reconstruction to a new frontier of capability.

SG59

***Toxoplasma* actin filament architecture supports rapid disassembly.**

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The tight conservation of eukaryotic actins underscores the fundamental role that the protein plays in maintaining cell shape and forming cellular structures. However, protozoa and other divergent eukaryotes have actins that show a greater degree of protein sequence deviations while still using the protein for essential functions. Actin plays a critical role in the pathogenicity of the apicomplexan parasite *Toxoplasma gondii*, where the protein functions in cell motility, invasion and egress, cargo transport, and material exchange. *T. gondii* contains one actin homologue which shares 83% sequence identity to skeletal actin and the parasite has far fewer actin regulatory proteins when compared to some other eukaryotic systems. Recent fluorescent microscopy work has demonstrated extensive filament networks within the parasite, but biochemical experiments in the literature show mixed results for the kinetic and structural properties of the filaments. In addition, long, unstabilized *T. gondii* actin filaments have only rarely been observed. Our studies *in vitro* show that actin from *T. gondii* assembles into long filaments that treadmill due to a low barbed-end assembly rate and a high pointed-end disassembly rate, and rapid nucleotide exchange within the free protomers.
Here, we use electron microscopy to determine the structures of stabilized and unstabilized actin filaments from *T. gondii*, confirming the existence of filaments tens of microns in length. While the overall filament structure remains very similar to that of skeletal actin, the reconstructions suggest a portion of the DNase loop from unstabilized filaments adopts an alternate conformation from that of skeletal actin, decreasing the number of hydrogen bonds and surface contacts along the longitudinal interfaces of the filament. In contrast, the DNase loop from the jasplakinolide-stabilized filament closely matches that of unstabilized skeletal actin. These structures help to explain the kinetics of the filaments

from *T. gondii* and the closely related actin 1 from *Plasmodium falciparum*, which shares 93% sequence identity with actin from *T. gondii*. with relatively few regulatory enzymes and a degree of sequence divergence, actin from *T. gondii* demonstrates how small evolutionary changes can give rise to alternative filament properties without gross changes in filament structure.

SG60

**The Hippo kinase cascade regulates a contractile cell behavior and cell density in a close unicellular relative of animals.**

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The genomes of close unicellular relatives of animals encode orthologs of many genes that regulate animal development. However, little is known about the function of such genes and signaling pathways in unicellular organisms or the evolutionary process by which these genes came to function in multicellular development. One such signaling pathway is the Hippo pathway, which regulates tissue size, cell proliferation, and oncogenesis in animals. The Hippo pathway is absent in most unicellular organisms but is found in the amoeboid organism *Capsaspora owczarzaki,* a close unicellular relative of animals that exhibits aggregative multicellularity through microvilli-microvilli adhesion. To understand the function of the Hippo pathway in unicellular organisms and to establish a simple system for studying this biomedically significant pathway, we developed gene knockout techniques and generated mutants for the *Capsaspora* Hippo pathway kinases Hippo (coHpo) and Warts (coWts). We find that, like in animals, coHpo and coWts cause cytoplasmic sequestration of the Hippo pathway nuclear effector coYki, indicating an ancient, premetazoan origin of this Hippo pathway regulatory mechanism. Strikingly, we find that these Hippo pathway kinases regulate cell density within multicellular structures in *Capsaspora:* loss of either kinase causes a large increase in the number of cells per volume within *Capsaspora* aggregates. We show that this effect on cell density is not due to differences in proliferation, but instead to cytoskeleton-dependent changes in the multicellular architecture of aggregates. As the role of the Hippo pathway in regulating cell density in the phenomenon of contact inhibition has been well established in mammalian tissue culture, our results demonstrate a shared and possibly ancient and conserved function of the Hippo pathway in cell density control in animals and *Capsaspora*. We present further data indicating that cytoskeletal properties of these *coHpo* and *coWts* mutant cells (specifically, a dynamic contractile behavior of the mutant cells) underlie these multicellular phenotypes. Visualization of the cytoskeleton in live contractile cells reveals stress fiber-like structures connecting the poles of contractile cells, suggesting that these contractile structures may have homology to those seen in mammalian cells such as myofibroblasts. Together, these results implicate cytoskeletal regulation but not proliferation as an ancestral function of the Hippo pathway and uncover a novel role for Hippo signaling in regulating cell density in a proliferation-independent manner.

SG61

**Evolutionary conservation and diversity of filamentous phage encapsidation.**

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Filamentous Ff bacteriophages (f1, fd, M13 etc.) are very popular biotechnology tools, and their assembly in the inner membrane of *Escherichia coli* has been well studied for 60 years. However, many other filamentous phages do not contain Ff homologues for viral assembly, and their packaging signals

(PS) and protein factors for encapsidation remain uncharacterized to date. *Xanthomonas* is a group of ~27 Gram-negative bacterial species as the major phytopathogens with economic importance. We and others have isolated a dozen filamentous phages infecting *Xanthomonas* pathogens. Unlike Ff coliphages, these phage DNAs integrate into their host chromosomes and replicate as prophages. Although the replication and coat proteins of *Xanthomonas* filamentous phages evolved from different origins, their PS sequences consist of a DNA hairpin with a conserved GGX(A/-)CCG(C/T)G stem and C/T-containing loop. Suppressor screening identified a novel nonstructural PSB15 (**PS**-**b**inding **15** kDa) protein essential for phage assembly. PSB15 is highly conserved among *Xanthomonas* filamentous phages but not found in Ff phages. Its N-terminal WAGFXF motif directly binds to PS DNA. PSB15 also interacts with phospholipid cardiolipin via its basic helix and C-terminus, so it can recruit PS DNA to bacterial inner membrane. The hydrophobic linker of PSB15 interacts with thioredoxin, a host protein required for Ff assembly. The mechanism of *E. coli* thioredoxin involvement in phage encapsidation is unknown so far. We found that *Xanthomonas* thioredoxin can form complex together with PS DNA and PSB15, but it releases PSB15-PS complex from the phospholipid membrane *in vitro*. Live cell TIRF imaging showed that loss of thioredoxin- or DNA-binding activity prolonged the dwell time of PSB15 at cell poles, suggesting that both activities may facilitate PSB15 dissociation from the inner membrane. Phage reproduction was significantly compromised when rescued with PSB15 mutations at PS-, cardiolipin-, or thioredoxin-binding motifs. To our knowledge, this is the first evidence showing *trans*-acting regulation of PS DNA association with the inner membrane for filamentous phage encapsidation. Our data also provide evolutionary insights into the diversity of filamentous phage life cycle.

SG62

**Resurrecting ancestral membranes in minimal living cells.**

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All cells are encapsulated by a lipid membrane which manages the relationship between life and its environment. Looking across the domains of life, we find a staggering diversity in the lipid composition of biological membranes. Since lipids determine the physical properties of membranes, which in turn influence their function, the evolution of membrane property and function is, at least indirectly, encoded in the diversification of lipid complexity. However, the principles required to account for the diverse range of lipid chemistries required for life have not been elucidated.
We hypothesize that all cell membranes share conserved properties encoded in their redundant yet diverse lipidomes, and that the specific biophysical properties of membranes required to occupy different functional niches were achieved through the evolution of diverse lipid chemistries. For example, all membranes converge on a narrow range of fluidity that is essential for bioactivity, whilst only certain organisms possess surface membranes that are more robust to physical and chemical extremes. We have developed minimal living membrane systems to understand how life employs the collective properties of lipids to build responsive organizational interfaces between cells and their environment. These systems are based on mycoplasmas, which due to their dependence on exogenous lipids, gives us the ability to tune their lipidome composition. For this presentation, I will report on our insights from recent advances that allow us to tune lipidome composition in the Minimal Cell (JCVI-Syn3.0) from fewer than 10 to more than 100 lipid species. Through this approach we reveal that cells can survive on as few as two lipids, and that lipidome complexity has profound consequences for cellular growth and robustness, and metabolic efficiency. By introducing ancient sterol analogues (hopanoids), we explore how the evolution of sterol structure could have directed the evolution of lipidome composition during the emergence of sterol-rich eukaryotic membranes. Further, we observe profound

effects resulting from subtle changes in lipid structure, such as the lipid chirality that distinguishes Archaea from the rest of life. By resurrecting ancestral lipidomes, we aim to reveal the conserved features of modern membranes and introduce a new paradigm for understanding why life needs so many lipids.

SG63

**Additional fitness consequences of rapidly evolving*wtf* meiotic drivers.**

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Killer meiotic drivers are selfish genetic elements that destroy gametes to favor their own transmission into more than 50% of the viable gametes produced by a heterozygote. The *wtf* genes are a large family of rapidly evolving meiotic drivers in fission yeasts. Each *wtf* driver gene encodes two proteins on overlapping transcripts: a poison and a corresponding antidote. In a *wtf* heterozygote, the poison attacks all developing gametes, while the antidote only rescues the *wtf*+ gametes. The persistence of active *wtf* drivers in the fission yeast genome likely relies on their ability to rapidly evolve to evade host suppression. We recently found that *wtf* genes possess hypermutable coding sequence repeats that facilitate their rapid evolution. Changes in the copy number of the coding sequence repeats simultaneously generates a novel poison and its corresponding antidote. We found that the coding sequence repeats were dispensable for protein function, so we posited they might be spacer domains that facilitate rapid evolution without otherwise affecting protein function. To test this idea, we generated mutants wherein the repeats where scrambled randomly, mutated to alanines, or swapped between fission yeast species. Contrary to our model, the domains were not merely spacers. Instead, the sequence of the repeat was important to generate a functional poison/antidote pair. Mutations in the repeats frequently generated a self-killing scenario where the poison allele was toxic but was not rescued by its corresponding antidote. This suggests the *wtf* genes can be mutated into self-killing alleles. Such alleles may arise in natural populations and add to the already significant fitness burden imposed on fission yeast by these genetic parasites.

SG64

**A bacterial expression cloning screen reveals tardigrade mitochondrial single-stranded DNA-binding proteins as potent desicco-protectants.**

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Tardigrades are known for their ability to survive remarkable extremes, but relatively little is known about the molecular underpinnings that allow for their extraordinary stress tolerance. We devised an expression cloning approach to screen for novel protectants from desiccation-tolerant tardigrades. We expressed cDNA libraries from the tardigrades *H. exemplaris* and *R. varieornatus* in *E. coli* and subjected the bacteria to desiccation. Sequencing the populations of surviving bacteria revealed enrichment of mitochondrial single-stranded DNA-binding proteins (mtSSBs) from both tardigrade species. Expression of mtSSBs in bacteria improved desiccation survival to similar levels as some of the best protectants known to date. The DNA-binding activity of the OB fold domain of mtSSB was necessary and sufficient to improve desiccation tolerance. In a eukaryotic cell, the tardigrade mtSSBs localized to mitochondrial DNA and could provide a modest benefit to desiccation survival. By comparing the efficacy of mtSSBs from other organisms, we provide evidence that desicco-protection is a conserved feature of mtSSBs.

More broadly, any OB-fold containing protein may act similarly to promote desiccation tolerance, likely by coating ssDNA to limit damage during desiccation. These results identify single-stranded DNA-binding activity as a potent contributor to extreme desiccation tolerance.

SG65

**Microtubule Organization and Centriole Remodeling in the Life Cycle of Chytrid Fungi.**

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Centrioles become microtubule organizing centers (MTOCs) by functionalization with pericentriolar material and accessory structures. This remodeling of the MTOC is important in the cell cycle and in development, where MTOCs adopt many forms and functions in differentiated cells. To study centriole and MTOC remodeling in an evolutionary context we are using the early-diverging chytrid fungus *Rhizoclosmatium globosum* that has centrioles yet also features of fungal MTOCs. Chytrids alternate in their rapid lifecycle between a ciliated zoospore stage with parallel unequal length centrioles as part of the basal body and a coenocytic sporangium stage with short equal orthogonal centrioles at the spindle pole. Zoospore centrioles have 'spur' structures that connect to a bundle of microtubules, while in the synchronous mitoses in the sporangium, centrioles participate in an MTOC with properties of both animal centrosomes and yeast spindle-pole bodies. We used RNA sequencing and high-resolution ultrastructure expansion microscopy to characterize the composition and architecture of centrioles and MTOCs during the fungal life cycle. In the zoospore, parallel centrioles are linked by centrin-based fibers and the spur MTOC consists of a focus of gamma-tubulin on the side of the mother centriole. During the transition from zoospore to sporangium, centrioles are protected and shorten, while axonemes retract and degrade. Overall, we find that the centriole cycle in chytrids is remarkably similar to that of syncytial animal cells in that centriole pairs separate from each other in early prophase, are segregated on the mitotic spindle, and mother and daughter centrioles disengage in late metaphase. Formation of the basal body and ciliogenesis are driven by a conserved transcriptional program. In the transition to a basal body, the distal centriole elongates before ciliogenesis begins and multiple microtubule bundles form that are later reduced in the resulting zoospores. This work illuminates key aspects of microtubule organization and remodeling, basal body assembly and disassembly, and the evolution of MTOCs, enhancing our understanding of these fundamental cellular processes.

SG66

**A fitness landscape instability governs the morphological diversity of tip-growing cells.**

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Cellular morphology affects many aspects of cellular and organismal physiology. This makes it challenging to understand the evolutionary basis for specific morphologies since the various facets of cellular physiology may exert competing selective pressures on this trait. The influence of these pressures, moreover, will depend on the mechanisms of cellular morphogenesis. To address this problem, we combined experimental cell biology with mechanics-based theory to analyze the morphological diversity of tip-growing cells from across the tree of life. We discovered that an instability in the convergent mechanism of "inflationary" growth shared by these cells leads directly to a bifurcation in their fitness landscape, which imposes a strong global constraint on their morphologies.

Additionally, we found that co-selection for cell size and elongation rate explains variation among observable morphologies. This analysis rationalizes the morphology-and provides quantitative insight into the ecology-of an enormous diversity of important fungal, plant, protistan, and bacterial systems. Additionally, our study elucidates a fundamental principle of evolutionary-developmental biology that would be difficult to rigorously demonstrate in more complex systems.

SG67

**Epithelial wound healing in *Clytia hemisphaerica* provide insights into extracellular ATP signaling mechanisms and P2XR evolution.**

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Epithelial wound healing involves the collective responses of many cells, including those at the wound margin (marginal cells) and those that lack direct contact with the wound (submarginal cells). How these responses are induced and coordinated to produce rapid, efficient wound healing remains poorly understood. Extracellular ATP (eATP) is implicated as a signal in epithelial wound healing in vertebrates. However, the role of eATP in wound healing *in vivo* and the cellular mechanism of eATP effects are poorly understood. Almost nothing is known about eATP signaling in basal metazoans. Here we show that eATP promotes closure of epithelial wounds *in vivo* in the cnidarian *Clytia hemisphaerica* (Clytia) indicating that eATP signaling is an evolutionarily ancient strategy in wound healing. Furthermore, eATP increases F-actin accumulation at the edges of submarginal cells. In Clytia, this indicates eATP is involved in coordinating cellular responses during wound healing, acting in part by promoting actin remodeling in cells at a distance from the wound. We also present evidence that eATP activates a cation channel in Clytia epithelial cells. This implies that the eATP signal is transduced through a P2X receptor (P2XR). Phylogenetic analyses identified four Clytia P2XR homologs and revealed two deeply divergent major branches in P2XR evolution, necessitating revision of current models. Interestingly, simple organisms such as cellular slime mold appear exclusively on one branch, bilaterians are found exclusively on the other, and many basal metazoans, including Clytia, have P2XR sequences from both branches. Together, these results re-draw the P2XR evolutionary tree, provide new insights into the origin of eATP signaling in wound healing, and demonstrate that the cytoskeleton of submarginal cells is a target of eATP signaling.

SG68

**The 'tail' of talin: Mechanical adaptation during the evolution of multicellular organisms.**

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The evolution of multicellularity must have been accompanied by mechanical adaptations, for example to resist the increased mechanical stresses observed during cell adhesion and cell-cell communication in higher organisms. Yet, how the mechanobiology of these cellular processes evolved during the transition from unicellular to multicellular organisms remains entirely unclear. Here, we investigate the molecular details of cell adhesion mechanics in mammalian cells and social amoeba *Dictyostelium discoideum,* which can exist in both unicellular and multicellular states. We focus our studies on the adhesion protein talin, which is known for its prominent role as an integrin activator and force transducer in metazoans. *D. discoideum* also expresses talin protein but lacks integrin receptors and is thought to bind Sib proteins to establish a mechanical linkage between plasma membrane and cytoskeleton. To determine whether talin-Sib linkages can indeed bear mechanical forces, we employed our previously developed, genetically

encoded FRET based molecular tension sensors. Our results indicate that talin linkages in *D. discoideum* (in-vivo), experience piconewton (pN)-scale forces in the range of 6-8 pN, similar to mammalian talin. When expressing a chimeric *D. discoideum* talin in talin-deficient fibroblasts, we observe that the talin-tail domain of *D. discoideum* is capable of recruiting vinculin, activating myosin-II and anchoring actin stress fibers. Consistent with our force measurements in *D. discoideum*, chimeric talin is able to carry mechanical loads similar to those observed across mammalian talin. However, talin from *D. discoideum* appears to fail at coordinating a regulated Focal adhesion turnover leading to severely impaired cell migration in fibroblasts. Together, our data indicate that the force-bearing function of talin is evolutionarily conserved, while its role as a regulator of cell adhesion and migration were acquired later and is unique across the metazoan timeline.

SG69

**The adhesion GPCR *cupid* regulates mating in the closest living relatives of animals.**

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All animals develop through the fusion of a differentiated sperm and egg. Although considered a key innovation, the evolution of gametes and gametogenesis in animals is poorly understood. Recently, evidence for sex has been described in choanoflagellates, the closest living relatives of animals. Under nutrient depletion, the model choanoflagellate *Salpingoeca rosetta* forms distinct cell types that aggregate, fuse, and undergo meiotic recombination. Additionally, the bacterium *Vibrio fischeri* can induce mating in *S. rosetta* cultures, suggesting that multiple environmental cues can trigger gametogenesis. Importantly, the signaling pathways underlying sexual reproduction in these different contexts have not been investigated. In this study, we report the discovery of an adhesion GPCR, named *cupid*, that regulates the switch from vegetative growth to sexual reproduction in *S. rosetta*. We found that the knock-out of *cupid* elicits the mating behavior observed in wild-type cells at the cellular and the transcriptional level, even in the absence of environmental mating cues (i.e. starvation or exposure to *V. fischeri*).The presence of specialized gametes in choanoflagellates raises the possibility that animal gametogenesis might have more ancient evolutionary origins. By presenting the first molecular characterization of sex in choanoflagellates, our finding holds the promise to reconstruct the evolution of gamete differentiation in animals.

## Subgroup: The Midbody and Midbody Remnant: From Abscission to Signaling Organelle

SG70

**Coordinated regulation of Citron kinase by Cdk1 and Aurora B regulates midbody formation.**

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At the end of cell division, the two daughter cells remain connected by an intercellular bridge that contains at its centre an organelle, the midbody, essential for the final physical separation of the two daughter cells (i.e., abscission). Recent studies have revealed that midbodies have also important functions post-mitosis, including cell fate, cell proliferation, tissue organization, and cilium and lumen formation. Despite the evidence of the involvement of the midbody in these important processes, our understanding of the mechanisms that regulate its formation, functions, and inheritance is very limited.The midbody is composed of an assortment of proteins arranged in a very precise and

stereotyped spatial pattern. The proper localization, regulation, and interactions of midbody proteins are essential for abscission and to prevent incorrect genome segregation. The large multifunctional kinase Citron kinase (CIT-K) plays a key evolutionarily conserved role in maintaining the orderly arrangement of midbody proteins. CIT-K exerts this role by directly interacting with, and regulating the localization of several midbody proteins, including anillin, the centralspindlin complex (an heterotetramer of KIF23/MKLP1 and RacGAP1), the chromosomal passenger complex (CPC, of which AURKB is the kinase subunit), and the kinesin KIF14. We have previously described a cross-regulation mechanism between AURKB and CIT-K that controls the assembly of the midbody by temporally regulating the association of CIT-K with both MKLP1/KIF23 and the CPC. Here, we report a novel cross-regulatory mechanism among mitotic kinases important for the formation and stability of the midbody. We found that Cdk1 and AURKB coordinately regulate CIT-K localization and association with its partners by phosphorylating two distinct residues located adjacent to or within the first coiled coil domain of CIT-K. Analyses using phospho-specific antibodies and phospho-mimetic and non-phosphorylatable mutants indicated that perturbing these phosphorylation events leads to incorrect localization of CIT-K, abnormal midbody formation, and accumulation of midbody remnants. Together, our findings indicate that coordinated regulation of CIT-K by Cdk1 and AURKB temporally regulates the association of CIT-K with its partners AURKB, centralspindlin, and KIF14, in order to finely regulate midbody formation.

SG71

**Coordination of distinct Anillin-dependent cytoskeletal elements during the contractile ring-to-midbody ring transition of cytokinesis.**

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During cytokinesis, an F-actin and myosin II-based contractile ring (CR) constricts to reduce the diameter of the cell. Upon closure, the CR disassembles but leaves behind some of its components in the form of a midbody ring (MR), which ultimately controls abscission. We have previously shown that the transition from CR to MR of *Drosophila* S2 cells requires the scaffold protein Anillin and is mediated through the opposing actions of Citron kinase (Sticky in *Drosophila*), acting with the Anillin N-terminus, and the septin cytoskeleton acting with the Anillin C-terminus. Here, we sought to better understand how these opposing actions of Anillin are coordinated during the CR-to-MR transition. In deplete-and-rescue, live cell imaging experiments, we examined the phenotypic consequences of separation-of-function mutants of Anillin that either prevent interaction with Sticky, or prevent the recruitment of septins to the CR. In each case, Anillin still localized robustly to the CR, but distinct defects in the CR-to-MR transition ensued and cytokinesis failed in characteristic ways, consistent with prior studies. Unsurprisingly, a double mutant Anillin, unable to bind Sticky or to recruit septins, was poorly recruited to the CR and was essentially non-functional for MR formation. However, co-expression of each Anillin allele in a trans-heterozygous combination rescued the defects observed upon expression of each allele alone and resulted in a successful CR-to-MR transition. Quantitative analyses of the co-expressed mutants fused to GFP or mCherry revealed distinct and separate behaviors of the two alleles. The data support the conclusion that distinct N- and C-terminal-dependent actions of Anillin during the CR-to-MR transition can be mediated by different molecules of Anillin and that there is no requirement for Anillin molecules to bind to Sticky and to engage with the septin cytoskeleton at the same time. Indeed, the results suggest that, rather than linking Sticky-dependent complexes to the septin cytoskeleton, Anillin may interact with each sub-set of interactors in a mutually exclusive manner, allowing distinct Anillin-dependent complexes to separate from one another. Thus, Anillin appears to be not only a linker of

cytoskeletal elements, but also a separator, which may explain how the closing CR thins as it transitions to a mature MR during the progression towards abscission.

SG72

**OSGN-1 is a conserved flavin-containing monooxygenase required to stabilize the intercellular bridge in late cytokinesis.**

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Cytokinesis is the last step of cell division and is regulated by the small GTPase RhoA. RhoA activity is required for all steps of cytokinesis, including prior to abscission when daughter cells are ultimately physically separated. Like germ cells in all animals, the *C. elegans* embryonic germline founder cell initiates cytokinesis but does not complete abscission, leaving a stable intercellular bridge between the two daughter cells. Here we identify and characterize *C. elegans* OSGN-1 as a novel cytokinetic regulator that promotes RhoA activity during late cytokinesis. Sequence analyses and biochemical reconstitutions reveal that OSGN-1 is a flavin-containing monooxygenase, a class of enzymes that transfer molecular oxygens to substrates using FAD and NAD(P)H as co-factors. Genetic analyses indicate that the monooxygenase activity of OSGN-1 is required to maintain active RhoA at the end of cytokinesis in the germline founder cell and to stabilize the intercellular bridge. Deletion of one of the human homologs of OSGN-1, OSGIN1, in HeLa cells results in a decrease of active RhoA at the intercellular bridge and in cleavage furrow regression in late cytokinesis, and thus leads to an increase in HeLa cell binucleation. These phenotypes can be rescued by expressing a catalytically-active (but not inactive) form of *C. elegans* OSGN-1, indicating that OSGN-1 and OSGIN1 are functional orthologs. Accordingly, we find that, like *C. elegans* OSGN-1, human OSGIN1 has monooxygenase activity *in vitro*. We propose that OSGN-1 and OSGIN1 are novel, conserved monooxygenase enzymes required to maintain RhoA activity at the intercellular bridge during late cytokinesis and thus promote its stability, enabling proper abscission in human cells and intercellular bridge stabilization in *C. elegans* germ cells.

SG73

**Regulation of cytokinesis by *O*-GlcNAcylation.**

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The abscission checkpoint ensures that the onset of cytokinetic abscission is synchronized with the completion of upstream mitotic events. Upon completion of mitosis, protein factors, including Aurora B and the Endosomal Sorting Complexes Required for Transport (ESCRT) pathway, assemble into a dynamic signaling hub at the midbody to fine-tune the timing and progression of abscission. This checkpoint arrests abscission in the presence of mitotic errors such as trapped DNA in the intercellular bridge, misformed nuclear pores, under-replicated DNA, and tension at the intercellular bridge. How cells integrate multiple different inputs to achieve faithful cytokinetic abscission is not well understood. Here we investigate the contribution of an intercellular glycosylation, known as *O*-GlcNAcylation to abscission regulation. *O*-GlcNAcylation is a post-translational modification of Ser/Thr residues with *O*-linked N-acetylglucosamine. *O*-GlcNac targets thousands of proteins and is involved in diverse cellular processes including signal transduction, gene expression, and protein degradation. Cellular *O*-GlcNAc flux is driven solely by two enzymes, *O*-GlcNAc transferase, OGT, and *O*-GlcNAcase, OGA. OGT concentrates with Aurora B at the midbody of dividing cells, and overexpression is linked with cytokinetic failure, indicating a role for *O*-GlcNAc in abscission regulation. Here we show that OGA and

OGT are required for maintaining an abscission checkpoint arrest under multiple checkpoint triggers. Using publicly available resources including the *O-GlcNAc Database* (www.oglcnac.mcw.edu), combined with our own mass spectrometry approaches, we have mapped *O*-GlcNAc sites onto multiple protein targets. Now with structure-function analyses, we are developing hypotheses for how *O*-GlcNAcylation may regulate key pathways and proteins that maintain faithful cytokinetic abscission.

SG74

**Connecting a unique organelle, the abscission checkpoint body, to the regulation of events at the midbody.**

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The last stage of cell division is regulated by the abscission checkpoint, which halts essential steps in membrane remodeling at the midbody that must occur to execute abscission. Abscission checkpoint activity is maintained under various conditions, including heightened intercellular tension between dividing daughter cells (Lafaurie-Janvore et al., 2013), replication stress earlier in the cell cycle (Mackay et al., 2015), chromatin bridges through the abscission site (Steigemann et al., 2009), and nuclear pore defects (Mackay et al., 2010). The checkpoint functions in part by preventing certain ESCRT (endosomal sorting complex required for transport) factors from assembling functionally at the midbody during cytokinesis, thereby averting their role in membrane fission at this site. Our previous studies indicated the early-acting ESCRT factor ALIX as a key target of this regulation. Unexpectedly, concomitant with its lag in localizing to the midbody, ALIX was detected in cytoplasmic bodies termed Abscission Checkpoint Bodies (ACBs), which are novel cytoplasmic organelles found to arise during cytokinesis under conditions that lead to sustained abscission checkpoint activity (Strohacker et al. 2021). ACBs harbor a unique coalescence of ESCRT factors, checkpoint signaling proteins, and, surprisingly, the RNA splicing factor SRRM2. We have since found that splicing factors SRRM1 and SON are also present at ACBs. We propose two models of how ACBs function to delay abscission: sequestration of ESCRT factors away from the midbody or prevention of specific RNA splicing events required for abscission. To probe these mechanisms, which are not mutually exclusive, we are testing: 1) whether ALIX mutants impaired for ACB targeting are free to localize to the midbody regardless of abscission checkpoint signals; 2) whether inhibition of RNA splicing delays abscission and, in particular, abrogates ALIX localization to the midbody at cytokinesis. Although there is not a previously established connection between RNA splicing and abscission, the data we will present indicates that RNA splicing is important for the abscission process to progress. This new insight adds to a growing appreciation of an intimate connection between mRNA and midbody form and function (Park et al., 2022; Farmer et al., 2022).

SG75

**DENND2B activates Rab35 at the intercellular bridge, regulating cytokinetic abscission and tetraploidy.**

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Cytokinesis relies on membrane trafficking pathways regulated by Rabs and guanine nucleotide exchange factors (GEFs). During cytokinesis, the intercellular cytokinetic bridge (ICB) connecting daughter cells undergoes abscission, which requires actin depolymerization. Rab35 recruits MICAL1 to oxidize and depolymerize actin filaments. We show that DENND2B, a protein linked to cancer and congenital disorders, functions as a Rab35 GEF, recruiting and activating Rab35 at the ICB. DENND2B's

N-terminal region also interacts with an active form of Rab35, suggesting that DENND2B is both a Rab35 GEF and effector. Knockdown of DENND2B delays abscission, leading to multinucleated cells and filamentous actin (F-actin) accumulation at the ICB, impairing recruitment of ESCRT-III at the abscission site. Additionally, F-actin accumulation triggers the formation of a chromatin bridge, activating the NoCut/abscission checkpoint, and DENND2B knockdown activates Aurora B kinase, a hallmark of checkpoint activation. Thus, our study identifies DENND2B as a crucial player in cytokinetic abscission.

SG76

**Differential role for VPS4 isoforms in cytokinetic abscission.**

**N. Elia**, I. Dvilansky, Y. Atlaras, D. Nachmias; Ben Gurion University of the Negev, Beer Sheva, ISRAEL.

Mutations in the human AAA-ATPase VPS4A cause severe Neurodevelopmental defects and Congenital Dyserythropoietic Anemia (CDA). VPS4 is a crucial component in cellular membrane remodeling, which drives the disassembly of ESCRT-III filaments during membrane fission. While most cells encode for a single VPS4 gene, human cells have two VPS4 paralogs, namely VPS4A and VPS4B but the functional differences between these paralogs is largely unknown. Here we set to investigate the role of the human VPS4 paralogs in the context of cytokinetic abscission. Using VPS4A and VPS4B knockout cell lines we found that these paralogs hold both overlapping and distinct roles in abscission. VPS4A depletion resulted in a severe abscission delay, that could be fully rescued by VPS4A expression but only partially rescued by VPS4B overexpression. Unexpectedly, a monomeric-locked VPS4A mutant also partially rescued the abscission delay in VPS4A KO cells and was found to bind the abscission checkpoint proteins CHMP4C and ANCHR. Depletion of VTA1, a co-factor of VPS4, disrupted the interactions between of VPS4A to ANCHR and resulted in accelerated abscission, strongly indicating a role for VTA1 in the abscission checkpoint. Collectively, our findings suggest a dual role for VPS4A in abscission, one that is canonical and can be compensated by VPS4B and another that is regulatory and is driven by its monomeric form. Our observations provide a potential mechanistic explanation for the neurodevelopmental defects and other related disorders reported in patients with mutated VPS4A despite the presence of a functional VPS4B paralog.

SG77

**The Specialized Regulation of Abscission and Midbody Remnants in the Developing Mammalian Brain.**

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

The midbody mediates cytokinetic abscission to sever the final connecting bridge between daughter cells after mitosis. Recent studies suggest that abscission regulation can influence cell proliferation, polarity, and fate. Through our analyses of mouse mutants in abscission genes, we have demonstrated that tight spatial and temporal regulation of abscission in neural stem cells (NSCs) is critical for proper brain growth and structure. NSCs are highly polarized and reside in a pseudostratified epithelium. Early NSCs undergo proliferative symmetric divisions, but later NSCs do asymmetric neurogenic divisions. In both of these types of division, abscission occurs at the apical membrane, at the same time and place that many fate-signaling events are happening. Surprisingly, we found that post-abscission midbody remnants (MBRs) persist on the apical membranes of dividing NSCs in vivo and in vitro. Intriguingly, they are more associated with early proliferative divisions than later neurogenic divisions. Whether they are causative or reflective of these daughter cell fate decisions remains unclear. It is possible that the MBR is a waste container that must be released to retain stemness, or that it is a signal vessel that promotes proliferation.

Next we tested whether the knockout of the midbody scaffold protein Cep55 would prevent the deposition of MBRs on the apical membrane. In these knockout brains, NSC abscission is delayed and sometimes fails, leading to increased apoptosis. Surprisingly, Cep55 knockout brains had even more abundant MBRs than control brains, regardless of developmental stage. This could be a manifestation of abnormal late abscission; or, it could be due to reduced MBR engulfment and disposal. We are building on these results to understand the roles of abscission and MBRs in regulating stem cell divisions and brain development.

SG78

**The midbody and midbody remnant are assembly sites for RNA and localized translation.**

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The midbody (MB) is a transient structure at the spindle midzone that is required for cytokinesis, the terminal stage of cell division. Long ignored as a vestigial remnant of cytokinesis, MBs are released post-abscission as extracellular vesicles called MB remnants (MBRs) and more evidence suggest that they can modulate cell proliferation, fate decisions, tissue polarity, neuronal architecture, and tumorigenic behavior. Here, we demonstrate that the MB matrix—the structurally amorphous MB core of unknown composition—is the site of ribonucleoprotein assembly and is enriched in mRNAs that encode proteins involved in cell fate, oncogenesis, and pluripotency, that we are calling the MB granule. Using a quantitative transcriptomic approach, we identified a population of mRNAs enriched in MBs and confirmed their presence in signaling MBRs released by abscission. The MB granule is unique in that it is translationally active, contains both small and large ribosomal subunits, and has both membrane-less and membrane-bound states. Both MBs and post-abscission MBRs are sites of spatiotemporally regulated translation, which is initiated when nascent daughter cells re-enter G1 and continues after extracellular release. MKLP1 and ARC are necessary for the localization and translation of RNA in the MB dark zone, whereas ESCRT-III was necessary to maintain translation levels in the MB. We present a model in which the assembly of RNP complexes are central to MB function and suggest the MBR serves as a novel mode of RNA-based intercellular communication with a defined biogenesis that is coupled to abscission, and inherently links cell division status with signaling capacity. To our knowledge, this is the first example of an autonomous large extracellular vesicle with active translation activity.

SG79

**The Molecular Machinery Governing Targeting on Midbody-Associated mRNAs during Cytokinesis.**

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Midbodies function during telophase to regulate the abscission step of cytokinesis. Until recently, it was thought that abscission regulating proteins, such as ESCRT-III complex subunits, accumulate at the MB by directly or indirectly binding to the MB resident protein, CEP55. However, recent studies have shown that depletion of CEP55 does not fully block ESCRT-III targeting to the MB. Here, we show that MBs contain mRNAs and that these MB-associated mRNAs can be locally translated, resulting in the accumulation of abscission-regulating proteins. We demonstrate that localized MB-associated

translation of CHMP4B (among other mRNAs) is required for its targeting to the abscission site and that 3' UTR-dependent CHMP4B mRNA targeting to the MB is required for successful completion of cytokinesis. Finally, we identify regulatory *cis*-elements within mRNAs that are necessary and sufficient for mRNA trafficking to the MB. We propose a novel method of regulating cytokinesis and abscission by MB-associated targeting and localized translation of selective mRNAs.

## MBoC Highlights: The Future of Cell Biology

SG80

**Endocytic organelle integrity in dendritic cells: when antigen makes a break for it!.**

**J. Canton**; Faculty of Veterinary Medicine, University of Calgary PO #939245, Calgary, AB, CANADA.

Endocytosis is the process whereby cells internalize extracellular material into a membrane-bound compartment called an endocytic organelle. These organelles acquire degradative properties and gradually convert their cargo, consisting of diverse macromolecules, into small exportable components including ions, amino acids, nucleosides and lipids. These are then exported from the lumen of the endosome into the cytosol via transmembrane proteins known as transporters and channels. There are exceptional instances, however, when large, endocytosed macromolecules, such as intact nucleic acids and proteins, need to be delivered into the cytosol. In these instances, it is likely that large, non-selective pores form in the limiting membrane of the endosomes. This is evident in immune cells called dendritic cells (DCs). DCs can transfer macromolecules including nucleic acids and proteins into the cytosol. Once in the cytosol, these macromolecules are either sensed by cytosolic receptors that elicit inflammatory signalling cascades, or in the case of proteins, can be processed for presentation to T cells in a process known as cross-presentation. This allows DCs to carry out essential roles in tissue surveillance. The problem is, despite the obvious biological importance of these processes, we know essentially nothing about these putative endosomal pores. Recently, we have employed novel imaging approaches to visualize, for the first time, these non-selective pores on large endocytic organelles called phagosomes. We linked pore formation to cross-presentation and therefore to DC function. The mechanism, however, has remained obscure. Now, utilizing state-of-the-art techniques in microscopy, I have gathered exciting preliminary evidence that a family of understudied apolipoproteins coordinate endosomal pore formation and therefore cross-presentation. This discovery of non-selective pore formation in the endocytic pathway of immune cells opens up interesting avenues for future research in cell biology and implications for both immunity and homeostasis will be discussed.

SG81

**Misregulation of inter-organelle contact site dynamics and function in neurodegenerative diseases.**

**Y. C. Wong**; Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL.

Cellular organelle dynamics and function are tightly regulated both spatially and temporally. An important site for such regulation occurs at inter-organelle membrane contacts, which dynamically form between different organelles throughout the cell to allow for direct organelle crosstalk and the bidirectional regulation of their dynamics and function. Using multiple super-resolution and live microscopy approaches, we recently showed that mitochondria-lysosome contacts are an important pathway for crosstalk between mitochondria and lysosomes. This site allows for the modulation of both organelles by coupling together the molecular machinery for Drp1 GTP hydrolysis and Rab7 GTP hydrolysis events on mitochondrial and lysosomal membranes at mitochondria-lysosome contacts,

resulting in the simultaneous control of both organelles' dynamics respectively. Moreover, these contacts further regulate additional downstream mitochondrial and lysosomal functions. Interestingly, mitochondria-lysosome contact dynamics and function can become misregulated at different steps in this pathway, leading to distinct neurodegenerative diseases including Parkinson's disease and Charcot-Marie-Tooth disease. Indeed, dysregulation of additional inter-organelle contact site dynamics may be critical drivers of the pathogenesis underlying multiple other neurological disorders such as Alzheimer's disease and Amyotrophic Lateral Sclerosis (ALS). Thus, future insights into inter-organelle contact site dynamics and regulation with super-resolution and live microscopy approaches will significantly advance our understanding of both cellular homeostasis and the etiology of major neurodegenerative diseases.

SG82

**The future of cell biology: integrating timing, location and concentration into pathway models.**

**N. L. Chanaday**; Department of Physiology, University of Pennsylvania - Philadelphia, PA, Philadelphia, PA.

Neurons are highly polarized cells that specialize in information transfer. Neurotransmission relies on the release of neurotransmitters from presynaptic terminals via regulated exocytosis of synaptic vesicles. After binding to postsynaptic receptors, these chemical neurotransmitters trigger an electrical signal, the action potential, that propagates along the neuron. These steps in synaptic transmission along with the downstream signaling cascades are triggered or modulated by calcium. Here we will show how the timing, intensity and localization of these calcium signals are crucial in determining the specific pathways and the plasticity mechanisms activated at individual synapses. We propose that to understand synaptic signaling mechanisms is not enough to uncover the identity of the molecules involved, but also include their temporal and spatial organization.

SG83

**Mapping the inner world of cells.**

**B. Huang**; University of California, San Francisco, San Francisco, CA.

Cellular processes are orchestrated by a large number of biomolecules in a spatially and temporally coordinated manner within a tiny volume. To uncover the underlying organizational principles and their functional relevance, we take microscopy visualization as the primary approach to systematically map their spatial localization, temporal dynamics and activity profiles. By combining small tags engineered from split fluorescence proteins and CRISPR/Cas9-mediated gene editing, we have enabled large-scale tagging of endogenous proteins in human cell lines for both microscopy visualization and biochemical analysis. Characterization of their live cell dynamics using epi-illumination light-sheet microscopy reveals intriguing phase condensation and de-condensation behavior of many proteins during the cell cycle. We have further developed the deep-learning framework to connect cellular images of proteins to their amino acid sequences.

SG84

**A practical approach to establishing a conceptual framework for holistic cell states and state transitions.**

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The cell is distinct from other complex systems in that it is the basic unit of life. In multicellular organisms, the same genome can give rise to many cell phenotypes, and for convenience these have often been classified as distinct cell types. Furthermore, a single cell type can be described as existing in multiple states (e.g., an epithelial cell can be quiescent, motile, apoptotic, etc.). Determining robust definitions for cell states is a long-standing challenge due to the heterogeneity of individual cells within a population and the continuous nature of variation in cell state landscapes. Traditionally, cell state was defined based primarily on what cells looked like, where they were located, and what function they exhibited. In the molecular era, the observables defining cell state expanded to include specific molecular markers, localizations of proteins within cells, and cellular structure-function relationships. Then in the post-genomic era, large-scale gene expression profiles and gene regulatory networks were folded in, resulting the current, largely molecular-focused, view of cell state. Throughout this history, cell state has always been defined by the cell phenotype (cell-intrinsic observables) and its environment (cell-extrinsic observables). Moving forward, we anticipate that the range of observables used to define cell states will evolve again, as single-cell imaging and analytics are advancing at a breakneck pace via the collection of large-scale, systematic cell image datasets and the application of quantitative image-based data science methods. Now is the time to integrate the spatiotemporal observables of the physical structure and organization of the cell with molecular observables, and to update the concept of a holistic cell state. We propose that cell observables can be conveniently divided into four categories related to the common modalities of data collection: 1) cell molecular census (all of the molecules in a cell), 2) cell organization (the spatio-temporal multi-scale arrangement of these molecules), 3) cell function (the actions and behaviors of a cell), with these three together representing the cell-intrinsic phenotype, and finally 4) the cell-extrinsic environment. In many cases, there is clear bi-directional feedback between all combinations of these four categories. We thus define a *stable holistic cell state* as one where these four categories of data observables of a cell can fluctuate, but ultimately mutually reinforce one another to maintain a stable state. We hypothesize that, in a cell state transition, at least one type of observable falls out of alignment with the others and then, due to the bi-directional feedback between observables, influences changes in the others to achieve a new mutually reinforcing stable state.

SG85

**Modulation of TDP-43 condensates rescues ALS/FTD disease phenotypes in multi-stressor paradigms.**

**J. D. Lai**, V. Yu; Neurological & Rare Diseases, Dewpoint Therapeutics, Boston, MA.

Over 97% of amyotrophic lateral sclerosis (ALS) patients exhibit TDP-43 proteinopathy. In ALS patient-derived iPSC motor neurons (iPSC-MNs), diverse cellular stressors trigger TDP-43 to phase separate into heterogeneous biomolecular condensates. Consequently, TDP-43-dependent functions such as pre-mRNA splicing of STMN2, as well as neuronal survival are negatively impacted. We performed an extensive small molecule phenotypic screen to uncover TDP-43 condensate-modifying drugs (c-mods) effective against multiple types of stress. We further screened these hits against stress in ALS iPSC-MNs. Collectively, these c-mods show dose-dependent inhibition of TDP-43 cytoplasmic condensates. C-mods elicit restoration of TDP-43 nuclear-cytoplasmic ratio, STMN2 expression, and the ratio of TDP-43-dependent alternatively-spliced transcripts across over 15 familial and sporadic ALS/FTD iPSC-MNs. C-mods also potently rescue stress-mediated neurite retraction in a dose-dependent manner. Importantly, this series displays specificity for departitioning TDP-43 from G3BP1-containing stress granules. A direct comparison against approved ALS treatments in a 43-gene microarray that represents a “TDP-43 loss-of-function score” show that c-mods better ameliorate TDP-43-specific changes in response to neuronal

stress. As a potential therapeutic, TDP-43 c-mods exhibit favorable pharmacokinetic and pharmacodynamic properties. Collectively, these data show that modulating TDP-43 condensates can rescue pathological phenotypes across diverse forms of ALS and FTD.

## Subgroup: Cells in the WILD: Environmental Influences on Cell Morphology and Behavior

SG87

**Hunting for design principles: using predatory ciliates to uncover strategies for building complex cell behaviors.**

**S. M. Coyle**, Z. Xu; Department of Biochemistry, University of Wisconsin, Madison, WI.

Within a volume of just a few picoliters, cells organize molecules and chemistry to build dynamic systems that move through, interact with and respond to their environment to perform complex behaviors like tiny robots. In Eukaryotes, predatory ciliates achieve familiar and easily scored animal-like behaviors—hunting for prey—at the single-cell level, thus providing a window into the design principles for how molecular structures, sensors, and activities can be integrated to build sophisticated microscale machines that perform specialized cell behaviors. Here we mine this diversity for fundamental systems biology design principles using two contrasting predatory ciliate models. The first, *Lacrymaria olor*, uses rapid morphology dynamics to manipulate a hunting proboscis that strikes, recognizes, and engulfs specific prey targets. The second, *Podophrya collini*, uses a fixed array of barbed tentacles to ensnare preys, triggering membrane fusion and rapid transport of prey cytoplasm into the cell body. In each case, specialized cellular structures with millisecond sensing and responding capabilities are used to recognize and capture targets. Using a combination of quantitative imaging, gait analysis, and molecular approaches we dissect how structure, sensing and activity are integrated to encode these behaviors.

SG88

**Crosstalk between metabolic enzymes and photo-sensation in *Stentor coeruleus*.**

V. Johnson, **M. M. Slabodnick**, K. Owusu-Nyantakyi, B. Farhi, L. Weyer Johnson, N. Phulwar; Knox College, Galesburg, IL.

The ability to sense and respond to light is critical for organisms throughout the tree of life. Interestingly, available data suggests that the ability to move towards or away from a light source (phototaxis) has evolved independently numerous times through a variety of cellular mechanisms. Heterotrich ciliates, like *Stentor* and *Blepharisma*, independently evolved the ability to phototax using specialized pigments packed into pigment granules held at the cell cortex. Although the species-specific pigments have been clearly implicated in the photosensation mechanism, the photoreceptor proteins required for signal transduction remain a mystery. We sought to identify the proteins packaged into the pigment granules, specifically those bound directly to the blue-green pigment called stentorin, in order to gain a better understanding of the photosensation mechanism in *Stentor*. We used mass spectrometry to identify proteins associated with pigment granules in *Stentor*, and then functionally characterized candidate genes using RNA interference and other assays. From these data we discovered that the glycolytic enzyme aldolase appears to be a major component of the pigment granules, possibly directly coupled to the stentorin pigment. Cells with reduced aldolase levels display defects responding to light, although how this enzyme contributes to the phototransduction pathway remains unclear. Gaining a better understanding of the pigment granules in *Stentor*, specifically how aldolase and

stentorin contribute to phototaxis, could yield insights into a novel photosensation mechanism that is potentially conserved in other Heterotrich ciliates.

SG89

**Cooperative hydrodynamics accompany multicellular-like colonial organization in the unicellular ciliate *Stentor*.**

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Evolution of multicellularity from early unicellular ancestors is arguably one of the most important transitions since the origin of life. Multicellularity is often associated with higher nutrient uptake, better defense against predation, cell specialization and better division of labor. While many single-celled organisms exhibit both solitary and colonial existence, the organizing principles governing the transition and the benefits endowed are less clear. Using the suspension-feeding unicellular protist *Stentor coeruleus*, we show that hydrodynamic coupling between proximal neighbors results in faster feeding flows that depend on the separation between individuals. Moreover, we find that the accrued benefits in feeding current enhancement are typically asymmetric- individuals with slower solitary currents gain more from partnering than those with faster currents. We find that colony-formation is ephemeral in *Stentor* and individuals in colonies are highly dynamic unlike other colony-forming organisms like *Volvox carteri*. Our results demonstrate benefits endowed by the colonial organization in a simple unicellular organism and can potentially provide fundamental insights into the selective forces favoring early evolution of multicellular organization.

SG90

**Movement through confined, disordered environments: exploring the space of bacterial motility.**

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Bacterial motility is central to several biological processes, including pathogenesis and biofilm formation. Accordingly, understanding how bacteria move through and interact with their surroundings has significant implications for human health and the environment. Though bacterial fitness often depends on the ability to navigate complex landscapes, most investigations of bacterial locomotion in the lab are performed in homogeneous environments. Recent work exploring bacterial swimming in 3D porous media reported notable deviations from the well-studied run-and-tumble dynamics in liquid media. However, the precise contribution of various environmental and physiological factors to bacterial locomotion remain unclear. Here, we track the movement of *E. Coli* through 2D structured environments, for which we can rigorously control the geometries to quantify how cell motility is impacted by both increasing levels of confinement (the mean spacing between obstacles) and disorder (the variance of spacing between obstacles). Our work creates a deeper understanding of how cellular movement is shaped by interactions with spatially complex and temporally dynamic mechanical cues that are ubiquitous in natural environments.

[*all authors contributed equally to this work and are listed alphabetically]

SG91

**How a non-motile cell swims.**

**A. G. Larson**, R. Chajwa, H. Li, M. Prakash; Stanford, Stanford, CA.

The daily vertical migrations of plankton play a crucial role in shaping marine ecosystems and influencing global biogeochemical cycles. They also form the foundation of the largest daily biomass movement on Earth. Surprisingly, amongst this diverse group of organisms, some single cell protists transit these depths exceeding 50 meters without employing flagella or cilia, and the underlying mechanisms remain poorly understood. It has been previously proposed that this capability relies on the cell's ability to regulate its internal density relative to seawater. Here, using Pyrocystis noctiluca as a model system, we demonstrate the primary mechanism for this density control is a rapid cellular inflation event, during which a single plankton cell expands its volume six-fold in less than 10 minutes. We diagram this event using concurrent velocity measurements and high resolution live imaging of actively migrating dinoflagellates minutes after they are collected from the ocean using a custom vertical tracking microscope. This self-regulated cellular inflation selectively imports fluid less dense than surrounding seawater, and can effectively sling-shot a cell and reverse sedimentation within minutes. This ability is made possible by a reticulated cytoplasmic architecture in Pyrocystis noctiluca that enables this rapid increase in overall cell volume without dilution of its cytoplasmic content. This vascular structure is described in detail using light-sheet microscopy as well as transmission electron microscopy. We further present a generalized mathematical framework that unifies cell-cycle driven density regulation, stratified ecology, and associated cell behavior in the open ocean. Our theory stitches together widely separated length and time scales, resulting in a minimal 1D model that incorporates both the physical and cell-physiological aspects of this reduced ecosystem in which gravitational pull can formally operate as a selection pressure in the evolution of marine plankton. Our study unveils an ingenious strategy employed by non-motile plankton to evade the gravitational sedimentation trap, highlighting how precise control of cell size was essential for survival in the ocean.

SG92

***Naegleria*, a model system for the "brain-eating amoeba", crawls quickly and persistently in confined environments that resemble routes to the brain.**

L. K. Fritz-Laylin, **K. Velle**; University of Massachusetts, Amherst, MA.

The evolutionarily divergent "brain eating amoeba" *Naegleria fowleri* has a ~95% case fatality rate, and primarily infects children. Despite this clear medical importance, we have little understanding of the mechanisms underlying *Naegleria* pathogenesis. This lack of knowledge is compounded by the large amount of evolutionary distance separating *Naegleria* from heavily-studied model systems. *Naegleria* is an opportunistic pathogen that normally lives in pond environments where it eats bacteria. To initiate an infection, *Naegleria* amoebae must crawl through narrow channels in the skull to reach the brain, where they continue to crawl in confinement to spread the infection. Model organisms like *Dictyostelium* and animal cells can use either branched actin networks or cytoplasmic pressure-dependent blebs to push out the front of the cell to crawl. We recently showed that *Naegleria* uses branched actin networks to crawl across flat surfaces, but it was unknown if and how *Naegleria* changes the way it crawls when in confined environments like the brain. Using quantitative microscopy, we now show that *Naegleria gruberi*—a non-pathogenic model for the related brain-eating amoeba *Naegleria fowleri*—switch from using actin-rich protrusions to using membrane blebs to crawl in confinement.

*Naegleria* also crawl faster and more persistently when confined between coverslips or in narrow microchannels. We find that in the absence of any chemical cue, *Naegleria* continue to probe the openings of microchannels until they get in, rarely disengaging from the microchannel interface. Once cells fully enter these channels, they crawl extremely quickly (up to 100 microns/min) in one direction for millimeters. This behavior may be beneficial to *Naegleria* in nature, where they crawl through three-dimensional pond sediments where they seek out bacteria on which to feed. To model these types of migratory interactions, we introduced *Naegleria* to sediment-like hydrogels, which cells readily entered and crawled through. Collectively, these data suggest that once *Naegleria* amoebae detect an opening to a narrow channel—similar to what they encounter during infection—cells will switch to blebbing motility to crawl quickly and persistently.

SG93

**The unconventional multicellularity of *Choanoeca flexa*.**

**T. Brunet**; Institut Pasteur, Paris, FRANCE.

The origin of multicellularity has been one of the main transitions in our evolutionary history. Traditionally, multicellular development has been classified into two main modalities: (1) clonal development, in which serial division of a single cell gives rise to an organism or a colony (as in the embryos of animals, plants, fungi and algae); (2) aggregative development, in which free-living single cells converge and adhere to each other in order to form a multicellular entity (as in social amoebae). Clonal development is more widespread and thought to be more evolutionarily stable than aggregative development, which is thought to be an "emergency response" to conditions of extreme environmental stress.

In the past few years, important insights into the origin of animal multicellularity have come from the study of choanoflagellates, the closest living relatives of animals. We have recently isolated a remarkable new species of choanoflagellate from splash pools on the island of Curaçao: *Choanoeca flexa*. *C. flexa* develops into multicellular sheets organized as polarized monolayers of cells that can invert their global curvature by collective contractility under the influence of environmental stimuli. Here, I will report on recent findings on the control of *C. flexa* multicellular development by the environment. *C. flexa* undergoes a unique type of development that combines aggregation of single cells and clonal expansion of colonies, thus blurring the dichotomy between aggregative and clonal multicellularity. *C. flexa* naturally lives in splash pools that undergo cycles of evaporation and refilling by waves, giving rise to extreme fluctuations in salinity. By a combination of field studies and laboratory experiments, we show that *C. flexa* transitions in and out of multicellularity under the control of environmental salinity fluctuations. We propose that its unusual development represents an adaptation to the extreme and fluctuating conditions experienced by *C. flexa*: aggregation allows fast establishment of multicellularity within the short windows of favorable environmental conditions that allow cooperative feeding and cell division, while unfavorable conditions cause a switch to a unicellular resistant form. This represents a novel example of an environmentally controlled life cycle, expands the option space for the evolution of multicellularity, and highlights the potential of emerging model systems to shed new light on fundamental and enduring biological questions.

SG94

**Echinoderm embryos to model environmental pressures on epithelial morphogenesis.**

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Embryos of many echinoderm species develop freely in sea water with a fertilisation envelope as the only protection from environmental insults. In the sea star *Patiria miniata* the fertilisation envelope not only protects, but also helps shape the early embryo, as the embryonic cells form a blastula by lining the inner surface of the envelope. This raises the possibility that the spatial constraints imposed by the envelope may influence epithelial morphogenesis, e.g. compaction, cell density and cell connectivity. Here we use live-imaging of the sea star embryo coupled with deep learning-based segmentation, to dissect the relative contributions of cell density, tissue compaction, and cell proliferation on epithelial architecture. We find that the 3D connectivity of cells within the tissue changes over time as epithelial compaction and cell density increase. Importantly, altering the spatial constraints acting on the embryos by mechanical compression results in precocious compaction, increased cell density as well as in changes in 3D packing. Moreover, the combined effects of i) the pressure imposed on the tissue by the compression and of ii) the occurrence of cell divisions within the tissue, affect 3D connectivity of embryonic cells. These results show how epithelial morphogenesis can be affected by changes in the spatial constraints acting on the embryo. For the sea star embryo, these spatial constraints are set by the size and material properties of the fertilisation envelope, which may be altered by the fluctuations in sea water salinity and pH that are now occurring due to climate change. Therefore, the sea star embryo is an excellent model to investigate how physical pressures due to environmental change affect epithelial morphogenesis. Current work is aimed at understanding whether and how sea water chemistry affects the fertilisation envelope and, consequently, epithelial morphogenesis during embryonic development of marine embryos.

SG95

**Reciprocal intra- and extra-cellular polarity enables deep mechanosensing through layered matrices.**

**C. Walter**; Washington University in St. Louis, St. Louis, MO.

Adherent cells migrate on layered tissue interfaces to drive morphogenesis, wound healing, and tumor invasion. While stiffer surfaces are known to enhance cell migration, it remains unclear whether cells sense basal stiff environments buried under softer, fibrous matrix. Using layered collagen-polyacrylamide gel systems, we unveil a migration phenotype driven by cell-matrix polarity. Here, cancer (but not normal) cells with stiff base matrix generate stable protrusions, faster migration, and greater collagen deformation due to ‘depth mechanosensing’ through the top collagen layer. Cancer cell protrusions with front-rear polarity produce polarized collagen stiffening and deformations. Disruption of either extracellular or intracellular polarity via collagen crosslinking, laser ablation, or Arp2/3 inhibition independently abrogate depth-mechanosensitive migration of cancer cells. Our experimental findings, validated by lattice-based energy-minimization modeling, present a cell migration mechanism whereby polarized cellular protrusions and contractility are reciprocated by mechanical extracellular polarity, culminating in a cell type-dependent ability to mechanosense through matrix layers.

SG96

**Coordinated wound responses in a regenerative animal-algal photosymbiotic metaorganism.**

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Animal regeneration requires coordinated responses of many cell types throughout the animal body. In animals carrying endosymbionts, cells from the other species may also participate in regeneration, but how cellular responses are integrated across species is yet to be unraveled. Here, we study the acoel *Convolutriloba longifissura*, which hosts symbiotic *Tetraselmis* green algae and can regenerate entire bodies from small tissue fragments. We show that animal injury leads to a decline in the photosynthetic efficiency of the symbiotic algae and concurrently induces upregulation of a cohort of photosynthesis-related genes. A deeply conserved animal transcription factor, *runt*, is induced after injury and required for the acoel regeneration. Knockdown of *runt* also dampens algal transcriptional responses to the host injury, particularly in photosynthesis related pathways, and results in further reduction of photosynthetic efficiency post-injury. Our results suggest that the *runt*-dependent animal regeneration program coordinates wound responses across the symbiotic partners and regulates photosynthetic carbon assimilation in this metaorganism.

## Subgroup: Deep Learning and Artificial Intelligence in Cell Imaging

SG97

**Deep Learning Segmentation for Large Scale Analysis of Cryo-Electron Tomography and VolumeEM.**

**J. Heebner**, C. Purnell, M. Grillo, M. Swulius; Penn State University - College of Medicine, Hershey, PA.

As cryo-electron tomography (cryoET) has gained popularity and become a more accessible technique, the ability of researchers to collect huge amounts of data has rapidly increased. Unfortunately, image analysis techniques have not kept pace. Though multiple methods have emerged to address the analysis gap and mine information, by and large the gold standard for segmentation of cryo-tomograms remains expert hand annotation. Deep learning is an appealing solution, but often a technically challenging one for researchers to implement. The need for workflows and software that are easy to stand up and get running is at an all-time high. To that end, we present a workflow that adapts the commercially developed software Dragonfly (Non-commercial licenses are free in most countries) for training convolutional neural networks (CNNs) to segment cryo-electron tomograms. We pair this workflow with simulated training data from our open-source software cryoTomoSim (CTS) to reduce the need for expert hand segmented training data. The CNNs trained in this manner are robustly inferable to other similar datasets and we demonstrate this by presenting NeuroSeg, a UNet that was trained on one real tomogram and one simulated tomogram, then used to fully segment nine features in 106 neuronal tomograms. By combining real and synthetic data, we have developed a workflow that allows for rapid training and robust segmentation of huge amounts of cryoET data. Furthermore, even without synthetic data, this workflow is applicable to many types of microscopy data including FIB-SEM, Serial block face, microCT, multi-channel fluorescence, etc. This workflow (and other workflows like it) has the potential to dramatically change the way we examine the huge datasets that our microscopes are capable of producing and unlock multitudes of new insights into the high-resolution arrangement of proteins and structures *in situ*.

SG98

**Streamlining deep-learning based 3D segmentation and application-appropriate validation of quantitative image-based assays.**

**G. Dunster**[1],. Allen Institute for Cell Science[1], M. P. Viana[1], J. Chen[2], S. M. Rafelski[1]; [1]Allen Institute for Cell Science, Seattle, WA, [2]Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, GERMANY.

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. Segmentation is an important step to extract meaningful measurements of the organization of intracellular structures from 3D microscopy images. Segmentations need to be accurate, consistent and reproducible to ensure biological interpretability across all aspects of a dataset (e.g., data collection days, microscope settings, experimental conditions). To address this challenge, we previously developed the Allen Cell & Structure Segmenter, a Python-based open-source toolkit for 3D segmentation. The first part of the Segmenter, a collection of classic image segmentation workflows together with a lookup table for workflow selection, was developed into a plugin for Napari to allow user-friendly access. The second part of the Segmenter is an iterative deep-learning workflow that begins with the segmentation outputs of the classic workflow and uses two straightforward “human-in-the-loop” curation strategies to convert these outputs into a set of 3D ground truth images for iterative model training without the need for manual painting. The network architectures were designed and tested specifically for 3D fluorescence microscope images. This deep-learning workflow is being developed into another Napari plugin with a user-friendly curation interface implemented using the open-source package CytoDL, which we are developing to simplify deep-learning experiment tracking and code maintenance. Next, the performance of the segmentation, or of other image analysis or deep-learning based image transformation algorithms, must be evaluated within the context of the specific biological application. There is no “one size fits all” workflow or software that, in one shot, covers all the interpretations a researcher may be targeting. Instead, biological interpretability of quantitative image-based assays requires “application-appropriate” validation. We are developing standardized approaches to streamline, to eventually automate this type of validation, and to guide biology researchers how to identify application-appropriate validation metrics for their specific interpretation goals.

SG99

**New uses for local shape descriptors in image analysis.**

**U. Manor**; University of California, San Diego, La Jolla, CA.

Segmentation of microscopy images is often plagued by difficulties determining borders between objects, whether they be plasma membrane borders in electron microscopy images of brain tissues, or fluorescence-labeled organelles moving past one another in living cells. Deep learning-based approaches have shown great promise in these kinds of tasks. One of the biggest bottlenecks in deep learning-based segmentation approaches is the requirement for large amounts of human-annotated/proofread training data, which is extremely time-intensive and therefore expensive to generate. Here we show that utilizing local shape descriptors ("LSDs", Sheridan et al., 2023) as an auxiliary learning task we can significantly reduce the required amount of annotations in order to generate useful 3D segmentation results. We also show that transformer-based architectures can be useful for improved tracking of organelles in live cell videomicroscopy data. We speculate that a combination of LSD-based

segmentation and transformer-based tracking approaches will serve as a useful tool for a broad range of biological imaging applications.

SG100

**PIXIMI: A web-based deep learning tool for biomedical image analysis.**

**A. Papaleo**, L. Moser, N. Gogoberidze, B. Cimini; BROAD INSTITUTE OF MIT AND HARVARD, Cambridge, MA.

Creation of machine learning networks for biological imaging tasks often suffers from a crucial gap: The subject matter experts who understand the images well are not typically computationally comfortable training neural networks, and lack simple ways getting started to do so. We present here Piximi (piximi.app), a free, open-source "Images to Discovery" web app designed to make it easy to train and deploy neural networks for image classification, object classification, and segmentation directly in the web browser, meaning users face no installation hurdles and can access web accessibility features such as in-browser translation and can be run on any device with a web browser, including mobile devices. Piximi allows users to upload 8-bit or 16-bit images that use common image formats such as png and tiff and supports arbitrary numbers of channels and z-planes. Project data, including images, annotations, categories, etc. can be saved to a project file, allowing for multi-session use and encouraging reproducibility/sharing. All data stays local on the user's machine, and training happens inside the browser; no data is transmitted to a central server unless explicitly stated. Due to this architecture, Piximi provides the option to be run offline from a locally hosted Docker container or on a user’s personal computer. After loading images into Piximi, users can label the images using any number of created categories. Loaded images can also be annotated via 9 in-app annotation tools, from simple bounding boxes to sophisticated superpixel-based color annotation. Models can be trained based on a small subset of the user’s data and then used to predict on a larger number of unclassified images, simplifying the identification of difficult-to-classify images. In an effort to provide robust functionality without overwhelming less computationally comfortable users, hyperparameters can be tuned in a simple interface and are hidden by default. Models trained by Piximi can then be exported and run on any other device/context that uses Tensorflow, meaning networks trained in-browser on smaller subsets of data can then be applied to arbitrarily large data sets that would not be appropriate for the browser.Users can upload image annotations in common specifications such as COCO and Stardist,or segment object(s) of their choosing using the app's built-in segmentation neural networks or, in some circumstances, an uploaded Tensorflow model from elsewhere. Identified objects can be subsequently classified using Piximi's classifier functionality. In summary, we believe this tool will thus help close the gap between scientists who want to use neural networks, and those who can, accelerating bioimage-based science in many domains.

SG101

**Visual interpretability of deep learning models in cell imaging.**

**A. Zaritsky**, O. Rotem, L. Ben Nedava; Ben-Gurion University of the Negev, Be'er-Sheva, ISRAEL.

With the rapid growing volume and complexity of modern biomedical visual data, we can no longer rely on humans' amazing capacity to identify visual patterns in biomedical images. Deep learning has emerged as a powerful technique to identify hidden patterns that exceed human intuition in complex cell imaging data. Extracting a deeper biological understanding, such as mechanistic description of complex phenotypes, require human interpretable explanation of the deep learning model’s decision

process, however, the non-linear entanglement of image features makes deep learning models a ‘black box’ that lacks straightforward explanations of which biologically meaningful image properties are important for the models’ decision.
I will present methods to "reversed engineer" deep learning models intended to highlight the image properties that critically define the model’s decision. First, a new generalized method toward systematic visual interpretability of deep learning image-based classification models that relies on counterfactual visual explanations using a disentangled latent representation to enable visually intuitive traversal of the latent space. We apply this method to decipher blastocysts morphological quality properties in the context of in vitro fertilization. Second, a method for interpreting image-to-image transformation models highlighting the essential information within an image toward maintaining accurate image-to-image transformation. We apply this method to reveal the spatial contexts driving generative models trained to map label-free images to organelle localization (“in silico labeling”).
Interpretable machine learning could enable computationally driven biological discovery: capitalizing on the machine’s unprecedented ability to automatically identify complex patterns within cell biological images, followed by human interpretation guided by visual explanation techniques. Using the power of the human visual system to understand machine learning would ultimately lead to the generation of new biological insight and testable hypotheses.

SG102

**Microscopy Image Restoration and Downstream Analysis - how AI is and will transform scientific image data analysis in the life sciences.**

**F. Jug**; Fondazione Human Technopole, Milan, ITALY.

The necessity to analyze scientific images is as old as the ability to acquire such data. While this analysis did initially happen by observation only, modern microscopy techniques enable us to image at unprecedented spatial and temporal resolutions, through the 'eyes' of many and very diverse imaging modalities.The unfathomable amounts of data acquired in the context of life science research cannot any longer be analyzed by manual observation alone. Instead, algorithmic solutions are helping researchers to study and quantify scientific image data.In the past years, our abilities to use artificial intelligence (AI) for the automated analysis of scientific image data gained significant traction, and many important analysis problems have now much improved solutions based on ANNs. At the same time, we start being aware of limitations that come with this new set of machine learning approaches. To overcome those it will require a community effort, and I will be happy to make this case in my talk.Additionally, I would like to give an update on some of the latest algorithmic developments from our and other labs. More specifically, I will talk about improved but easy to use image restoration and segmentation methods and the efforts of our community to store, share, and run AI based methods via the BioImage Model Zoo -- a Horizon Europe funded infrastructure we are currently establishing.

SG103

**Omega: Harnessing the Power of Large Language Models for Bioimage Analysis.**

**L. A. Royer**; Chan Zuckerberg Biohub, San Francisco, CA.

We introduce Omega, a sophisticated conversational agent that leverages the power of Large Language Models (LLMs), such as ChatGPT, for image processing and analysis tasks. Omega comes as a user-friendly Python-based plugin for napari, which simplifies the task of cell imaging by holding a conversation in natural language instructions with users to perform intricate image processing and

analysis tasks such as segmenting cell nuclei and subsequent quantifications.
Notably, Omega's innovative features encompass generating detailed, step-by-step plans for tasks like nuclei segmentation in an image, creating user interface widgets on-demand, and correcting its own code, thereby augmenting its utility and convenience for researchers. Furthermore, the Omega integrates renowned cell and nuclei segmentation algorithms such as cellpose and StarDist, along with our leveraging denoising software Aydin for image clarity.
While the power of this tool brings the potential to revolutionize the field of cell imaging, it also carries the need for cautious adoption, considering the known limitations of LLMs. For example, the risk of hallucinated facts and trivial reasoning errors by the model necessitates unambiguous task descriptions by the user. Future improvements could extend Omega's capabilities to understand and produce speech or combine it with image-centric models to augment its utility.
In conclusion, Omega offers not just a tool for task completion but also an interactive platform for user education in image processing and analysis. Its multilingual abilities hold the promise of democratizing cell imaging and making it more accessible to researchers worldwide, irrespective of their coding skills or English language proficiency. This advance represents a significant stride towards a future where AI meets biological research, making scientific exploration faster, more accessible, and more intuitive.

SG104

**Paint to track: A functional maps framework for tracking 3D cell biological surfaces.**

**M. Driscoll**[1], R. Magnet[2], A. Aqeel[1], M. Ovsjanikov[2]; [1]University of Minnesota, Minneapolis, MN, [2]École Polytechnique, Palaiseau, FRANCE.

Cells shape and reshape themselves as they accomplish diverse functions in vivo. Numerous algorithms have been developed to locally track the cell edge in 2D microscopy movies, enabling discoveries ranging from cellular search strategies to the intracellular signaling hierarchies governing cell shape change. However, robust 3D surface tracking algorithms have yet to be developed. Since extending currently used 2D cell edge tracking algorithms into 3D is infeasible, a new computational strategy is required.
To track detailed 3D cell surfaces across large movements, we developed a computational framework based on functional maps, which were originally invented in computer graphics to solve shape correspondence problems between pairs of deformable 3D surfaces. Rather than directly mapping points on a surface to corresponding points on another surface, in a functional maps approach, shapes are analyzed by considering real-valued functions defined on, and maps between shapes are then derived between these functions. To enhance interpretability, we framed the tracking parameters as physical conservation laws, enabling physical modeling or extraction of physically inspired quantities. Furthermore, since cells can be associated with a variety of functions, not simply by those dependent on position, cell surfaces can be locally tracked across large movements via other features, such as local curvature or the localization of a fluorescence marker. Our computational framework will pave the way for deep learning pipelines that run in biologically inspired geometries, such as the cell surface. We demonstrate the capabilities of our framework by tracking the surfaces of cells with diverse morphologies and imaged via multiple microscopy modalities. To illustrate the biological utility of 3D surface tracking, we apply our framework to track morphological motifs, including blebs and filopodia, compare surface velocities to local rates of change in underlying signaling activity, and extract time series of actin filament polymerization at the surface of a blebbing cell. By rendering accessible the analysis of 3D dynamic morphology and its associated intracellular signaling, we anticipate that our framework will initiate a wave of biological investigation into the function of 3D cell organization.

SG105

**Self-supervised Foundation Model Captures High-dimensional Morphology Data from Single Cell Brightfield Images.**

K. Saini, K. Jacobs, S. Zhang, **R. Carelli**, A. Wong-Thai, C. Luengo, V. Lu, A. Mavropoulos, A. Jovic, J. Mei, T. Vollbrecht, S. Boutet, M. Salek, M. Masaeli; Deepcell, Inc, Menlo Park, CA.

Many methods for imaging and sorting cells rely on biomarkers that may perturb cells and create selection bias. To eliminate dependence on biomarkers and enable broader assessment of cells, we developed REM-I, a platform that characterizes cells based on high-dimensional morphology. Live cells are captured with brightfield high resolution imaging and analyzed in real-time by self-supervised deep learning models to generate quantitative AI embeddings, which represent high-dimensional descriptions of cell morphology. One key technical challenge in building this platform was developing an AI model to extract features from cell images of diverse human cells without prior knowledge of cell types, cell preparation, or other application-specific knowledge for an exploratory approach. Accordingly, we developed the Human Foundation Model (HFM), which is a hybrid architecture that combines self-supervised learning (SSL) and morphometrics (computer vision) to extract 115 dimensional embedding vectors representing cell morphology from high-resolution REM-I cell images. SSL produces a foundation model with high generalization capabilities that enables hypothesis-free sample exploration and efficient generation of application-specific models. Meanwhile, computer vision extracts features that represent measurable concepts (e.g., cell size, shape, texture, intensity) and improves model interpretability. Here, we describe how the HFM self-supervised backbone model was trained, the discriminatory power added by supervised tasks, and validation of the reproducibility and generalization capabilities of the resulting model. We also report on the Axon data suite, a user-friendly tool designed for researchers to analyze data and create custom reusable workflows. This includes the ability to store and manage data, visualize data as low-dimensional projections, and train classifiers to recognize and sort cell populations on the REM-I instrument. To enable compatibility with user-preferred downstream analysis pipelines, the tool provides data export options for images, plots, and embeddings. Our approach allows users of all computational skill levels to access and interpret AI-enabled morphological profiling. Potential applications of the REM-I platform include heterogeneous sample evaluation, tumor cell enrichment, cell state characterization, and multi-omic integration.

SG106

**Towards foundation models for cellular image analysis.**

**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 deep learning have made it easier to convert imaging data into interpretable and actionable information. Recent advances in deep learning and image labeling methodologies have placed the construction of foundation models - deep learning models that perform essential tasks across diverse datasets - within reach. In this talk, I present my lab’s progress toward creating foundation models for cellular image analysis and how these models have changed our approach to performing image-based measurements.

## Subgroup: Energy and Metabolism in Time and Space

SG107

**Glycolytic Metabolon Assembly on Mitochondria via Hexokinase O-GlcNAcylation.**

H. Wang[1], **G. Pekkurnaz**[1], J. Vant[2], R. Sánchez[1], L. Micou[1], A. Zhang[1], V. Luczak[1], S. Yu[1], M. Jabbo[1], S. Yoon[1], A. Abushawish[1], M. Ghassemian[1], E. Griffis[1], A. Singharoy[2]; [1]University of California San Diego, La Jolla, CA, [2]Arizona State University, Tempe, AZ.

Glucose, as the primary source of cellular energy, is metabolized through glycolysis, a process initiated by the rate-limiting enzyme, Hexokinase (HK). In tissues with high metabolic demands, such as the brain, the prominent isoform is HK1, which primarily localizes to the outer mitochondrial membrane. This localization is crucial for the efficient coupling of glycolysis and oxidative phosphorylation, thereby optimizing energy generation. This study uncovers a novel molecular mechanism that regulates HK1 activity and its mitochondrial localization, mediated by the metabolic sensor enzyme, O-GlcNAc transferase (OGT). OGT catalyzes the reversible post-translational modification of serine and threonine residues on proteins with a GlcNAc sugar moiety, known as O-GlcNAcylation. The activity of OGT is regulated by intracellular UDP-GlcNAc concentrations, modulated by glucose flux through the hexosamine biosynthetic pathway. Our research reveals that HK1 undergoes dynamic O-GlcNAcylation at its regulatory domain, with increased O-GlcNAcylation occurring in response to upregulated OGT activity. Our findings demonstrate that O-GlcNAcylation of HK1 promotes its mitochondrial localization, facilitates the assembly of a glycolytic metabolon on the outer mitochondrial membrane, thereby enhancing both glycolytic and mitochondrial ATP production rates. Mutations in the O-GlcNAcylation site of HK1 lead to decreased ATP production rates and dysregulated presynaptic vesicle release in neurons. Our study thus reveals key molecular pathways that couple neuronal metabolism to mitochondrial function via OGT and how their dysregulation can contribute to neurological disorders.

SG108

**Robust control of ATP levels by allosteric regulation of glycolysis.**

**D. Titov**; University of California, Berkeley, CA.

Glycolysis is a conserved metabolic pathway that produces ATP and biosynthetic precursors. Here, we use a mathematical model to investigate how the control of glycolytic enzymes through allostery and mass action accomplishes various tasks of ATP homeostasis, such as controlling the rate of ATP production, maintaining high and stable ATP levels, and ensuring that ATP hydrolysis generates energy. Our model uses enzyme rate equations with >150 biochemical parameters estimated from experiments to accurately predict glycolysis activity in living cells. We find that allostery is required for the robust maintenance of high and stable ATP levels by preventing a futile cycle between ATP-producing and ATP-consuming enzymes of glycolysis, while mass action alone is sufficient for regulating the rate of ATP production and ensuring that ATP hydrolysis generates energy. Our results suggest that the functions of allostery are distinct from its postulated role in controlling metabolic pathway rates.

SG109

**A spatial map of hepatic mitochondria uncovers functional heterogeneity shaped by nutrient sensing signaling.**

S. Kang, **N. Porat-Shliom**, R. P. Cunningham, C. B. Miller, C. M. Cultraro, J. Hernandez, L. M. Jenkins, A. Lobanov, M. Cam; National Cancer Institute, NIH, Bethesda, MD.

In the liver, mitochondria are exposed to different concentrations of nutrients due to their spatial positioning across the periportal (PP) and pericentral (PC) axis. How these mitochondria sense and integrate these signals to respond and maintain homeostasis is not known. Here, we combined intravital microscopy, spatial proteomics, and functional assessment to investigate mitochondrial heterogeneity in the context of liver zonation. We found that PP and PC mitochondria are morphologically and functionally distinct; beta-oxidation and mitophagy were elevated in PP regions, while lipid synthesis was predominant in the PC mitochondria. In addition, comparative phosphoproteomics revealed that mitophagy and lipid synthesis are regulated by phosphorylation in a zonated manner. Furthermore, we demonstrated that acute pharmacological modulation of nutrient sensing through AMPK and mTOR shifted mitochondrial phenotypes in the PP and PC regions of the intact liver. This study highlights the role of protein phosphorylation in mitochondrial structure, function, and overall homeostasis in hepatic metabolic zonation. The findings have important implications for liver physiology and disease.

SG110

**The subcellular localization of mitochondria regulates gene expression patterns.**

N. Shannon, **B. Cunniff**; Pathology and Laboratory Medicine, University of Vermont, Burlington, VT.

The subcellular architecture of mitochondria is highly dynamic and variable across cell types and tissues. The local positioning and activity of mitochondria within cells provides a platform for localized signaling events governed by mitochondrial derived signals, including ATP, calcium, and reactive oxygen species (ROS). Our group has characterized the subcellular distribution of hydrogen peroxide ($H_2O_2$) in the context of varying mitochondrial architectures using the $H_2O_2$ fluorescent biosensor HyPer7. Deletion of the mitochondrial adapter protein Miro1 in mouse embryonic fibroblasts (MEFs) leads to perinuclear constriction of mitochondrial populations, leaving the periphery of these cells devoid of mitochondria. $H_2O_2$ levels map to sites of highest mitochondrial density and therefore $H_2O_2$ levels are significantly lower in the cell periphery of Miro1 KO MEFs, compared to wild type MEFs or in Miro1 KO MEFs expressing Myc-tagged Miro1. Additionally, $H_2O_2$ levels in the nucleus of Miro1 KO MEFs are significantly higher compared to Miro1 WT or Myc-Miro1 expressing MEFS. Because $H_2O_2$ can impact gene-expression patterns through changes in transcription factor activity, chromatin organization and direct oxidation of DNA we evaluated gene-expression patterns in the context of subcellular mitochondrial positioning. We conducted RNA-sequencing of Miro1 WT, Miro1 KO and Myc-Miro1 MEFs and identified differentially expressed genes in Miro1 KO MEFs compared to Miro1 WT MEFs that are rescued when Myc-Miro1 is re-expressed into Miro1 KO MEFs. Gene ontology pathways represented by these differentially expressed genes include Alzheimer's signaling, Angiogenesis, p38 MAPK, EGF signaling and integrin signaling. To further evaluate the role of perinuclear mitochondrial positioning in regulating gene expression we employed an optogenetic system to artificially redistribute mitochondria to the cell periphery or to the perinuclear space in Miro1 KO and Myc-Miro1 MEFs, respectively. Optogenetic relocalization of mitochondria rescued some of the observed gene-expression changes. Additionally, expression of the mitochondrially targeted $H_2O_2$ scavenger catalase partially impacted similar gene and protein expression patterns. Together, these data provide the first data set evaluating gene-expression

changes in the context of subcellular mitochondrial architectures using Miro1 deletion and optogenetic systems. Ongoing and future studies aim to elucidate the molecular signaling supporting these observed changes to gene-expression dependent on subcellular mitochondrial architectures.

SG111

**Live-imaging reveals metabolic rewiring in skin stem cells that drive tolerance to oncogenic mutations *in vivo* by two distinct cell competition strategies.**

**A. Hemalatha**, R. Perry, V. Greco; Yale University, New Haven, CT.

Metabolic rewiring downstream of growth signals is a major driver of regulated and aberrant growth. To understand how oncogenic mutations are sensed and their aberrant activity suppressed in the skin stem cells, we asked how metabolic states change *in vivo* when wild-type (WT) cells interface with mutant cells. We leveraged two oncogenic mutations that employ different cell competition strategies: Wnt signaling activated mutant βcatGOF cells are eliminated and outcompeted by WT cells; $Hras^{G12V}$ mutant cells outcompete WT cells yet are integrated into functional epidermis. Using two techniques:1. optical redox imaging to image endogenous levels of NADH and FAD, adapted *in vivo* in skin of live mice over time and 2. $^{13}C_6$- glucose tracer- mass spectrometry to measure metabolic fluxes from epidermis directly, we tracked spatio-temporal changes in the metabolic states of skin stem cells as it adapted to the mutations.We discover: 1. There is a ***rapid redox (NADH/FAD) drop*** in the stem cells in response to the mutations that precede other tissue aberrancies making it one of the first observable responses to the oncogenic insult. 2. Redox ratios and their temporal dynamics are ***a measure of cell fitness and predicts*** the ability of a mutant cell to persist or get eliminated from the stem cell compartment. The βcatGOF cells that maintain low redox ratio are eliminated and $Hras^{G12V}$ cells that recover their redox after a transient drop persist and expand within the skin stem cell compartment 3.The βcat and Hras mutants that are ultimately tolerated by the tissue display ***metabolic rewiring contrary to Warburg effect***, a fundamental concept in cancer metabolism, wherein cancer cells upregulate glycolysis in favor of glucose oxidation despite the ATP deficit. Instead, we discovered that both winner and loser mutations upregulate flux through glucose oxidation to mediate the observed redox changes. Interestingly, in the winner Hras mutant, more lactate is made from pyruvate uncoupled from TCA cycle flux. 4. Inhibiting glucose oxidation dramatically inhibits the tissue morphological changes downstream of βcat and Hras mutations and reverses the cell competition outcome such that the loser βcatGOF cells are no longer effectively eliminated and winner $Hras^{G12V}$ cells lose their proliferative advantage. Hence, metabolic rewiring downstream of oncogenic growth factor signaling is necessary for the aberrant phenotypes downstream of these mutations and the tissue responses that suppress/ tolerate them. Surprisingly, we find that a similar redox drop is observed in newly formed Wnt-positive mesoderm cells during normal gastrulation of mouse embryos, suggesting that the metabolic machinery downstream of growth factor signaling is conserved across normal development and aberrant oncogenic activation.

SG112

**Non-invasive long-term imaging of microbial metabolic imaging by Raman methods.**

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The metabolic composition of cells strongly affects their patterns of gene expression, cell cycle progression, behavioral strategies, and allocation of resources between processes such as protein expression ribosomogenesis and the formation of storage lipids and carbohydrates. For single-celled

microbes, in particular, phenotypic and metabolic heterogeneity may be strongly linked, especially under stressful conditions. Study of such heterogeneity and its consequences over time would strongly benefit from the ability to monitor metabolic state non-invasively in single cells over time. I will discuss our adaptation of Raman methods to track glucose incorporation rates (by stimulated Raman scattering) and overall metabolic dynamics (by spontaneous Raman microscopy) over several generations in budding yeast cells under various environmental conditions. These measurements lay a basis for the systematic study of the role of metabolic heterogeneity in the survival of microbes under harsh environmental and genomic stresses.

SG113

**Spatiotemporal regulation of glycolysis at synapses during energy stress.**

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Neurons are polarized cells that can extend the length of an animal, forming synapses far from the neuronal cell body. The activity states of synapses vary across time and space resulting in transient changes in energy demand. How neurons regulate local energy metabolism at synapses to meet energy demand and sustain synaptic function is not well understood. We have previously shown that in neurons of the nematode *C. elegans*, energy stress caused by transient hypoxia causes glycolytic enzymes to dynamically re-localize within the cytoplasm near synapses. To measure glycolysis at a sub-cellular scale, we adapted a fluorescent biosensor, called HYLight, that can temporally detect changes in a glycolytic metabolite at single synapses. This enables the genetic dissection of mechanisms of metabolic flexibility that sustain energetically vulnerable processes at synapses *in vivo*. One factor that can enable or restrict metabolic flux is nicotinamide adenine dinucleotide (NAD+), a coenzyme that serves as an electron acceptor in numerous biochemical pathways, including glycolysis. Accumulation of its reduced form, NADH relative to its oxidized form NAD+, can be a driver of reductive stress and is associated with several metabolic diseases. We found that **L**actate **D**e**H**ydrogenase (LDH-1) and **G**lycerol-3-**P**hoshphate **D**e**H**ydrogenase (GPDH-2), two enzymes that can regenerate NAD+ from NADH, are required for synaptic vesicle recycling during energy stress. We observe that GPDH-2 localizes to synaptic regions, and its localization is dependent on synaptic vesicle transport machinery. Using a biosensor to measure changes of the glycolytic metabolite, fructose 1,6-bisphosphate (FBP), we see that patterns of FBP consumption are disrupted in *gpdh-2(-)* and *ldh-1(-)* mutants, consistent with altered glycolytic flux. We aim to manipulate NAD+ levels using heterologous expression of a bacterial NADH oxidase, LbNox to determine the effects of maintaining elevated NAD+/NADH ratios on different aspects of neuronal health, particularly at synapses.

SG114

**Investigating the Relationship Between Mitotic Oscillator Properties and Free Energy Dissipation in Cell Cycles during Early Embryonic Development.**

**S. Wang**, U. Kadiyala, L. Yourston, D. Walker, Q. Yang; Biophysics, University of Michigan, Ann Arbor, MI.

This study aims to investigate the relationship between the properties of the mitotic oscillator, such as speed and precision, and free energy dissipation in cell cycles during early embryonic development. The regulation of the oscillator relies heavily on a circuit centered around the cyclinB-CDK1 complex. Sustaining such oscillations with high accuracy in this far-from-equilibrium system incurs significant energetic costs. Previous theoretical studies have proposed a link between oscillator accuracy and

energy dissipation, but experimental characterization of this relationship has been challenging. Here we developed a high-throughput microfluidics system to create and measure thousands of oscillators in diverse energy landscapes. Water-in-oil micro-droplets encapsulating *Xenopus laevis* egg extracts function as artificial cells with fully intact mitotic circuit components. Systematic manipulation of ATP levels and network topology in individual droplets allowed for quantitative assessments of diverse cell cycle dynamics. Our findings revealed a non-monotonic response in the oscillator's behavior to changing energy availability. When exploring the energy landscape supporting CDK1 bistability, the oscillator exhibits maximal speed and minimal phase diffusion at an intermediate ATP level, but is not able to maintain this high-performance overtime. As ATP level approaches both upper and lower bounds, the speed decreases, and the system undergoes a progressively faster dephasing rate, but continues to maintain a higher degree of regularity. Furthermore, manipulating the network topology by removing the energy-intensive Wee1/Cdc25 regulations on CDK1 has an impact on energy dissipation and subsequently on oscillator performance. The research shows optimal energy budget is necessary to promote cell cycle rate and precision and provides valuable insights into the crucial role of the free energy budget in governing the mitotic oscillator's performance.

SG115

**Organization of Cytoskeletal Networks Creates Non-Equilibrium Energy Gradients Through Space and Time.**

**A. I. Duarte**[1], H. Lee[1], R. A. Banks[2], S. Hirokawa[3], V. Galstyan[4], C. Ji[5], V. Gomez[1], M. Tajik[6], H. Postma[7], M. Thomson[1], R. Phillips[1]; [1]California Institute of Technology, Pasadena, CA, [2]Chan Zuckerberg Biohub, San Francisco, CA, [3]Institut de Biologie du Développement de Marseille, Marseille, FRANCE, [4]AMOLF, Amsterdam, NETHERLANDS, [5]Massachusetts Institute of Technology, Cambridge, MA, [6]Vienna Center for Quantum Science & Technology, Vienna, AUSTRIA, [7]California State University, Northridge, Northridge, CA.

Active matter systems consume fuel to form organized, dynamical structures and patterns. These ordered states do not exist in the absence of an energy source. To gain insight into the energetic cost to form and maintain structure, we investigate the assembly of an ordered aster from a disordered, homogeneous mixture of microtubules and their attendant kinesin motors. This self-organization occurs due to optogenetically-controllable crosslinking of motor proteins that walk on microtubules and hydrolyze ATP. Here, we perform the first careful measurement of ATP consumption, and power usage, through space and time on an in *vitro* cytoskeletal network. We additionally develop reaction-diffusion models and corresponding finite elements simulations to predict how a given motor profile results in non-equilibrium ATP distributions. Comparing the theoretical versus measured ATP profiles provides insight into the dynamics of self-organization and properties of the motors. Our experiments reveal spatial gradients in ATP consumption. The concentration of ATP depletes over time, first in the center of the aster and then propagating outward. This is inversely related to the concentration of motors, which are most concentrated in the aster center. Our theoretical models qualitatively match the experimental depletion profiles of ATP. We also find that power expenditure correlates with aster contraction, with largest changes in aster radius accompanying the greatest power usage. These results suggest that loading of motors, due to high motor densities at small aster radii, creates slower contraction. This work is a first step toward understanding the role of energy consumption through space and time to produce organization in this active matter system. More broadly, our work provides a case study towards the larger effort of developing generalized theories of non-equilibrium systems connecting energy, entropy, and order.

SG116

**Coupling energy metabolism to morphogenesis in the developing lung.**

**B. Lemma**, S. Zhang, S. Topiwala, D. Roman, K. Goodwin, A. Kosmrlj, C. Nelson; Princeton University, Princeton, NJ.

Energy at a molecular scale is required to fuel cellular activity that gives rise to tissue morphogenesis. Despite a deep understanding of cellular energy metabolism, it is unclear whether energy metabolism is patterned during organogenesis in a way that correlates with and/or drives gene expression or mechanical forces. We are measuring the spatial patterns of energy metabolism, primarily through the lens of mitochondrial activity in the early embryonic chicken lung. At this stage of development, the chicken lung is comprised of tubes of simple columnar epithelium surrounded by a loose mesenchyme. To generate the first several branches off the dorsal surface of the primary bronchus, subsets of epithelial cells undergo actomyosin-driven apical constriction. The first three of these branching events are highly stereotyped in time and location; we therefore focused on this geometrically ideal system to study how energy metabolism relates to changes in tissue morphology. We imaged mitochondrial density, membrane potential, glucose concentration and ATP concentration in the epithelium during branching in cultured lung explants. We found increased mitochondrial activity in the epithelium at the emerging branches. Additionally, we measured oxygen consumption rates to set physical bounds on the levels of total mitochondrial respiration. Our work suggests that patterns of energy metabolism are connected to patterns of morphogenesis of multicellular tissues. This work is funded in part by an NIH Director's Pioneer Award (HD111539) and the NSF Postdoctoral Research Fellowships in Biology Program under Grant No. (2305831)

## Subgroup: Feedback Loops and Spatiotemporal Patterning

SG117

**Unraveling Attractor States: Rhythmic Contractile Behavior and Oscillatory Activation of Rho GTPase during Mitosis.**

C. Tong, **M. Wu**, X. J. Xu; Yale University, New Haven, CT.

Non-linear circuits play a pivotal role in governing the organization and dynamics of living systems. One example is the rhythmic contractile behavior that occurs in the cortex of living cells, which is essential for cells to change shape. In mitosis, precise activation of contractility in time and space is essential for cell division. Our recent research indicates that the pulsatile activation of Rho GTPase on the plasma membrane of mitotic cell is regulated by two coupled networks that involve both lipid metabolism and nonvesicular lipid transport. These two networks operate on different time scales and are linked in a way that generates both simple oscillations with different periodicities and complex mixed-mode oscillations. We thus propose that the phosphoinositide-Rho GTPase signaling network is poised at the edge of chaos, and small changes in the kinetic parameters of the phosphoinositide metabolism network enable cells to rapidly transition into various ordered states. The identification and visualization of these oscillations offer unprecedented opportunities to investigate signal transduction in the living systems using a dynamical systems framework.

SG118

**Mechanics and organization of actin subpopulations control Ras/PI3K activation in migrating cells.**

**J. Kuhn**, P. Devreotes; Johns Hopkins University, Baltimore, MD.

To produce forward motion, many cells must create and maintain two distinct actin architectures: a cell "front" with polymerizing, primarily branched actin and a cell "back" containing contractile actomyosin networks with more linear and crosslinked actin bundles. Signaling molecules like activated Ras and PI(3,4,5)P3 (PIP3) control the size and location of the cell front and are critical for normal motility. These signaling and cytoskeletal networks face a fundamental problem: while the molecules in them turn over within seconds, cells must maintain distinct fronts and backs for minutes or hours. While we understand many of the parts involved in this process, how they function as an ensemble to generate processive motion is not well-understood. Particularly, little is known about how the organization and mechanics of the cytoskeleton influences upstream signaling networks. This has been difficult to study because many cytoskeletal proteins are essential or redundant. To address this, we designed a system to acutely inhibit Myosin assembly by recruiting a Myosin Heavy Chain Kinase to the membrane in a model of amoeboid migration (*Dictyostelium discoideum*). We observed that lowering Myosin assembly increases Ras activation and sensitivity to chemotactic stimulus. We then probed how actin network morphology affects signaling by treating cells with inhibitors of actin nucleation and assembly. In *Dictyostelium* and human neutrophil-like HL60 cells, inhibiting Arp2/3 branched actin nucleation dramatically lowers PIP3 levels. Disassembling the remaining F-actin or Myosin bipolar thick filaments reverses this decrease, showing that the remaining actomyosin network was suppressing front signaling. Conversely, activating Arp2/3 by raising the concentration of G-actin increases Ras activation. Finally, global or local activation of RacE, a *Dictyostelium* RhoA homolog which increases linear actin abundance and crosslinking, inhibits Ras activation. Taken together, our results show that the organization and mechanics of distinct actin networks control cell signaling and provide an attractive mechanism for how cells can maintain front-back polarity. This integration between cytoskeletal and signaling networks may also explain how moving cells respond to mechanical cues and has important implications for the control of oncogenes like Ras in highly invasive cancer cells.

SG119

**Defining the regulation of Rho GAP, RGA-3/4 on cortical excitability.**

**S. Kim**, W. M. Bement; University of Wisconsin–Madison, Madison, WI.

Cortical excitability, the ability of cell cortex to generate propagating waves of actin assembly and Rho GTPase activity, has been implicated in many essential biological processes such as cell locomotion and morphogenesis. During cytokinesis in amphibian and echinoderm embryos, propagating waves of Rho activation and inactivation, and complementary waves of F-actin assembly and disassembly are amplified and focused at the equatorial cortex by the mitotic spindle. Such GTPase waves are proposed to result from a fast positive feedback loop based on Rho and the Rho GEF, Ect2, that advances the wave front and a delayed negative feedback loop based on F-actin and the Rho GAP, RGA-3/4, that trails behind the wave to shut it off. Several important mechanistic details are lacking, however, in our understanding of cytokinetic cortical excitability. In particular, the relationship between F-actin and RGA-3/4 is not well understood, nor has RGA-3/4 been characterized in any detail. To further our knowledge about RGA-3/4, we have performed structure and function analysis in *Xenopus* oocytes. The oocytes are quiescent in terms of cortical excitability due to low expression levels of cytokinetic regulators, including RGA-3/4. RGA-3/4, when expressed alone in *Xenopus* oocytes, localizes to and

reorganizes the cortical F-actin network into interlocking cables. This activity is independent of its GAP activity but requires interaction with Rho. Additionally, the first 43 amino acids as well as IG$^{232,233}$ residues are necessary for F-actin reorganization. When co-expressed with Ect2 in *Xenopus* oocytes, RGA-3/4 induces cortical excitability. In contrast to F-actin reorganization, cortical excitability requires GAP activity as well as the portions of the protein needed for F-actin reorganization. Interestingly, an RGA-3/4 mutant that comprises the GAP domain and regions required for F-actin reorganization fails to support cortical excitability - instead, it generates static patterns of Rho activity. Complete induction of cortical excitability requires a minimum of ~120 aas of the region downstream of the GAP domain (aa234-350). Overall, this study shows how RGA-3/4 interacts with F-actin and regulates cortical excitability, providing more insights into the fundamentals of the delayed negative feedback loop in cytokinetic cortical excitability.

SG120

**Coupled Excitable Networks Regulate Cellular Waves and Morphology: Insights from Mathematical Models.**

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Cell migration is a dynamic and complex process involving intricate regulatory networks. Traveling waves are observed at the basal surface of migrating cells providing clues about the underlying mechanisms that orchestrate cellular locomotion. These waves are indicative of coupled excitable networks, where the interaction between two distinct systems plays a crucial role in regulating cell movement. The first, known as the Cytoskeletal Excitable Network (CEN), acts as a fast regulatory mechanism that influences the cytoskeleton. The second system, referred to as the Signal Transduction Excitable Network (STEN), operates at a slower scale and controls the activity of signal transduction species involved in cellular responses. CEN and STEN are interconnected through a sophisticated network of interactions. A feedforward connection from STEN to CEN allows STEN to modulate the activity of CEN, shaping the cytoskeleton's responsiveness to external chemical stimuli. Additionally, feedback loops from CEN to STEN contribute to a dynamic interplay between the two systems, facilitating further regulatory control, including the sensing of mechanical signals. The level of cellular activity is directly influenced by the size of the threshold in these excitable networks, a parameter determined by the strength of the feedforward and feedback loops. To gain deeper insights, we developed a mathematical model to explore the influence of these loop strengths on the observed waves and cellular morphology. Through computational simulations, the model demonstrates that altering loop strengths leads to distinct phenotypes in both the observed waves and cellular morphology. We also use the model to show how to improve the efficiency of directed cell migration by controlling signal fluctuations. Understanding the relationship between network architecture and cellular behavior is critical for elucidating the fundamental principles that govern cell migration. Our study sheds light on the intricate dynamics of coupled excitable networks and their role in regulating cellular waves and morphology during migration. These findings provide valuable insights into the fundamental mechanisms of cell movement and offer potential avenues for exploring novel strategies to manipulate cellular behavior in various physiological and pathological contexts.

SG121

**Self-organized cytoskeletal patterning in an artificial cell cortex.**

**J. Landino**; Geisel School of Medicine at Dartmouth, Hanover, NH.

The cell cortex, comprised of the plasma membrane and an underlying meshwork of filamentous actin (F-actin), is remodeled during a variety of essential biological processes including cell adhesion, cell migration, and cell division. Previous work has shown that prior to large-scale remodeling the cell cortex is dynamically patterned with subcellular waves of F-actin assembly and disassembly, a phenomenon termed “cortical excitability”. In developing embryos, cortical excitability is generated through coupled positive and negative feedback, with rapid activation of F-actin assembly driven by the small GTPase Rho, followed in space and time by inhibition of Rho activity. These feedback loops are proposed to serve as a mechanism for amplification of active Rho signaling at the cell equator to support furrowing during cytokinesis. Investigating the mechanisms that support and regulate cortical patterning is currently limited by a lack of technical approaches that can bridge our understanding of biochemical feedback signaling and cortical pattern formation, including the molecular regulation of signaling molecules, membrane dynamics, and cytoskeletal remodeling. A breakthrough in this gap in knowledge has been the development of an “artificial cortex”, made from supported lipid bilayers (SLBs) and *Xenopus* egg extract, which successfully reconstitutes active Rho and F-actin dynamics in a cell-free system. This reconstituted system spontaneously develops two distinct types of self-organized cortical dynamics: singular excitable Rho and F-actin waves, and non-traveling oscillatory Rho and F-actin patches. Like *in vivo* cortical excitability, patterning in the artificial cortex depends on Rho activity and F-actin polymerization. We also find that SLB fluidity directly influences the propensity for pattern formation in the artificial cortex, which suggests dynamic membranes support cortical patterning. The role of membrane dynamics and composition in regulating self-organized cortical patterns is largely uncharacterized. Our preliminary investigations into SLB-dependent regulation of pattern formation have revealed that lipid composition organizes specific types of patterning. Specifically, phosphatidylethanolamine (PE) supports repeating excitable waves not observed with other SLB compositions. This opens the door for future studies on the relationship between the cell membrane and cytoskeletal patterning within the cell cortex. The artificial cortex is a novel system for investigating the biophysical and biochemical regulation of self-organized patterning.

SG122

**Dissecting crosstalk and feedback mechanisms in leukocyte migration.**

**S. R. Collins**; University of California, Davis, Davis, CA.

Cell migration is fundamental for development, immune responses, and cancer metastasis. Leukocytes are a striking example, as they navigate persistently while following chemical signals to target locations. The Rho GTPase signaling network plays a central role in this process by promoting stable cell polarity while integrating inputs from receptors. These GTPases have characteristic roles in controlling the cytoskeleton, and they exhibit pattern-forming abilities that help segregate cell front and rear activities. Feedback and crosstalk connections are thought to be central to this pattern forming ability, and for shaping signaling dynamics downstream of receptors. However, the molecular wiring of these connections in leukocyte chemotaxis remains unclear. To address this challenge, we have assembled optogenetic tools and spectrally compatible biosensors to control one network component while monitoring the activity of a second in live migrating cells. Using this approach, we identified feedback and crosstalk connections regulating Cdc42, including a central role for actin in these connections. We

find that actin assemblies are necessary for maintaining local signal transmission from receptors to Cdc42, and that actin modulates crosstalk connections to promote stable polarity.

SG123

**Wnt and EGFR Signaling Pathways Spatially and Temporally Link the Cell Cycle and Differentiation of Blood Progenitors.**

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Hematopoiesis, the process of blood cell formation, is a highly complex and dynamic developmental program that requires precise temporal and systemic control mechanisms. During homeostasis, a critical balance is maintained between myeloid-like progenitors and their differentiated progeny, which function to mitigate stress and innate immune challenges. The molecular mechanisms that help achieve this balance are not fully understood. Using genetic dissection in *Drosophila*, we find that spatial and temporal integration of Wnt and EGFR signaling pathways is critical in modulating hematopoietic progenitor cell fate decisions, cell cycle control, and cellular differentiation. During development, a Wnt6/EGFR-signaling network simultaneously controls progenitor growth, proliferation, and differentiation. Unlike G1-quiescence of stem cells, hematopoietic progenitors are blocked in the G2 phase by a beta-catenin-independent Wnt6 pathway that restricts Cdc25 nuclear entry and promotes cell growth. Wnt6 levels in the large pool of progenitors are specifically controlled by a non-autonomous Hedgehog signal generated from the niche. On the other hand, canonical beta-catenin-dependent Wnt6 signaling is spatially confined to mature progenitors through localized activation of the tyrosine-kinases EGFR and Abl, which promote nuclear entry of beta-catenin and facilitate exit from G2 and subsequent differentiation in G1 phase. Furthermore, we find that a high level of E-cadherin at the plasma membrane inhibits nuclear translocation of beta-catenin. Together, these interconnected multifunctional signaling pathways combine transcription-dependent and -independent forms of both Wnt6 and EGFR signaling to create a direct link between cell-cycle control and differentiation. We expect that this unique combinatorial strategy of Wnt and RTK signaling pathways will operate in contexts broader than blood development and in a variety of species.

SG124

**Wave chemotaxis in neutrophils.**

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In crawling cell chemotaxis, the attractant receptor occupancy is translated and amplified intracellularly so that formation of the expanding leading edge and the contracting trailing ends is directed along the perceived gradient. Such strictly spatial means of chemotactic sensing however is insufficient to understand aggregating/swarming chemotaxis where the chemoattractant signals themselves are self-generated and relayed between the migrating cells in a manner akin to action potential conductance. In *Dictyostelium*, cells migrate towards self-secreted cAMP that is relayed between the cells in the form of propagating waves to form fruiting bodies. In neutrophil swarming, chemotaxis towards relayed LTB4 drives cell migration to the site of inflammation. There cells are met with a task of reading out traveling pulsatile stimulus that is composed of a rising phase (wave front) and the attenuating phase (wave back) which are spatially reverse in orientation and time. While this problem has been analyzed in detail in *Dictyostelium*, and the role of rectified temporal response and the memory effect have been clarified, how neutrophils move in response to such moving stimulus if at all is still under explored. Here, using a

microfluidics wave generator, we demonstrate conditions where neutrophil-like HL60 cells can migrate towards the propagating attractant signal. I will discuss how the cells are able to make a spatially asymmetric response based on the live-cell FRET measurement and optogenetic perturbation of the Cdc42 activity, as well as pharmacological perturbation of Rac, PI3K and RhoA.

SG125

**Deciphering Spatial and Mechanical Coordination of Biological Oscillators through Synthetic Biology.**

**Q. Yang**; University of Michigan, Ann Arbor, MI.

Early embryonic development relies on precise intracellular timing driven by complex biochemical and genetic networks known as biological clocks. These clocks coordinate through intercellular communications to form collective patterns. It remains elusive how clocks and patterns maintain precision against stochasticity and how they coordinate with chemical and mechanical signals in environments.

I will discuss unpublished work exploring mitotic cycles and segmentation clocks, two crucial cell-autonomous oscillators governing early developmental patterning. The spatial coordination of mitotic oscillators is proposed to create waves in two forms: canonical trigger or bistable waves, and recently proposed sweep waves, where the bistability bifurcates to a stable high state via a global increase in activation rate, giving faster speeds than the classical Luther's formula. Little work exists studying the time dependence of mitotic waves. Using a newly developed Cdk1 FRET sensor and reconstituting 1D mitotic waves in Xenopus extracts with and without nuclei, we experimentally demonstrated two types of traveling waves: fast sweep or phase waves transitioning into slower trigger waves. Moreover, we found heterogeneous activation (by nuclei) can speed up the transition, reducing the entrainment time. These motivated a novel experimental development of directed waves using the CSF extracts as a high-activity Cdk1 source, supporting a unified mechanism of entrainment predicted by theory.

We also established single-cell assays using dissociated presomitic mesoderm (PSM) cells from zebrafish tailbuds. Through biomechanical perturbations using microposts, we uncovered the role of surface rigidity, mechanical cues, and biochemical gradients in regulating segmentation clock dynamics, underscoring mechanobiology in spatiotemporal patterns of somitogenesis.

SG126

**Recording morphogen signals reveals origins of gastruloid symmetry breaking.**

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Living systems can break symmetries to transform a homogeneous state to a state with distinct spatial domains. Biological symmetry breaking has been proposed to rely on biochemical Turing circuits, amplification of stochastic noise, or externally supplied cues, but these potential mechanisms remain difficult to distinguish. Here we use synthetic “signal-recording” gene circuits to study symmetry breaking in gastruloids, a simplified model of early mammalian embryogenesis. We find that an ordered sequence of BMP, Nodal, and Wnt signaling predicts cells’ future position along the anterior-posterior (A-P) axis, even before any of these morphogen signals are themselves polarized. Our study reveals that A-P axial polarization does not appear to require a biochemical Turing-like circuit or externally supplied cues, and instead arises through early signaling variability followed by a cell sorting process to form distinct anterior and posterior domains.

SG127

**Coupling of proliferation and differentiation through activation and delayed inactivation of CDK and ERK kinases.**

**S. Sharma**, H. Berger, T. Meyer, M. N. Teruel; Weill Cornell Medicine, New York, NY.

During terminal cell differentiation, permanent cell cycle exit and irreversible differentiation commitment both occur in the G1-phase of the cell cycle[1]. However, whether and how the two events are directly linked remains unclear. Here we measured live-cell activity reporters for CDK4/6 and CDK2, the main kinases that drive G1-to-S-phase progression, together with a reporter for PPARG, the master transcriptional regulator of adipogenesis, simultaneously expressed in thousands of single cells undergoing adipogenesis[1,2,3]. We found that a prolonged drop in CDK activity correlates with a sharp rise in PPARG levels and subsequent differentiation commitment, supporting previous work from us and others showing that PPARG directly drives the expression of the CDK inhibitor p21. However, cell-to-cell variability in the dynamics of CDK inactivation also revealed that many cells, that eventually differentiate, exit the cell cycle days before increasing PPARG levels, raising the conundrum of how these cells exit the cell cycle without increasing PPARG to drive p21 expression. We found that proliferation-driven increases in cell density drive a p21-independent route to cell cycle exit through a coupled increase in expression of the cell cycle inhibitor p27 and decrease expression of Cyclin D, thus providing at least two distinct and cooperative paths to regulate cell cycle exit during terminal differentiation. Given the correlation between CDK inactivation and increased PPARG expression found in our live-cell imaging experiments, the application of CDK inhibitors, while significantly inhibiting proliferation, unexpectedly, had only a small effect on promoting differentiation commitment. This suggested that cell density promotes differentiation through mechanisms extending beyond cell cycle inhibition. Remarkably, ERK signaling pathway inactivation strongly promoted PPARG expression and differentiation commitment and occurs naturally at high cell density. Thus, suppression of ERK activity is a critical step that coordinates cell cycle exit in the G1-phase, occurring through CDK inactivation, with differentiation commitment. References - 1. Zhao M, ⋯, Teruel MN, *Cell Reports*, 2020 ,2. Spencer S, ⋯, Meyer T, *Cell*, 2013, 3. Yang HW, ⋯, Meyer T, *Elife*, 2020

SG128

**Deconstructing the human segmentation clock *in vitro*.**

**O. Pourquie**[1,2,3]; [1]Genetics, Harvard Medical School, Boston, MA, [2]Pathology, Brigham and Women's Hospital, Boston, MA, [3]Harvard Stem Cell Institute, Cambridge, MA.

The spine is characterized by the periodic arrangement of vertebrae along the anterior-posterior (AP) axis. This segmental or metameric organization is established early in embryogenesis when pairs of embryonic segments called somites are rhythmically produced by the presomitic mesoderm (PSM). The tempo of somite formation is controlled by a molecular oscillator known as the segmentation clock. The segmentation clock generates a complex signaling pulse of Notch, Wnt and FGF activity involved in the rhythmic production of somites. This oscillator has been well characterized in model organisms, where it has been shown to tick with a species-specific period. Virtually nothing is known on human somitogenesis as it proceeds between 3- and 5-weeks post conception when embryos are extremely difficult to access. We have developed protocols to differentiate human pluripotent stem cells (ES/iPS) *in vitro* into PSM cells. Single cell RNA-sequencing comparison of these human cells differentiating *in vitro* with mouse embryo PSM reveals that they faithfully recapitulate the PSM differentiation sequence *in vitro*. Using our *in vitro* system as a proxy for human somitogenesis, we were able to demonstrate

that human iPS reporter cells harboring a HES7 fluorescent reporter differentiated to PSM exhibit 5-hour oscillations, thus identifying the human segmentation clock. We have also succeeded in generating PSM organoids from human iPS cells that can sequentially form somites exhibiting a normal antero-posterior pattern *in vitro*. Our work using these *in vitro* systems led to a novel understanding of the role of the segmentation clock in formation of the anterior and posterior somitic compartments. Our work provides a framework to study early stages of human somitogenesis which are poorly accessible in the embryo. I will discuss how these novel *in vitro* systems provide us with powerful tools to dissect the segmentation of the embryo and the control of developmental timing.

## Subgroup: Nuclear Mechanobiology

SG129

**An energy flow organizes the oocyte nucleus across scales.**

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Cells remodel their cytoplasm using cytoskeletal motors. Motor activity generates random forces that stir the cytoplasm, agitating and displacing membrane-bound organelles like the nucleus in somatic and germ cells. The energy of these forces is transmitted inside the nucleus, yet its consequences on liquid-like biomolecular condensates residing in the nucleus remain unexplored. Here, we probe experimentally and computationally diverse nuclear condensates, that include nuclear speckles, Cajal bodies, and nucleoli, during cytoplasmic remodeling of female germ cells named oocytes. We discover that growing mammalian oocytes deploy cytoplasmic forces to timely impose multiscale reorganization of nuclear condensates for the success of meiotic divisions. These cytoplasmic forces accelerate nuclear condensate collision-coalescence and molecular kinetics within condensates. Inversely, disrupting the forces decelerates nuclear condensate reorganization on both scales, compromising condensate-associated mRNA processing and consequently hindering oocyte divisions that drive female fertility. We establish that cytoplasmic forces can reorganize nuclear condensates in an evolutionary conserved fashion in insects. Our work implies that cells evolved a mechanism, based on a flow of energy coming from cytoplasmic force tuning, to functionally regulate a broad range of nuclear condensates across scales.

SG130

**Atypical Nuclear Phenotypes in Confined Cancer Cells.**

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The physically diverse environment that a metastasizing cancer cell experiences has been shown to induce functional changes through signaling pathways and selection pressure. Strong confinement that cancer cells encounter in the metastatic process can challenge the integrity of the cell nucleus - the largest organelle in the cell. Nevertheless, the most frequent damage - nuclear blebs and nuclear envelope ruptures - are rapidly repaired, ensuring an overall stability of the nucleus. Submitting a panel of cell lines to mechanical confinement we have identified, however, a novel nuclear phenotype suggesting a strong instability. This phenotype was observed at a low frequency in most cells but was found to be vastly predominant in a set of cell lines immortalized from circulating tumor cells (CTCs). Instead of the repeated blebbing observed in most cells upon strong confinement - a succession of nuclear envelope rupture and repair events - these atypical nuclei fold into a “sickle-shape”, which has not been reported before. These nuclei display large openings in the nuclear envelope and strong chromatin condensation, but the cells remain alive and are devoid of apoptotic markers. The formation of “sickle-shape” nuclei can be completely blocked by the drug blebbistatin, which inhibits myosin II. Analysis of nuclear envelope composition and perturbation experiments in cells showing classical phenotypes versus “sickle-shape” nuclei point to the conjunction of multiple factors required for these atypical nuclei to occur: The combination of low lamin A/C and low vimentin levels together with a high level of contractility. This suggests a working model in which strong acto-myosin contractility induced by confinement would tear apart the envelope of mechanically fragile nuclei. It is still unclear whether the “sickle-shape” phenotype is purely detrimental to cell survival under strong confinement conditions, explaining the known short life span of CTCs; or whether it could have been selected as an advantage for a specific set of mechanical constrains in blood circulation. Our work might provide novel insights on the least researched step of the metastatic cascade - circulation in the blood. It also reveals a particular cellular state in which the nuclear envelope becomes highly unstable, potentially suggesting novel therapeutic approaches.

SG131

**Nuclear mechano-osmotic regulation of gene transcription and cell state.**

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Change in cell state and lineage are associated with substantial changes in cell and nuclear morphology. For example, embryonic stem cells exhibit dramatic nuclear flattening upon exit from pluripotency, a morphological change necessary for this transition, implicating nuclear morphology as an important regulator in cell fate decisions. Extrinsic deformation, through altering intranuclear pressure, has been associated with changes in nuclear volume and envelope tension. However, how nuclear volume and tension in the NE control genome architecture and gene expression is not well understood, and molecular mechanisms gated by changing forces within the NE are undefined. We investigated the dynamic regulation of the cell response to changes in osmotic pressure and nuclear envelope tension using human induced pluripotent stem cells (iPSCs) as a model system. To characterize cellular response to extrinsic deformation, we performed paired single-cell RNA and ATAC sequencing after transient compression and compared the immediate response to the expression profile after 24 hours of recovery. We found significant but reversible transcriptional and chromatin changes after just 30 minutes of mechanical compression. The changes were related both to cell identity as well as stress response genes. To understand the mechanisms of these two responses, we investigated the acute

transcriptional mechanoresponse with nascent RNA sequencing and enhancer analysis and identified key regulatory elements and targets of both mechano- and osmo-sensitive nascent transcription and enhancer commissioning specific for cell state transitions and the mechanoadaptive response. Surprisingly, these rapid effects led to long lasting transcriptional changes that persisted long after confinement was released and were strictly dependent on the biochemical context in which the mechanical stimulus was provided. Our findings thus indicate that the response to rapid nuclear compression/confinement can be broken into two components: osmotic stress that drives transcriptional reset, and nuclear envelope tension that drives specific transcriptional activity. We found that the relative contribution of these components is dependent on the availability of pluripotency growth factors, which stabilize enhancer elements that have lasting effects on the transcriptional state. Collectively, these investigations reveal how cells sense dynamic changes in their tissue environment and translate these into changes in transcription and thereby cell state.

SG132

**Fibrillar adhesions govern the timescales of mechano-adaptation via the vimentin cytoskeleton.**

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Cell function is driven by both chemical and mechanical signals, occurring at timescales from seconds to days. To regulate the timescales of their response, cells need effective mechanisms that control how these signals reach the nucleus. Such regulatory mechanisms are well described for fluctuating chemical signals, but whether analogous mechanisms exist for mechanical signals is still unknown. Here we demonstrate that the formation of fibrillar adhesions locks the nucleus in a mechanically active conformation, setting the mechanical response timescale to that of fibrillar adhesion remodelling (~1 hour). This process encompasses both mechanical deformation and associated mechanotransduction (such as via YAP), in response to both increased and decreased mechanical stimulation. The underlying mechanism is the anchoring of the vimentin cytoskeleton to fibrillar adhesions and the extracellular matrix through plectin 1f, which maintains nuclear deformation. Our results reveal a mechanism to regulate the timescale of mechanical adaptation, effectively setting a low pass filter to mechanotransduction.

SG133

**Investigating the Immediate Impact of Lamin B1 Loss on Nuclear Architecture and Gene Expression Using an Auxin-Inducible Degron System.**

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The nuclear lamina (NL) is a filamentous protein meshwork composed of lamins and associated proteins that underlies the nuclear envelope. Lamins have major roles in mechanically reinforcing the nucleus as well as organizing the genome. The mammalian NL is composed of the A-type lamins (Lamin A/C) and the B-type lamins (Lamin B1 and Lamin B2). Mutations in genes encoding for lamins are linked to several “laminopathy” diseases, but it remains unclear how each lamin isoform contributes to NL function. Our

ability to address this question has been limited by the fact that the lamin proteins have a long lifetime, especially in non-dividing cells. As a result, it has not been possible to study lamin function with temporal precision. We are using the auxin inducible degron (AID) system to rapidly deplete each lamin isoform. We can degrade each lamin isoform to undetectable levels within 10 hours (less than one cell cycle). We find that degrading one lamin isoform has no effect on the protein levels of the remaining isoforms. We are now working to define how Lamin B1 (LB1) contributes to the two major proposed functions of the NL. Previous studies have established that depletion of LB1 results in changes to nuclear shape and size, an increase in nuclear blebbing and nuclear rupture events, and lamin A/C (LAC) meshwork disorganization. We have failed to observe significant increases in nuclear size, nuclear blebbing or nuclear rupture events in our AID LB1-depleted nuclei. These findings suggests that these nuclear architecture defects are not immediate consequence of LB1 depletion. We verified that lamin A/C disorganization is an immediate consequence of LB1 depletion. Actin depolymerization via Cytochalasin D treatment ameliorates the effects of LB1 depletion on LAC meshwork organization. We hypothesize that LB1 maintains proper NL organization by resisting actin forces on the nucleus. We performed RNAseq after acute depletion of LB1 to query the immediate impact of LB1 loss on gene expression. We found that 16hrs after LB1 depletion, there are over 150 differentially expressed genes (DEGs). This number increases to over 350 DEGs 96hrs after LB1 depletion. These results suggest that gene expression changes precede any NL architectural defects. Ongoing experiments will further elucidate immediate impacts of LB1 depletion on nuclear architecture and gene expression.

SG134

**Propagation of Mechanical Agitation along the Chromatin Fiber in Live Cells.**

**I. V. Surovtsev**, E. Clarke, M. Saah, R. Ogasawara, T. Moss, N. Campbell, S. G. J. Mochrie, M. C. King; Yale School of Medicine, New Haven, CT.

The nucleus plays a unique role in the response of eukaryotes to mechanical inputs by simultaneously sheltering the genome from insults, transducing mechanical signals and translating these signals to modulate the gene expression pattern. Understanding and disentangling of these different aspects requires quantitative knowledge of how mechanical forces applied to the nuclear exterior are transmitted and propagated inside the nucleus. The mechanical response of the nuclear envelope to applied stress has been shown to depend on chromatin and its association with the nuclear periphery. These sites of chromatin tethering likely play key roles in mechanotransduction. However, we lack a quantitative framework for the physical and genomic length scales over which forces are transduced from these attachment points. To address this knowledge gap, we have developed a live cell assay that utilizes the natural agitation of the centromeres by cyclic, microtubule-dependent motion of the spindle pole body (SPB) in the fission yeast, *S. pombe*. Using fluorescent microscopy and high-precision single particle tracking of individual chromosomal loci, we measure the correlation in motion between the agitation point (the centromere) and a distant 'probe' locus in a library of strains with varied genomic distances between the centromere and 'probe' locus. Our results surprisingly show that this mechanical agitation propagates along the chromatin far from the source - to, at least, several hundred kilobases away - much father then the current estimates of chromatin persistence length (250-6,000 bp). Combining the measured correlation of SPB and chromatin locus motion as a function of genomic distance with the theory of semi-flexible polymers, we can arrive at an estimate of the persistence length (rigidity) of the chromatin in its native, nucleoplasmic, environment. These new insights into chromatin rigidity and correlation in the dynamics between distant chromatin loci will inform quantitative models of how mechanical stress can be directly transmitted to the genome, including the

length scales, both physical and genomic, over which we expect the chromatin is capable of responding directly to mechanical stress exerted on the nuclear envelope.

SG135

**Reorganization of solid-like chromatin drives nuclear response to chemo-mechanical environmental cues.**

**A. Kant**, V. B. Shenoy; Department of Material Science and Engineering, University of Pennsylvania, Philadelphia, PA.

Genomic remodeling as a result of a diverse range of chemo-mechanical signals from the nuclear microenvironment enables the cell response to such signals. However, the biophysical principles linking the environmental cues and the corresponding intranuclear chromatin reorganization are not yet fully uncovered. To construct such a link built on fundamental non-equilibrium thermodynamic principles, we have developed a meso-scale coarse-grained model of nucleus-wide chromatin spatial organization into densely compacted heterochromatin domains and loosely packed euchromatin regions. The model incorporates the energetics of chromatin-chromatin interactions coupled with conservative diffusive kinetics of nucleoplasm and epigenetic marks, giving rise to emergent phase separation of chromatin. In addition, epigenetic reactions drive a non-conservative interconversion between the two phases. Further, we treat chromatin as hyperelastic solid-like polymer with a compaction-dependent shear modulus. Our model can be generalized to capture solid-solid, liquid-solid, and liquid-liquid phase separation of chromatin. At a steady state, our model predicts formation of stable domains of compacted heterochromatin. By accounting for elasticity and epigenetic kinetics, we theoretically determine the size scaling of the stable heterochromatin domains. Our theoretical predictions and numerical simulations match in-vivo observations after epigenetic modulations. We find that solid-like behavior of chromatin contributes to the nuclear mechanical properties such as its mechanical stiffness and volume, as has been reported experimentally. Our model also predicts that application of hyper(hypo)-osmotic shocks leads to a thermodynamic preference for the hetero(eu)chromatin phase, with larger (smaller) compacted domains. Similarly, upon application of tensile (compressive) uniaxial and biaxial mechanical stress, we predict a prevalence of the eu(hetero)chromatin phase, corresponding changes in size-scaling of condensed domains. Our predictions are quantitatively validated by new and existing high-resolution in-vivo imaging of nucleus as well as chromatin sequencing data. Our predictions mechanistically explain in-vivo nuclear and chromatin behavior under a diverse set of dynamic microenvironmental chemo-mechanical cues. We believe that our model lays the foundation for understanding the role of chromatin organization in nuclear mechanosensing.

SG136

**Breaking the LINC - reducing mechanical stress on the nucleus improves cardiac function and survival in *LMNA*-related dilated cardiomyopathy.**

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Mutations in the *LMNA* gene, encoding the nuclear envelope (NE) proteins lamin A and C, are responsible for a plethora of human diseases (laminopathies), often specifically affecting striated muscle. The most prevalent laminopathy is *LMNA*-related dilated cardiomyopathy (*LMNA*-DCM). The molecular mechanisms leading to pathogenesis remains largely unresolved, leaving patients with limited and ineffective treatment options. Cardiac muscle biopsies from patients and mice models of *LMNA*-

DCM provide anecdotal evidence of nuclear defects; however, it remains unclear whether nuclear damage causally contributes to disease progression. The heart is exposed to constant mechanical stimulation, we therefor hypothesize that *Lmna* mutations reduce nuclear stability, resulting in transient NE rupture in cardiac muscle cells, damaging the DNA and promoting pathogenesis. To test this hypothesis, we generated a mouse model with inducible, cardiac specific deletion of lamin A/C. In adult mice, this resulted in the rapid development of severe dilated cardiomyopathy and sudden death within 30 days of lamin A/C deletion. Lamin A/C deleted hearts exhibited severely reduced ejection fraction, left ventricle thinning and dilatation, interstitial fibrosis, increased incidence of NE rupture in cardiac myocytes, and cardiac myocyte loss. Combining bulk and single-nucleus RNA sequencing techniques reveled a shift in several of the left ventricle resident cell populations, as well as activation of pathways involved in DNA damage response, cell death, innate immunity, and inflammation at early disease stages. To reduce the mechanical load on cardiac myocyte nuclei and thereby decrease mechanically induced damage, we disrupted the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex, which physically connects the cytoskeleton to the nucleus, by expression of a dominant negative nesprin construct in our inducible lamin A/C deletion model. This resulted in a significant rescue in both cardiac function and overall survival (to >300 days). Collectively, these results support the idea that mechanical stress on the more fragile *Lmna* mutant nuclei induces NE ruptures, decreasing cardiac myocyte viability and promoting pathogenesis. Finding effective ways to reduce the mechanical load sensed by cardiac muscle nuclei can pave the way towards effective therapeutic approach to treat *LMNA*-DCM.

SG137

**Fat Droplet-Nucleus Interactions & Rupture.**

**I. Ivanovska**, D. Discher; University Pennsylvania, Philadelphia, PA.

The nucleus in many cell types is a stiff organelle, but fat-filled lipid droplets (FDs) in cytoplasm can be seen to indent and displace the nucleus. FDs are phase-separated liquids with a poorly understood interfacial tension γ that determines how a FD interacts with other organelles. Here, micron-sized FDs remain spherical as they indent the nucleus and peri-nuclear actomyosin, causing local dilution of Lamin-B1 independent of Lamin-A,C and frequently triggering nuclear rupture. Focal accumulation of the cytosolic DNA sensor cGAS at the rupture site is accompanied by sustained mislocalization of DNA repair factors to cytoplasm, increased DNA damage, and delayed cell cycle. Macrophages show FDs and engulfed rigid beads cause similar indentation dilution. High Lamin-A,C tends to limit rupture but additional stress can drive rupture. Throughout, spherical shapes of small FDs indicate a high γ, which we measure for FDs mechanically isolated from fresh adipose tissue as ∼40 mN/m. This value greatly exceeds that of biocondensates, but is typical of oils in water and sufficiently rigid to perturb cell structures including nuclei.

SG138

**Stress-induced fragmentation of the endoplasmic reticulum enables effective nuclear mechanotransduction.**

**Z. Shen**[1,2], P. Niethammer[1]; [1]Cell Biology, Memorial Sloan Kettering Cancer Center, New York, NY, [2]Gerstner Sloan Kettering Graduate School of Biomedical Sciences, New York, NY.

Nuclear membrane mechanotransduction (NMMT) allows cells to sense and rapidly adapt to their physical tissue microenvironment. Reportedly, NMMT triggers inflammatory wound detection and migration mode-switching of various cell types when their nuclei become critically compressed or

swollen. This involves the rapid translocation of mechanosensitive peripheral membrane proteins, many of which participate in inflammatory lipid metabolism, to the stretched nuclear membrane (NM). How the NM develops and stably maintains tension upon physical perturbation rather than dissipating it by drawing surface from the connected endoplasm reticulum (ER) is a central conundrum of NMMT. By quantitative confocal imaging of two genetically encoded fluorescent stretch sensors, cPLA2-mKate2 and ALPS-mKate2, we show that cell swelling and confinement promote NMMT through $Ca^{2+}$ dependent ER fragmentation. Our studies indicated ER fragmentation can work in parallel with previously established mechanism of nuclear lamina and nuclear-associated cytoskeletons to modulate "nuclear mechanosensitivity" and downstream mechanosensitive signaling. Thus, the stress-induced reduction of accessible ER membrane reservoirs caused by cell-extrinsic physical perturbation or cell-intrinsic necrotic changes are a previously unacknowledged priming condition for NMMT.

## Subgroup: Science and Art: Bridging Two Creative Universes

SG139
**Integrative Illustration and Modeling from Atoms to Cells.**
**D. S. Goodsell**; the Scripps Research Institute, La Jolla, CA.

The scale level between molecular structure and cellular ultrastructure, termed the cellular mesoscale, is still largely inaccessible to direct experimental visualization. Models and artistic conceptions of the detailed molecular structure of whole cells are currently accessible using an integrative approach that combines experimental data from microscopy, structural biology, genomics, and proteomics. Artistic conceptions of the cellular mesoscale are useful for science outreach and education, and as a prelude to generation of 3D molecular models of whole cells.

SG140
**Visualisation facilitates communication in arts and in science.**
**G. Pigino**; Structural Biology Centre, Human Technopole, Milan, ITALY.

My empirical observation is that many microscopists also have an artistic inclination. In our free time, many of us paint, draw, make prints, take photos, make videos, make ceramics, sculptures, or other crafts. We are intrigued by visual aesthetics, being it an abstract painting, a beautiful landscape, the intricate morphology of a cell or the 3D structure of a protein. I fell in love with the complex structure of the ciliary axoneme many years ago. But if visual arts can please our eyes and souls, they can also help us to better understand our science and make it more accessible to others as well. My lab makes extensive use of animations and illustrations to share our findings with other scientists or a lay audience. We investigate the biology of motile and primary cilia with a combination of structural biology and cell biology approaches. In this presentation, I will show how we combine different imaging techniques, from cryo-ET to live cell imaging or expansion microscopy to shed light on the molecular process involved in cilia assembly and function. I will also present to you a fun art and science project in which we used Chlamydomonas phototaxis to make the cells collectively print their own self-portrait.

SG141

**Artistic Inspirations when Exploring, Explaining and Communicating Scientific Data.**

**H. Jambor**; Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, GERMANY.

Scientists and artists strive to convey their ideas and discoveries resulting from meticulous observations or inner reflections respectively. They achieve this with visualizations, which can make complex concepts accessible but are subject to prejudiced interpretation by viewers. For effectively communicate scientific findings with visuals, we can draw inspiration from the time-honored practices of the arts.
I will present three exemplary stories on how we can evoke artistic traditions to effectively communicate scientific data. In the first case, I show how we employ visual data representations to explore and recognize patters, shapes and forms in images and how adjustments and analyses help us to make these more accessible to us and to our audiences. Artistic elements, such as color, shape, and composition, but also abstraction techniques, can be used to highlight specific data points, trends, or outliers, and deemphasizing noise, to make it easier for viewers to interpret and understand the information.
Secondly, I will touch on how one dataset can give rise to diverse perspectives and subjective interpretations, and how this diversity can be evoked through with visualizations. I outline for an exemplary dataset how a workflow corset can be used to systematically iterate visualizations, which each give researchers the possibility to explain a different angle of a dataset. In turn, this creative approach can further the understanding also of the scientsits own interpretation of data.
In my third example I show how we use simple visualizations and storytelling to inform patients about the course of their cancer treatment. We evaluated the visual elements in isolation for their clarity, tested them in a complex information context, and surveyed them in clinical use with patients. By weaving medical data into a personal narrative, data is transformed into a compelling narrative that is understandable and memorable to patients.

SG142

**Accelerating Cell Biology with Scientific Illustration & User Experience Design.**

**T. P. Do**,. Allen Institute for Cell Science, G. T. Johnson; Allen Institute for Cell Science, Seattle, WA.

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 utilize an open science approach to develop a variety of assays, data, and computational tools available at allencell.org. As an in-house Scientific Illustrator and User Experience (UX) Designer, I create visually compelling and accurate presentations of complex scientific concepts, facilitate multidisciplinary collaboration, and optimize the experience of using and accessing our science and tools. I will demonstrate how we use best methods in scientific illustration (SI) to produce effective communication material and to translate abstract mental models into concrete visual aids, which benefits content experts during the early conceptualization stages of writing grants or papers, providing a means to clarify, organize, and articulate their thoughts more effectively.
Our UX design process involves similar interactions, but with more diverse groups of researchers, engineers, project managers, and users throughout a project's lifecycle, which I will compare and contrast with SI. We employ several UX methods to gather valuable insight about the tools and our users to inform our design decisions and guide the development process. I will highlight transferable skills

between these two disciplines that can open doors to exciting opportunities. I will also show how each field has its own strengths that can enhance the outcomes of the other, such as making UX interactions more engaging by applying the storytelling and aesthetic skill of an illustrator, or approaching the audience from a UX perspective when drafting visualizations.
I will use examples from our website throughout the talk to illustrate the importance of deliberate, flexible design and workflows, as well as the necessity of clear guidance and communicative media to ensure accessibility and usability of our data and practices for all users, regardless of their current skill level or resources. I will conclude by sharing upcoming initiatives, such as integrating UX best practices into institute projects beyond our UX team, and enhancing the efficiency of our SI process amidst a continuously evolving landscape of tools.

SG143
**A return to the practice of drawing in the biological sciences.**
**M. Mir**; Stowers Institute for Medical Research, Kansas City, MO.

Biology has entered an age where advances in imaging technologies have made data acquisition easier and more detailed than ever. But perhaps we are overlooking subtle, but important details in the process. The field of cell biology sits on a foundation of early illustrations - precise recordings of microscopic views from a time when they could only be captured with the human hand. It is well established that illustrations are important in education, but we rarely hear about how the process of drawing offers its own insights. What might we learn by the careful observation that drawing requires? Here, I introduce methods for incorporating drawing into your research practice and describe situations where drawing has led to valuable insights in the sciences. The development of new technologies does not mean that we should leave older ones behind, but instead use all of our tools in combination to deepen our understanding of the natural world.

SG144
**Bringing Molecular Stories to Life: Visualizing Research in Biochemistry and Molecular Biotechnology for Science Literacy & Outreach.**
**L. Martínez-Nuñez**; UMASS Chan Medical School, Worcester, MA.

Effective communication of scientific concepts is essential for fostering scientific literacy and societal engagement. Illustration has proven to be a highly impactful and engaging tool for visually compelling complex scientific ideas. 3D illustration adds realism, depth, visual impact, and versatility when telling scientific stories. As part of the Biochemistry and Molecular Biotechnology Outreach headquarters, my goal is to increase the delivery of the scientific knowledge our researchers produce. I create visual stories to spark curiosity and cultivate scientific literacy within and outside the department. By raising public awareness of scientific research in biochemistry and molecular biotechnology, we aim to enhance public understanding of remarkable advancements in healthcare, drug development, antibiotic resistance, and more. I utilize open-source molecular coordinates in combination with molecular visualization software, 3D Blender, and molecular nodes to create striking and impactful 3D scientific visuals. Additionally, as part of the Structure-Based Drug Design core facility at UMass Chan, I have collaborated with researchers from various institutions to create visuals for their scientific publications. Some of the most impactful stories include visuals showcasing the development of new therapeutics, antiviral drugs, autoimmune disease, and rare disease molecular mechanisms, the intriguing tale of an ultra-stable phage with an exceptionally long tail, and an exciting narrative about a multi-subunit

tethering complex involved in membrane trafficking. By combining artistry with scientific accuracy, illustrations can bridge the gap between complex concepts and the public, sparking curiosity and fostering a deeper understanding of scientific phenomena.

SG145
**The Art of Basic Science.**
**M. O'Reilly**; O'Reilly Science Art, LLC, Boston, MA.

There are numerous ways that art can support the advancement of science but many people still put science and art at opposite ends of a spectrum. Since becoming a scientist 25 years ago, I have watched the emergence of technologies and careers that make it easier than ever for science and art to enhance one another. As a science communicator for over a dozen years, I continue to work on, explore, and write about these topics, from science illustration in the age of drag-and-drop libraries and AI to how scientists can embrace their own creativity. I write a monthly newsletter called the Art of Basic Science that explores the role of art in the advancement of science. I am happy to share from my experience whatever will complement the subgroup.

SG146
**Ideas can flourish in the spaces where art and science meet.**
**C. Prinz**; Biology, University of Washington/Howard Hughes Medical Institute, Seattle, WA.

Visual media for scientific work should convey its meanings by catching the reader's eye and revealing the key findings in a succinct and accurate way. The challenge for a person sitting at the art-science interface is: how can one distill the complex scientific findings of a research project into a small piece of artwork without losing the depth and focus of the research? Speaking from personal experience, sometimes when developing illustrations, cartoons, or schematics for a paper/talk, the ideas seem to flow out of my pen onto the page. More often though, I find that one has to attempt a few ideas and make iterative progress via multiple rounds of communication with researchers before landing on a final product that is both accurate/meaningful and striking. This back and forth can yield wonderful results for figures and papers. Additionally, it can also open up a space for learning, refinement, and communication of ideas. Although the artist nearly always takes away some new level of scientific understanding of the systems, concepts, and models at play; the scientist also learns from the art and artistic process such as how to better and effectively communicate a complicated idea to audience outside the field, or may gain greater insight themselves into the very project they are researching. For instance, when working on a set of camera lucida-style drawings of zebrafish skin from an electron micrograph, adding distinct colors to the different epithelial layers revealed that the uppermost layer was still intact (consistent with previous data), but was being contorted and stretched in a way that had not been initially apparent from the micrographs alone. As a researcher and an artist, I would be delighted to share a bit about my own experiences creating figures and illustrations for scientific papers and presentations for both my own work and in collaboration with other scientists over the past 4 years. I have worked on projects in which I have been a direct experimentalist and artist also where I was strictly collaborating with other researchers in an artistic role. I will compare my experiences and learning curves over time and as an artist in different capacities.

SG147

**Beauty in the beast: Using pottery for increasing appreciation of invertebrates.**

**D. Ozpolat**; Biology, Washington University in St. Louis, Saint Louis, MO.

I am afraid of most insects and spiders. If an insect other than a house fly or butterfly approaches me and catches me off guard, I scream. But fear is only one layer of my complex experience and relationship with these creatures. After the initial scream, comes love, admiration, and curiosity. I have learned to open my heart and the non-amygdala parts of my brain to insects and spiders. So much so that, I make sculptures of them using ceramics, I have them on my dinner plates as patterns. I also happen to be a biology professor, who works on another group of underappreciated but adorable invertebrates: segmented worms. and I am on a mission! I want to invite everyone to try having this rich relationship with these animals. You feel fear and initial disgust perhaps? No problem, you are human after all. Can you take a step closer and look at these organisms with a fresh eye, like an alien who has freshly landed on Earth, unburdened with the evolutionary, social, and cultural baggage? Through my art, and my research, I create conversations to help build more complex relationships with these (lovable) creepy crawlies.

SG148

**Visualizing Killer Immune Cells with Microscopes and Hot Sculpted Glass.**

**A. Ritter**; Altos Labs, Redwood City, CA.

In the last decade, new cancer therapies have been developed that train immune cells called cytotoxic T cells to destroy tumors. Modern live-cell microscopy imaging of real cytotoxic T cells killing cancer has provided new insights into the cell biology underlying this important process. The resulting videos and images could inspire new therapeutics and may also give hope to patients receiving cancer immunotherapy (and their families). Physical manifestations of this biology in creative media like sculpted glass could provide another way for patients and other lay people to connect to the cell biology and therapeutic potential of T cells.

SG149

**Sculpting Science with Art.**

**B. Welm**; Surgery, University of Utah, Salt Lake City, UT.

The union of art and science combines two seemingly distinct fields. Art, with its expressive and subjective nature, offers a unique lens through which scientific concepts can be visualized, communicated, and interpreted. While science provides a framework of rationality and empirical exploration of the natural world that can move artistic endeavors in new directions. When art and science converge, they foster a profound exchange of ideas and perspectives, resulting in awe-inspiring and thought-provoking creations. These creations provide a bridge between diverse communities, stimulate dialog, and facilitate engagement between scientists and the public. Dr. Bryan Welm is an Associate Professor in the Department of Surgery at the University of Utah and breast cancer researcher at the Huntsman Cancer Institute. His passions for exploration and discovery led him down both scientific and artistic paths. As an artist, he blends rational and imaginative concepts into sculptures that convey processes leading to discovery in scientific research. He has created sculptures of nucleic acids, proteins, drugs, model organisms, and cells, which often integrate disparate concepts of the microscopic world and human experience. Dr. Welm also incorporates his sculptures in science outreach and

engagement activities to empower a sense of wonder and inspire future generations to explore careers in STEM.

SG150
**Designing Video Games for Science Communication: Appealing to the Non Fiction Gamer.**
**M. Stegman**; Molecular Jig Games, LLC, Baltimore, MD.

We have researched learning and attitude changes in players of several video games, including *Immune Attack*, *Immune Defense* and currently, *Strategic Repair Protocol*. Our goal is to help adults become familiar and more confident with the biochemistry and cell biology concepts relevant to infectious disease, nutrition and mental health. Our approach is to start with a simulation of a molecular or cellular process that is otherwise difficult to understand. We create some manner of player agency and then present series of levels that progressively challenge the player to learn the simulation. We have shown that *Immune Attack* players remember names, functions and appearances of cells and proteins that they need to use to win the game. Additionally, players felt more confident than non-players in their ability to understand diagrams that looked similar to the game. Therefore, we made *Immune Defense* look similar to diagrams of cells that adults might find in newspapers. We iterated on each level of *Immune Defense* until >75% of (online and in person) players voluntarily play through the first 4 levels, evidence that the levels are keeping them in the Flow state. However, players looking at *Immune Defense* for the first time often say, “This is science, I should know this,” which is not optimal first impression. So, while designing our current game, *Strategic Repair Protocol*, we drew inspiration from *Microscopya*, by Beata Mierzwa and added some whimsy: We gave our players Steampunk styled machines to manipulate. Additionally, David Goodsell’s colorful cellscapes inspired our Art Noveau environment. Our players do not say “Oh this is science” right away. Rather, we found that players play first, accepting our description of the game as a “non-fiction puzzle game.” After playing, however, players are intrigued and more than half of them ask us what the simulation represents. We have thus engaged non-scientists with the process of altering enzyme activities by optimizing the concentrations of substrates and products and/or the locations of the enzymes. Our results demonstrate that non-scientists, and indeed even non-gamers, can be engaged in learning about molecular and cellular systems when the simulations have been designed to engage players using game design principles. We are testing what kind of materials players will choose to investigate after playing, and devising tools to test player learning about enzymes.

## Subgroup: Water Dynamics and Pressure Regulation in Cells and Tissues

SG151
**Cytoplasmic flows and nuclear positioning in early Drosophila embryos.**
**S. Di Talia**[1], C. Hernandez-Lopez[2], A. Puliafito[3], M. Vergassola[2]; [1]Duke University - Durham, NC, Durham, NC, [2]Ecole Normale Superieure, Paris, FRANCE, [3]University of Turin, Turin, ITALY.

Cytoplasmic flows are widely emerging as key functional players in development. In early Drosophila embryos, flows drive the spreading of nuclei across the embryo. I will present our efforts combining hydrodynamic modeling with quantitative imaging to develop a physical model of cytoplasmic flows and their impact on development. I will introduce a two-fluid model that features an active actomyosin gel and a passive viscous cytosol. Gel contractility is controlled by the cell cycle oscillator, the two fluids being coupled by friction. In addition to recapitulating experimental flow patterns, our model explains observations that remained elusive, and makes a series of new predictions. Thus, our two-fluid model

explains flows and nuclear positioning in early Drosophila , while making predictions that suggest novel future experiments.

SG152

**Nuclear folds act as nucleus hydrostatic pressure regulators.**

**R. Rollin**[1], A. Williart[1], M. Nava[2], D. J. Muller[2], D. Cuvelier[1], P. Sens[1], J. Joanny[1], M. Piel[1]; [1]Institut Curie, Paris, FRANCE, [2]ETH Zurich, Zurich, SWITZERLAND.

Cells and their nuclei experience a large range of mechanical stresses in different biological contexts such as cell migration or growth in confined environments. A growing body of evidence suggest that the changes in nuclear volume accompanying those stresses may enable mechanotransduction, for example by affecting the liquid-liquid phase transitions in the nucleus. Yet, the interplay between mechanical stresses and changes of the nucleus volume, i.e water fluxes at the nuclear envelope (NE), is not clear. To get insights in this fundamental question, I will disclose non-published data recording the force and the volume response of Hela cell nuclei during a uniaxial compression both at the population level (static confiner experiments) and at the single nucleus level (AFM experiments). The response is non-trivial and exhibits two clear regimes of deformation. A first “safe” regime, at low confinements, for which the nucleus volume remains constant, the nucleus surface smoothens, and the force required to confine nuclei remains small. Followed by a regime of nucleus volume loss and sharp force increase (up to NE rupture and bleb appearance). Interestingly, this non-trivial volume response can be closely recapitulated within an osmo-mechanical model of the nucleus that we developed previously called the nested Pump-Leak model (Rollin et al. Elife 2022). The model proposes that at low confinements the NE tension is buffered by the folds which act as membrane reservoirs, hence impeding water outflux of the nucleus. Upon folds disappearance, the tension then increases due to the stretching of the lamina, leading to nucleus hydrostatic pressure increase and subsequent volume loss, with a sharp threshold between the two regimes, due to the stiffness of the nuclear lamina. To justify this ad-hoc nonlinear elastic constitutive equation for the NE, I will further provide both experimental and theoretical evidence showing that these folds originate from a well-known mechanical instability. Stiff elastic sheets bound to compliant substrates buckle upon compressive stresses. Compression in the system {Lamina, Chromatin} under study is further explained by the competition between the NE formation just after mitosis and the geometrical transition induced during chromosome decondensation. Together, our combined experimental and theoretical study of confined nuclei demonstrates new important roles of the nuclear folds, both for the pressure regulation in the nucleus, the nucleus volume homeostasis, the structural integrity of the NE and the nuclear size scaling, with potential implications for genome organisation and mechanosensing.

SG153

**increased intraluminal pressure in liver disease changes hepatocyte polarity and function.**

**C. Mayer**[1], S. Nehring[2], M. Kücken[3], U. Repnik[4], S. Seifert[1], A. Sljukic[1], J. Delpierre[1], H. Morales-Navarrete[1], S. Hinz[5], M. Brosch[2], B. Chung[6], T. Karlsen[6], M. Huch[1], Y. Kalaidzidis[1], L. Brusch[3], J. Hampe[2], C. Schafmayer[5], M. Zerial[1]; [1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, GERMANY, [2]Department of Medicine I, Gastroenterology and Hepatology, University Hospital Carl-Gustav-Carus, Dresden, GERMANY, [3]Center for Information Services and High-Performance Computing, Technische Universität Dresden, Dresden, GERMANY, [4]Central Microscopy, Department of Biology, Christian-Albrechts-Universtät zu Kiel (CAU), Kiel, GERMANY, [5]Department of General Surgery, University

Hospital Rostock, Rostock, GERMANY, [6]Norwegian PSC Research Center, Oslo University Hospital Rikshospitalet, Oslo, NORWAY.

Hepatocytes in the liver transport bile in small channels, the bile canaliculi. As bile is an osmotically active fluid, impaired bile flow increases pressure inside the canaliculi. Although this phenomenon is important for many liver pathologies, we have only limited knowledge of how hepatocytes respond to increasing intraluminal pressure. During development, hepatocytes form apical bulkheads inside the canaliculi, which increase canalicular resistance to intraluminal pressure. Here, we investigate whether apical bulkheads also support canaliculi in the adult liver. We observed the accumulation of apical bulkheads in mouse models and patients with impaired bile flow. However, upon persistent high pressure apical bulkheads can be lost. Apical bulkhead loss upon persistent high pressure leads to the formation of abnormally dilated canaliculi and finally structures resembling "liver cell rosettes". Rosettes have been described to form in many liver diseases, but until now the underlying cause was unknown. Using primary hepatocytes and 3D organoids, we showed that high canalicular pressure - and not bile acid signaling - leads to the loss of apical bulkheads and the formation of rosettes. Through 3D imaging techniques in patients with primary sclerosing cholangitis (PSC), we found that these rosettes are segments of epithelial-like tubes, marking a change in hepatocyte cell polarity. The hepatocytes forming rosettes in PSC patients also show reduced activity of many metabolic enzymes, which may contribute to the progression of the disease. In summary, we found that apical bulkheads serve as protective structures for hepatocytes against the effects of impaired bile flow and high pressure. Their loss is associated with changes in the liver's architecture, hepatocyte polarity and their metabolic function. Our findings prove that hepatocytes can actively sense and respond to intraluminal pressure by a yet unknown mechanosensory mechanism.

SG154

**Hepatocyte apical bulkheads are dynamic mechanical elements that protect bile canaliculi against elevated pressure.**

**M. P. Bebelman**[1], M. J. Bovyn[1,2], Y. Kalaidzidis[1], P. A. Haas[1,2], M. Zerial[1]; [1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, GERMANY, [2]Center for Systems Biology Dresden, Dresden, GERMANY.

Hepatocytes are specialized liver epithelial cells with multiple apical and basal surfaces. During liver development, small lumina form between the apical surfaces of juxtaposed hepatocytes. Later, these expand into narrow tubules, called bile canaliculi (BC), which fuse into a 3D network where bile is secreted and transported. BC elongation is enforced by apical bulkheads, membrane extensions that traverse the canalicular lumen and connect opposing apical membranes. Here, we show that apical bulkheads are mechanical elements that support BC formation by counteracting elevated luminal pressure. Using STED super-resolution microscopy, we found that apical bulkheads contain a contractile actomyosin cortex and are connected by adherens junctions, suggesting a mechanical function. Microinjection of fluid into the BC of hepatocytes *in vitro* revealed that apical bulkheads persist at high luminal pressures. Laser ablation of individual apical bulkheads directly expanded the surrounding apical membrane, demonstrating that they are under tension. A mechanical BC model, parameterized using experimentally measured laser ablation data, revealed that apical bulkheads double the luminal pressure BC can hold. Consistently, imaging of embryonic liver tissue showed that most apical bulkheads are localized in nascent BC that are not yet connected to the network and therefore have elevated luminal pressure. Finally, lattice light-sheet microscopy revealed that apical bulkheads are dynamic

structures that form and retract through the remodelling of the junctional belt. In conclusion, our findings demonstrate that apical bulkheads are dynamic mechanical elements that protect the BC network against elevated luminal pressure.

SG155

**Quantifying turgor pressure in budding and fission yeasts based upon osmotic properties.**

**J. LEMIERE**, F. Chang; Cell and Tissue Biology, University of California, San Francisco, San Francisco, CA.

Walled cells, such as plants, fungi, and bacteria cells, possess a high internal hydrostatic pressure, termed turgor pressure, that drives volume growth and determines cell shape. Rigorous measurement of turgor pressure, however, remains challenging, and reliable quantitative measurements, even in budding yeast are still lacking. Here, we present a simple and robust experimental approach to access turgor pressure in yeasts based upon the determination of isotonic concentration using protoplasts as osmometers. We propose three methods to identify the isotonic condition - 3D cell volume, cytoplasmic fluorophore intensity, and mobility of a cytGEMs nano-rheology probe - that all yield consistent values. Our results provide turgor pressure estimates of 1.0 ± 0.1 MPa for *S. pombe*, 0.49 ± 0.01 MPa for *S. japonicus*, 0.5 ± 0.1 MPa for *S. cerevisiae W303a* and 0.31 ± 0.03 MPa for *S. cerevisiae BY4741*. Large differences in turgor pressure and nano-rheology measurements between the *S. cerevisiae* strains demonstrate how fundamental biophysical parameters can vary even among wildtype strains of the same species. These side-by-side measurements of turgor pressure in multiple yeast species provide critical values for quantitative studies on cellular mechanics and comparative evolution.

SG156

**Taming Hippo's and Hydrostatic Pressure.**

**C. G. Hansen**; Institute for Regeneration and Repair - Centre for Inflammation Research, University of Edinburgh, Edinburgh, UNITED KINGDOM.

Cells need to rapidly and precisely react to multiple mechanical and chemical stimuli in order to ensure precise context-dependent responses. This requires dynamic cellular signalling events that ensure homeostasis and plasticity when needed. We have previously shown that YAP and TAZ, the co-transcriptional regulators of the Hippo signalling pathway control cell volume. Using quantitative label-free digital holographic imaging, combined with genome editing, biochemical assays and confocal imaging, we are able to analyse the temporal cellular response to hydrostatic pressure. Upon elevated cyclic hydrostatic pressure, the cell responds by rapid, dramatic and reversible changes in cellular volume. We find that cells without YAP and TAZ have lower plasma membrane tension and present evidence that YAP/TAZ drive the cellular response to hydrostatic pressure, a process that is at least partly mediated via clathrin-dependent endocytosis. Additionally, upon elevated oscillating hydrostatic pressure, YAP/TAZ are activated and induce TEAD-mediated transcription and expression of cellular components involved in dynamic regulation of cell volume and extracellular matrix. This cellular response confers a feedback loop that allows the cell to robustly respond to changes in interstitial fluid pressure. I will present recent data highlighting the complex cellular interplay centred on the cellular response to hydrostatic pressure. www.gramhansenlab.com

SG157

**Deciphering the Role of Hydrostatic Pressure in Cellular Functions and Behaviors.**

**Q. Ni**[1], Z. Ge[2], J. Fu[3], S. Sun[2]; [1]Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD, [2]Mechanical Engineering, Johns Hopkins University, Baltimore, MD, [3]Department of Physics, Johns Hopkins University, Baltimore, MD.

Mammalian cells inhabit a fluid environment and thus are continually subjected to hydrostatic pressure. The intracellular hydrostatic pressure is slightly higher than outside the cell, creating an outward hydrostatic pressure gradient ranging from 500-2000 Pa. Thermodynamically, this hydrostatic pressure gradient is balanced with an osmotic pressure gradient at the cell surface. Disrupting this balance results in water and ion flux, leading to immediate alteration of cell volume and electrophysiology. On the other hand, hydrostatic pressure is mechanically balanced by cytoskeletal contractility and membrane tension to preserve cell shape. Therefore, hydrostatic pressure is one of the fundamental physical cues in cellular environments. Embryonic development and tumorigenesis involve changes in hydrostatic pressure, and alterations in blood or interstitial fluid hydrostatic pressure are associated with diseases. Despite its importance, the effects of hydrostatic pressure on cell mechanical and electrophysiological properties remain poorly understood. In this work, we systematically investigate the impact of hydrostatic pressure on cell size, migration, growth, electrophysiology, and epigenetics. We reveal that changes in hydrostatic pressure by as low as 500 Pa can significantly modify cellular behaviors. Interestingly, cells employ distinct mechanisms to respond to positive and negative pressure changes: negative pressure triggers cytoskeletal activation of the Sodium-Hydrogen exchanger 1(NHE1), while positive pressure triggers potassium leakage via potassium channels. Our study provides novel insights into how cells sense and respond to hydrostatic pressure and underscores its importance in cell biology, with implications for understanding pressure-related physiological and pathological processes.

SG158

**Neutrophils actively swell to potentiate rapid migration.**

**T. L. Nagy**, J. Strickland, O. D. Weiner; Biochemistry & Biophysics, University of California, San Francisco, San Francisco, CA.

While the involvement of actin polymerization in membrane protrusion is well-established, we have a more limited understanding of the role of transmembrane water flow in cell motility. Here we investigate the role of water influx in neutrophil migration. These cells undergo directed movement to sites of injury and infection. Chemoattractant exposure increases cell volume and potentiates neutrophil migration, but the causal link between these processes is not known. Using a genome-wide CRISPR screen, we identify the regulators of the chemoattractant-induced neutrophil swelling, including NHE1, AE2, PI3K-gamma, and CA2. Through NHE1 inhibition in primary human neutrophils, we show that cell swelling is both necessary and sufficient for rapid migration following chemoattractant stimulation. Our data demonstrate that cell swelling complements cytoskeletal inputs for chemoattractant-induced potentiation of migration.

SG159

**Intracellular pH is dynamic during cell division and dysregulated pH drives cytokinesis defects.**

**K. A. White**; Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN.

Transient increases in intracellular pH (pHi) (7.2-7.6) are linked to normal cell behaviors while constitutively increased pHi is an emerging hallmark of cancer. However, the role for spatiotemporal pHi dynamics in regulating both normal cell processes and cancer progression remains unclear. We recently found that single-cell pHi oscillates dynamically (0.16±0.07 pH units) throughout the mammalian cell cycle. Time-lapse microscopy of single cells showed pHi decreases at G1/S, increases during S phase, decreases at S/G2, and increases again at G2/M before rapidly acidifying during mitosis. Importantly, this sinusoidal pattern of pHi dynamics is linked to cell cycle timing regardless of where cells start in the cycle. These single-cell pHi dynamics are necessary regulators of cell cycle progression as high pHi elongates G1 phase while low pHi shortened it. We next tested whether the observed acidification at mitosis is required for successful cell division. Interestingly, we found that raising pHi increased cytokinesis defects, including cleavage furrow failure, double nucleation, and >2 daughter cell formation in both transformed and normal cells. We also found that high pHi significantly increased rates of entosis (cell engulfment by its neighbor). Taken together, our data suggest that spatiotemporal pHi dynamics regulate single-cell cell cycle progression and that constitutively increased pHi is a sufficient driver of cytokinesis defects, polyploidy, and entotic phenotypes in genetically normal cells. Future work will identify the pH-sensitive proteins that regulate pHi-driven cell cycle progression and determine whether dysregulated pHi dynamics can drive cancer initiation via dysregulation of pH-sensitive mitotic regulators leading to genomic instability, cytokinesis failures, and entotic events.

SG160

**Advection of sol generates hydrodynamic forces during microtubule aster movement.**

**T. Mitchison**, L. Bai; Harvard Medical School, Boston, MA.

The cytoplasm of animal cells comprises a gel made of entangled cytoskeleton and endomembrane networks percolated by a sol made of water, small molecules and soluble macromolecules that span a large range of sizes. If these components move collectively, continuum approximations such viscoelasticity may suffice to describe the emergent mechanics. If the sol moves relative to the gel, more complex physical models are required. We previously used poroelastic formalism to explain slow equilibration of hydrostatic pressure gradients across tissue culture cells, driven by actomyosin or osmotic forces, and proposed an effective pore size of approximately 30nm for the network component of cytoplasm (PMID: 17921219, PMID: 19690051). When cytoskeletal networks move, this small pore size can cause advection of sol. The mechanical implications of advection have not been widely discussed but could be important in any situation where the cytoskeleton moves collectively. For example, Deneke et al showed that actomyosin networks in syncytial drosophila embryos advect sol near the plasma membrane, causing pressure gradients that move nuclei in the opposite direction internally (PMID: 30982601). Our current focus is on centrosome positioning during early cleavage divisions in frog eggs, which we model in an extract system. After mitosis, microtubule asters move apart in response to pulling forces from dynein and actomyosin, carrying centrosomes and nuclei with them. We found that all the cytoplasmic networks in asters moved collectively as a gel. Moving asters advected sol, as revealed by tracking the motion of a small molecule probe inside asters and passivated beads outside. We observed flow of sol into the gap between paired sister asters as they moved apart after mitosis. We propose that the outward-moving asters advect sol, generating a pressure gradient

that transports sol into the gap between them. This inward flow at the midplane supports aster growth by supplying soluble subunits. During cytokinesis in somatic cells, advection of sol by separating asters may generate high pressure at the poles, which facilitates blebbing, and low pressure in the midzone, which facilitates furrow ingression.

## Hacking Human Cellular Systems at Scale - Disease Modeling & Drug Discovery

SG162

**Human Pluripotent Stem Cell, Organoids and Disease Modeling.**

**S. Chen**[1,2]; [1]Department of Surgery, Weill Cornell Medicine, New York, NY, [2]Center for Genomic Health, Weill Cornell Medicine, New York, NY.

Organoids are miniaturized, *invitro*-grown organs that closely resemble the functional, structural, and biological complexity of*in vivo* organs. They can be derived from either human pluripotent stemcell (hPSCs) or adult tissues. Notable, hPSC-derived organoids offer enhancedcomplexity and scalability compared to those derived from adult tissue-derivedorganoids. In the early years of my scientific career, we conducted highthroughput/content chemical screens and identified several small moleculespromoting hPSC differentiation toward pancreatic β cells, pancreatic ductalepithelial cells, cardiac pacemaker cells, colon organoids, and more. Weestablished an hPSC platform encompassing over 14 types of hPSC-derived cellsand organoids, including endoderm derivatives, such as pancreatic endocrine,colonic, small intestinal, alveolar, airway, liver organoids, mesodermderivatives, such as cardiomyocytes, endothelial cells, pacemaker cells,macrophages, etc, and ectoderm derivatives, such as cerebral organoids. We are interested inapplying this multi-organoid platform to study common diseases. Compared totraditional *in vitro* models, human organoids more accuratelyrecapitulate the intricate cellular components and three-dimensional tissuearchitecture, making them increasingly popular for modeling human diseases.While most previous organoid-based disease modeling has focused on rarediseases caused by single gene/mutation, common diseases affect entirepopulations, involve multiple organs, and are associated with complex genetics.In our previous studies, we have used this multi-organoid platform todemonstrate the key concepts of applying organoid to study common diseases,including isogenic hPSCs to study the individual genetic variants identifiedfrom population-based genome-wide associate studies (GWAS), induced pluripotentstem cell (iPSC)-based GWAS, multiple organoids models to examineorgan/tissue-specific host response, and immune-host mini-tissue to mimic theimmune cell-mediated host response.Our current focus is on applying the hPSC-derived organoid platform tosystematically investigate the impact of genetic and environmental factors ondisease progression, with the aim of developing precision medicine for commondiseases.

SG163

**Neurodevelopmental Disease Endophenotypes in Rett Syndrome Patient-Derived Organoids and Their Rescue by a Small Molecule Epigenetic Modifier.**

**S. Kato**[1,2]; [1]Herophilus, San Francisco, CA, [2]UCSF, San Francisco, CA.

We developed a multi-modality screenable Rett brain organoid model that captures the genetic background of Rett patients and the human biology of X chromosome inactivation. Phenotypic screening of a library of small molecules led to the identification of an epigenetic modifier that produces durable reactivation of MeCP2 in dividing astrocytes. This reactivation leads to rescue of synaptic density and structural deficits in surrounding neurons, concordant with observations in Rett patients. This program

demonstrates the utility of the new generation of complex human in vitro models for mechanistic biology studies and early stage drug discovery.

SG164

**Searching For the Boundary Between Health and Als- Hacking Human Cellular Systems At Scale Disease Modeling & Drug Discovery.**

**S. Altschuler**; UCSF, San Francisco, CA.

Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressing and highly heterogeneous disease. The time between diagnosis and death is typically 2-3 years, most ALS patients have no known genetic cause, and even within defined genetic subtypes there is significant variation in clinical presentation. These challenges underscore the importance of obtaining information to personalize clinical decisions quickly after diagnosis. Here, we discuss recent efforts to rapidly identify ALS disease signatures from patient biopsies soon after diagnosis.

SG165

**Hepatobiliary Development and Disease in a Dish.**

**T. Takebe**; University of Cincinnati, Cincinnati, OH.

Remarkablydivergent cell types form tightly embedded connections in our body. Completeloss of interconnectedness leads to devastating clinical conditions, includinglethal organ failure end-points. For example, biliary atresia (loss ofhepato-biliary connection: inter-organ), is the most prevailing cause ofirreversible liver failure for which existing cures are currently absent. Inhepatology, our lab seeks to understand how the living system createsinterconnectedness across strata, how interconnectedness plays a role inorchestrating tissue development and maturation, and how biological alterationsin interconnectedness threaten our health. The past threedecades of embryology research, together with evolving genetics and genomicstoolsets, have revealed a high-resolution developmental roadmap tounderstanding how lineage specification occurs through intricatetranscriptional regulation. with the Zorn/Wells lab, we have revealed howparacrine signals from the neighboring mesenchyme direct embryonic foregutendoderm towards hepato-pancreatic-biliary (HBP) fate. By pursuing developmentalroadmap, we recently showed that it is possible to synthesize the entire HBPpattern *in vitro* from human pluripotent stem cells (hPSCs). The HBPmulti-organ model established a functional connection of the bile duct withintissues. In disease modeling, we have developed "*en masse* phenotypingplatform" that allows for large-scale population-scale phenotypic analysesand can help to elucidate the contributions of personalized mechanistic traitsto metabolic liver diseases. Here I will summarize our ongoing studies toinvestigate human liver development and disease through the lens of “interconnectedness”biology. Our goal is to define personalized mechanisms as a fundamental steptoward developing evidence-based, rational solutions for mechanism-directeddiagnostic and therapeutic investigation.

## New Frontiers in Host-Pathogen Interactions: From Immune Defense to Disease and Therapeutic Strategies

SG166

**Deciphering Malaria Biology with the Use of Potent Aspartyl Protease Inhibitors: A Robust Academic Industry Collaboration Leading to A Clinical Development Compound.**

**D. Olsen**; Merck, West Point, PA.

A phenotypicscreen of an aspartyl protease inhibitor library identified a hit molecule withsingle digit nanomolar potency. Resistance selection followed by whole cellgenome sequencing pointed to plasmepsin X as the target protein. Intracellularand biochemical evaluations validated the target and also determined that moreadvanced program leads were also hitting a second target with picomolarpotency. Dual targeting yielded leads with very high barrier to the selectionof resistance and in vitro and in vivo efficacy against all 3 stages (liver,blood and sexual/mosquito) of the parasite lifecycle. The cross-species conservationof the active sites of the molecular target enzymes plasmepsins IX and X isconsistent with observed inhibitory activity against various plasmodium speciestested including P. berghei, *P.falciparum*, *P. vivax* and *P. knowlesi*. Finally, a structure guidedmedicinal chemistry program optimized potency, pharmacokinetics andpharmacodynamics properties resulting in the identification of a clinical developmentcompound MK-7602.

SG167

**Targeting Fusobacteria in the Tumor Microenvironment.**

T. Wyche[1], A. Byford[2], S. Xu[2], M. Zielstorff[2], T. Sana[1], L. Lieberman[1], J. Zhang-Hoover[2], **S. Kasper**[1]; [1]Merck Research Laboratories, Cambridge, MA, [2]Merck Research Laboratories, Boston, MA.

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States. The vast majority of CRCs are believed to be caused and/or driven by environmental factors. There is mounting evidence for the role of the microbiota in CRC initiation and progression. Recent studies have uncovered specific microorganisms that are enriched in the CRC tumor microenvironment (TME), with *Fusobacterium nucleatum* being the most consistently identified. Since *F. nucleatum* was first discovered in the TME roughly a decade ago, it has been shown to be associated with worse overall survival, increased metastatic events, and immune suppression in patients. In preclinical studies, *F. nucleatum* has been shown to damage host cell DNA, induce cancer cell proliferation, promote metastasis, and alter host immunity/inflammation. In this talk, we will describe our efforts to develop *in vitro* and *in vivo* models to characterize the role of *F. nucleatum* in tumor progression. We will highlight a high-throughput 3-dimensional tumor spheroid/bacteria co-culture model that we developed to interrogate these host-microbe interactions, and which could serve as a phenotypic screening platform for *Fusobacterium*-targeted approaches. We will share our findings from these models, including *in vitro* changes in host gene expression related to metastasis, abundance of oncogenic metabolites, cytokine production, and tumor volume (*in vivo*), and discuss these in the context of the published literature. Lastly, we will summarize the opportunities and challenges of targeting bacteria in the CRC TME as a therapeutic strategy.

SG168

**Activating Cellular Pathways to Cure Chronic Hepatitis B: From the Bench to the Clinic.**

**K. Carter**; Evotec, Princeton, NJ.

Type-I interferons (IFN-I) have shown limited ability tocure patients with chronic hepatitis B (CHB), thus more effective treatmentsare needed to achieve cure in a significant proportion of patients. We havedemonstrated that the simultaneous stimulation of CD40 and IFN-I pathways invitro and in vivo HBV infection models increased the antiviral efficacycompared to IFN-I alone. The combination of IFN-I and CD40L, on primary humanhepatocytes (PHH) and in AAV/HBV-infected mice, showed a significant increasein anti-HBV activity when compared to either CD40L or IFN-α alone. Fusion ofIFN-I molecules to an anti-CD40 agonistic mAb yielded a bifunctional moleculeactive on both CD40 and IFNR reporter cells capable of delivering bothactivities to HBV infected hepatocytes in vivo. The fusion molecule is able toreduce viral products from HBV-infected PHH treated for a period of 4 daysafter infection at picomolar concentrations without cytotoxicity. Stimulationof CXCL10 release and anti-HBV activity in infected PHH treated for 1 dayfollowed by washout period of 3 days was maintained. These results demonstratethe feasibility of combined stimulation of CD40 and IFN-I pathways with asingle bifunctional molecule to achieve potent activity against HBV.

SG169

**Identifying SARS-CoV-2 Entry Factors through Photo-Proximity Labeling.**

**M. Saeed**; Boston University Chobanian, Boston, MA.

Deciphering themolecular details of how a virus gains entry into cells can improve ourunderstanding of viral pathogenesis. SARS-CoV-2, the causative agent of theCOVID-19 disease, uses ACE2 as a receptor to enter cells. Yet the fullrepertoire of cell surface proteins that contribute to viral entry is unknown.We developed a photocatalyst-based **viral**-host protein microenvironment **map**pingplatform (ViraMap) to probe the molecular neighborhood of the SARS-CoV-2 spikeprotein on the human cell surface. Application of ViraMap to ACE2-expressingcells captured ACE2, the established co-receptor NRP1, and several novel cellsurface proteins. We systematically analyzed the relevance of these candidateproteins to SARS-CoV-2 entry by knockdown and overexpression approaches inpseudovirus and authentic infection models and identified PTGFRN and EFNB1 asbona fide viral entry factors. Our results highlight additional host targetsthat participate in SARS-CoV-2 infection and showcase ViraMap as a powerfulplatform for defining viral interactions on the cell surface.

## Subgroup: Cell Biology across Boundaries: ASCB-EMBO-MBSJ Joint International Workshop

SG170

**A new regime of ultra-low gene expression noise.**

**S. Hauf**[1], D. E. Weidemann[1], J. Holehouse[2], A. Singh[3], R. Grima[4]; [1]Virginia Tech, Blacksburg, VA, [2]Santa Fe Institute, Santa Fe, NM, [3]University of Delaware, Newark, DE, [4]University of Edinburgh, Edinburgh, UNITED KINGDOM.

Gene expression is inevitably accompanied by stochastic variation in gene products (“noise”). Gene expression noise must be minimized to allow for the reliable execution of cellular functions. However, noise cannot be suppressed beyond an intrinsic lower limit. For constitutively expressed genes, this limit

has generally been believed to be Poissonian, meaning that the variance in mRNA numbers cannot be lower than their mean. We found mRNA variances significantly below this limit for several genes in fission yeast that are important for cell division. Such low variance cannot be explained by the classical gene expression model for low-noise genes. Our transatlantic collaboration between experimentalists and modelers developed a revised model that is consistent with these data. The revised model explains this ultra-low noise by assuming multiple rate-limiting steps in transcription and RNA degradation. Interestingly, in this model, nuclear RNA export and RNA degradation influence gene expression noise differently than in the previously characterized models for Poissonian or super-Poissonian noise. Our results re-define the lower limit of eukaryotic gene expression noise and suggest gene expression characteristics that allow for ultra-low noise.

SG171

**RNA recoding in squid optimizes kinesin function in response to ocean temperature.**

**S. Reck-Peterson**, K. Rangan; University of California, San Diego, La Jolla, CA.

Intelligent cephalopods, such as octopus, squid, and cuttlefish, are unusual in that they extensively edit their messenger RNAs (mRNAs) using adenosine deaminases acting on RNA (ADARs). This RNA editing leads to recoding—changes to the amino acid sequence of proteins—for over half of the transcriptome of these animals. Here, we showed that intracellular transport-related mRNAs are some of the most highly recoded mRNAs in cephalopods. This prompted us to use the transport system as a model to ask if mRNA recoding might be adaptive for these animals. We hypothesized that ADAR activity and RNA recoding might be particularly responsive to changes in ocean temperature. To test this hypothesis, we worked with wild squid hatchlings. We placed live hatchlings in seawater of varying temperatures for 24 hours and then sequenced their kinesin-1 mRNAs. We found that kinesin-1 is more extensively recoded in animals that lived in cold seawater. Next, we performed in vitro motility assays using a chilled or heated microscope objective using recombinant proteins that mimicked recoding at different seawater temperatures. Strikingly, we found that recombinant kinesin-1 motors that mimicked recoding in the cold had enhanced motor activity in the cold compared to those that were not recoded. Our results suggest that recoding could be an adaptive strategy for these animals to thrive in a range of ocean temperatures. We also performed an extensive analysis of yeast cytoplasmic dynein-1 and human kinesin-1 to determine if sites of recoding altered the motile properties of these highly conserved motors. We found that many of the mutations we made subtly altered dynein or kinesin motor activity, but none of the mutations ablated motor activity. Our results suggest that the RNA “editome” of cephalopods could serve as a guide for identifying regulatory regions of other conserved proteins.

SG172

**Collective induction of oogenesis via long-range cytoplasmic streaming in C. elegans.**

**F. Motegi**, K. Kimura; Institute for Genetic Medicine, Hokkaido University, Sapporo, JAPAN.

The successful production of gametes, oocyte and sperm, is a critical prerequisite for organisms to generate offspring. During oogenesis, germ cells undergo enlargement through cytoplasmic exchange through intracellular bridges with neighbouring cells. The process of pro-oocyte enlargement involves dynamic reorganization of the plasma membrane and apoptosis of adjacent germ cells, although the precise physiological role of cytoplasmic exchange in pro-oocyte selection and induction remains incompletely elucidated.
In this study, we investigated the mechanisms underlying oogenesis in C. elegans and found that the

process is stimulated via long-range cytoplasmic streaming driven by collective suction of the cytoplasm in enlarging oocytes. The germ cells establish actin-dependent cytoplasmic streaming in the cytoplasmic bridges (Wolke et al., 2007) and initiate anisotropic reorganization of the plasma membrane, leading to the preferential development of pro-oocytes located on the external surface of the U-shaped gonadal tube (Hirsh et al., 1976). Even though approximately half of the germ cells undergo apoptosis (Gumienny et al., 1999), inhibiting apoptosis through ced-4(RNAi) did not impede cytoplasmic streaming, and the germ cells still underwent anisotropic morphogenesis. The shape of the gonad tube, particularly the macro-scale curvature of germ cells and their intracellular bridges, appears to play a crucial role in oogenesis, as manipulation of the gonad into a straight form severely compromised anisotropic morphogenesis of germ cells. Cytoplasmic streaming emerged as a promising candidate responsible for the enlargement of pro-oocytes on the external surface. Further investigations using local manipulation of cytoplasmic mechanics revealed that cytoplasmic streaming from distal germ cells towards pro-oocytes is mediated through collective suction of the cytoplasm by enlarging oocytes on the external surface of the U-shaped tube.
In conclusion, our findings support a model of non-cell-autonomous induction of oogenesis, where long-range streaming of the cytoplasm contributes to oogenesis by promoting anisotropic expansion of pro-oocytes adjacent to enlarging oocytes. This research sheds light on the complex interplay of cellular mechanics governing macro-scale control of oogenesis.

SG173

**Centrosomes and Cilia in development, Evolution and Disease.**

**M. Bettencourt-Dias**; Fundação Calouste Gulbenkian, Oeiras, PORTUGAL.

**Centrioles in development, disease and evolution**Centrioles are critical structures for cilia and centrosome assembly. Cilia are evolutionarily conserved structures with many sensory and motility-related functions. The centrosome is the major microtubule organizing center of animal dividing cells. Here, I will discuss efforts from our lab in understanding how centriole number, structure and function is regulated in different tissues and organisms, to accomplish such diverse functions such as proliferation, fertility, and movement.

SG174

**The form and function of syncytial cells.**

**A. Gladfelter**; Duke University, Durham, NC.

Syncytial cells are found throughout the biosphere from single-celled fungi and amoeba to the muscles and placenta of mammals. What functions emerge from this form of organization where many nuclei share a common cytoplasm? This talk will focus on a comparison of organizing principles seen in the multinucleate cells of fungal mycelium and the syncytiotrophoblast cell of the human placenta. Both cell types achieve large sizes and complex shapes with hundreds to billions of nuclei sharing a common cytoplasm. Additionally, both display nuclear autonomous behaviors such that nuclei express different genes or progress through the cell cycle asynchronously despite sharing a common cytoplasm. I will discuss how these systems can serve as powerful models for cytoplasm organization and RNA regulation. Finally, I will present a conceptual model for how nuclei in a syncytial cell can behave as independent computational units to integrate spatially and temporally variable signals. I will propose that a multinucleate state enables robust decision-making and optimization on the scale of a single cell.

SG175

**Mechanisms of spindle assembly in medaka fish embryos.**

**T. Kiyomitsu**; OKINAWA INST. OF SCI. & TECH., Onna-son, JAPAN.

Key mechanisms and concepts of spindle assembly have been established using somatic cultured cells and Xenopus egg extracts. However, mechanisms of embryonic spindle assembly remain poorly understood in vertebrate embryos. Here, we established live functional assay systems in medaka fish (*Oryzias latipes*) embryos by combining CRISPR knock-in with an auxin-inducible degron technology. In contrast to somatic spindles, we found that early embryonic spindles have unique structural features to achieve faithful chromosome segregation. I will discuss how a chromosome-derived Ran-GTP gradient and other conserved key players coordinately assemble functional spindles in medaka embryos.

## Subgroup: Recent Insights into the Structure, Function, and Dynamic Properties of Vimentin Intermediate Filaments

SG176

**Unusual Dynamics of Vimentin Intermediate Filament Visualized with SunTag Labeling.**

**B. Ranganathan**[1], W. Yeo[2], A. Serpinskaya[1], H. Zhang[2], V. I. Gelfand[1]; [1]Cell and Developmental Biology, Northwestern University, Chicago, IL, [2]Biomedical Engineering, Northwestern University, Chicago, IL.

Intermediate filaments (IFs) are integral components of the cytoskeleton, conventionally considered as static 'mechanical cores' of eukaryotic cells. Previously, we utilized the photoconversion technique to demonstrate that Vimentin Intermediate Filaments (VIFs) undergo rapid transport along microtubules, driven by the kinesin-1 motor. In this study, we adopted the SunTag labeling approach, developed by Ron Vale's group, to investigate the dynamics of VIF with higher precision. For this purpose, we engineered vimentin molecules fused with 24 copies of the GCN4 peptide epitope. The GCN4 epitope specifically binds to a single-chain antibody known as scFV-GCN4, which is coupled with super-folded green fluorescent protein (sfGFP). By utilizing the vimentin-SunTag technique, we successfully generated bright and photostable dots on individual VIF, enabling precise tracking of VIF dynamics over extended periods in time-lapse movies. Our findings revealed that VIFs exhibit high dynamics throughout the cell. The transport of VIF particles was significantly reduced when microtubules were depolymerized or in the absence of kinesin-1, indicating their dependance on microtubule-based transport mediated by kinesin-1. Remarkably, within a vimentin bundle, individual dots displayed transport in opposite directions, with varying velocities. This striking behavior suggests that individual VIFs within a bundle can move independently, representing a previously unknown characteristic of VIF dynamics. In conclusion, the Vimentin-SunTag labeling approach proved to be a valuable tool for studying the behavior of vimentin dynamics. This approach allows us to gain a deeper understanding of the biological significance of vimentin intermediate filament dynamics and their role in various cellular processes.

SG177

**Vimentin intermediate filaments are critical for 3D cell migration and nuclear translocation.**

**A. Patteson**; Syracuse University, Syracuse, NY.

Cell migration is a critical process underlying proper tissue maintenance. While a soft nucleus allows a cell to squeeze through small pores, the resulting physical stress can lead to nuclear damage and genomic variability. We have shown that the cytoskeletal intermediate filament protein vimentin

protects against DNA damage during migration. Here, we present a mechanical model in which vimentin modulates force transmission in a substrate-stiffness dependent manner, helping fine-tune the cell's response to different matrix stiffnesses. Further, we present new results on how vimentin mediates the microtubule network and mediates extracellular matrix remodeling to facilitate cell motility.

SG178

**Vimentin filament entanglement patterns ER shape and subcellular distribution.**

**A. Moore**, J. Lippincott-Schwartz; Howard Hughes Medical Institute, Janelia Research Campus, VA.

The endoplasmic reticulum (ER) is a membrane-enclosed organelle that plays central roles in the maintenance of eukaryotic cell health and survival. The ER forms an elaborate, contiguous network that extends from the nuclear envelope to the cell edge. Despite this global continuity, the ER manifests an astonishing diversity of nanoscale membrane structures, including flat sheets, curved tubules, and a nuanced continuum of shapes therebetween. These complex geometries are maintained through the coordination of ER shaping proteins, which fine-tune nanoscale membrane curvature, and cytoskeletal interactions that stabilize and position them within the cytoplasm. Here, using advanced light and electron microscopy techniques, we describe a novel, cytoskeleton-stabilized ER subdomain, which we term ER tubule clusters. These clusters represent dense 3D enclaves of confined, aggregated ER tubules nestled amid otherwise sparse peripheral ER. Tubule clusters, distinct from matrices or fenestrated ER sheets, form at the cell edge and migrate inwards, ensnaring and incorporating increasing ER tubule mass as they travel. Dense, mature clusters exhibit an increased surface area-to-volume ratio, partially excluding luminal content while acting as sinks for curvature stabilizing membrane proteins. Critically, we find that ER-tubule clusters are entangled with and scaffolded by dense vimentin filament knots. Using cryogenic structured illumination microscopy correlated with focused ion beam scanning EM, we mapped and computationally reconstructed ER tubule clusters, identifying close apposition of vimentin filament bundles with the ER surface. We further show that vimentin filaments are scaffolded on the ER surface through plectin-mediated tethering by the LINC complex protein nesprin-3. Prolonged expression of nesprin-3 enhances ER-vimentin coupling, while disruption of the interaction or depletion of vimentin by siRNA eliminates the ER tubule clusters and promotes expansion of planar ER geometry. Together, our results provide the first evidence for vimentin-ER entanglement in the maintenance of ER structure and affirm the important function of vimentin intermediate filaments in organizing subcellular membrane positioning.

SG179

**Role of vimentin intermediate filaments in Epithelial-to-Mesenchymal transition.**

**S. Sivagurunathan**[1,2], A. Vahabikashi[1,3], H. Yang[4], J. Zhang[5,6], K. Vazquez[6,7], D. Rajasundaram[8], Y. Politanska[9], H. Valencia[9], J. Notbohm[5,6,7], M. Guo[4], S. Adam[1], B. Cimini[2], R. Goldman[1]; [1]Department of Cell and Developmental Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL, [2]Imaging platform, Broad Institute, Cambridge, MA, [3]Department of Bioengineering, Northeastern University, Boston, MA, [4]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, [5]Biophysics program, University of Wisconsin-Madison, Madison, WI, [6]Department of Engineering Physics, University of Wisconsin-Madison, Madison, WI, [7]Department of Mechanical Engineering, University of Wisconsin-Madison, Madison, WI, [8]Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA, [9]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Vimentin, a Type III intermediate filament (VIF) cytoskeletal protein, regulates the mechanical and migratory behavior of cells. It is a well-established marker for the epithelial to mesenchymal transition (EMT). We previously showed that when MCF7 breast epithelial cells are induced to express vimentin by transient transfection, there are rapid changes in cell shape, motility and adhesion reflecting changes that take place in the EMT. However, the influence of vimentin expression on other EMT markers has not been extensively studied. To address this, MCF7 epithelial cell lines expressing vimentin from a cumate-inducible promoter were created. These cells assemble extensive VIF networks, display significant organizational changes in the endogenous keratin IF networks and a loss of desmosomes. These changes are accompanied by significant reductions in intercellular forces quantified by monolayer traction force and stress microscopy. In contrast, the cytoplasm of MCF7 cells induced to express vimentin is stiffer than uninduced cells as measured by micro-rheology assays. The expression of vimentin also upregulates many genes involved in cell migration as determined by RNA sequencing. Interestingly, TWIST1, a EMT related transcription factor is activated only in cells expressing VIFs and not in cells expressing the Y117L vimentin mutant which halts polymerization at the unit length filament (ULF) stage. This suggests that fully polymerized VIF structures are required to regulate the expression of genes involved in the EMT. Collectively, our results show that the vimentin levels expressed in our studies influence the EMT process by inducing a hybrid-EMT-like state through the activation of genes regulating cell migration. Supported by NIGMS.

SG180

**Low complexity domains assembled into a complex helical vimentin structure.**

**O. Medalia**[1], M. Eibauer[1], R. Goldman[2], S. Köster[3], S. Sivagurunathan[4]; [1]University of Zurich, Zurich, SWITZERLAND, [2]Northwestern University, Chcago, IL, [3]Universität Göttingen, Germany, GERMANY, [4]Northwestern University, Chicago, IL.

In mesenchymal cells, vimentin is an integral component of the cytoskeleton that play crucial rolls in the cell's structure and function. Vimentin provide cells with specific mechanical properties and contribute to the mechanical robustness of cells. Due to its complex architecture, the structure of vimentin filaments, as well as other IFs has remained elusive. To determine the structure of vimentin, we combined *in situ*, *ex-vivo* and *in vitro* structural approaches. More specifically, we applied cryo-focused ion beam milling, cryo-electron microscopy and tomography, to obtain a 3D structure of vimentin filaments. It assembles into a densely-packed and highly-ordered helical structure, composed of 40 polypeptides in cross- section, organized into 5 octameric protofibrils. Surprisingly, the intrinsically disordered head domains assemble and provide unique structure that retains the integrity of the filaments, while the tail domains bridge between adjacent protofibrils. Our work provides the first atomic model of assembled IFs that have a similar features as other cytoplasmic IFs.

SG181

**Vimentin Filament Precursor Droplets and Stress Fiber Stabilization.**

**A. Basu**, T. Krug, D. A. Weitz; Harvard John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA.

Liquid-liquid phase separation (LLPS) is increasingly being recognized as a key player in cytoskeletal organization ranging from Nephrin clusters in actin nucleation[1] to centrosomes in the case of microtubes[2]. Though relatively little is known for the intermediate filaments[3,4], it has been shown that Vimentin forms precursor puncta before maturing into Vimentin Intermediate Filaments (VIFs) and VIFs

reversibly dissolve into puncta during cell division and attachment. In this work, we employed a cell line with mutated Vimentin that prevents formation of VIFs to explore the physical properties of precursor puncta and their interaction with other cellular elements. These precursors were revealed to be LLPS condensates by their selective and reversible dissolution upon 1,6-Hexanediol treatment. They also dissolved upon application of osmotic stress while actin fibers were relatively undisturbed. In vivo shape fluctuations of these droplets were used to estimate their surface tension and their merging and splitting behavior was used to estimate their viscosity; both surface tension and viscosity values were consistent with other biological condensates. We observed extensive colocalization of the Vimentin droplets with actin stress fibers resulting in oscillatory motion of colocalized droplets compared to the random motion of the free ones. Similar colocalization was also observed at low concentration of Vimentin in cells which eventually formed complete VIFs upon increasing concentration. This co-localization is reminiscent of the physical phenomenon of liquid droplets wetting a filament; wetting of stress fibers were further confirmed by elongation of droplets along the fibers and inefficient recognition droplet coated stress fibers by F-actin specific dyes. Wetting of stress fibers with Vimentin droplets affected actin treadmilling as evidenced by the stabilization of stress fibers against Cytochalasin B and Latrunculin A treatment. The liquid-like nature of droplets and their tendency to wet stress fibers can be immensely important in understanding the growth of VIFs from droplet precursors using the stress fibers as a template.

1.Case, L. B., Zhang, X., Ditlev, J. A. & Rosen, M. K. Stoichiometry controls activity of phase-separated clusters of actin signaling proteins. *Science* **363**, 1093-1097 (2019)2.Raff, J. W. Phase Separation and the Centrosome: A Fait Accompli? *Trends Cell Biol* **29**, 612-622 (2019)3.Wu, H. *et al.* Vimentin intermediate filaments and filamentous actin form unexpected interpenetrating networks that redefine the cell cortex. *Proceedings of the National Academy of Sciences* **119**, (2022)4.Zhou, X. *et al.* Transiently structured head domains control intermediate filament assembly. *Proceedings of the National Academy of Sciences* **118**, (2021)

SG182

**Behavior of Vimentin and Keratin Filaments at High Strains.**

**S. Köster**[1], C. Lorenz[2], R. W. Style[3], S. Klumpp[1]; [1]University of Goettingen, Goettingen, GERMANY, [2]Cornell University, Ithaca, NY, [3]ETH Zurich, Zurich, SWITZERLAND.

The cytoskeleton, which determines the mechanical properties of the cell, consists of three types of protein filaments: actin filaments, microtubules, and intermediate filaments. Among them, intermediate filaments are the most flexible and most stretchable, exhibiting a fascinating nonlinear force-strain behavior. Interestingly, intermediate filament proteins are expressed in a cell-type dependent manner and may play a role in defining the mechanics of different cell types. A particularly interesting pair of intermediate filaments is vimentin and keratin as the “switching” between them is a hallmark of the epithelial-to-mesenchymal transition (EMT). Using optical tweezers, we characterized the response of vimentin and keratin filaments to strain and relaxation and were able to theoretically model this response by considering the strictly hierarchical structure of the filaments. This unique architecture consists of multiple alpha-helical domains arranged in parallel, and nonequilibrium transitions between folded and unfolded states. Combining the experiments and the model, we conclude that intermediate filaments serve as "safety belts" and "shock absorbers" for the cell, preventing damage during high-speed impacts while remaining flexible, for example, during cell motility. We also find that keratin and vimentin filaments behave distinctly differently according to their roles in stationary and motile cells,

respectively: during repeated stretching, vimentin filaments soften but maintain their length, while keratin filaments lengthen but maintain their stiffness.

SG183
**An interpenetrating network model to understand the cytoskeleton.**
**M. Guo**, H. Yang; MIT, cambridge, MA.

Under many physiological and pathological conditions such as division and migration, cells undergo dramatic deformations, under which their mechanical integrity is supported by cytoskeletal networks (i.e. intermediate filaments, F-actin, and microtubules). Recent observations of cytoplasmic microstructure indicate interpenetration among different cytoskeletal networks. In this talk, I will present our recent works performing micromechanical experiments in living cells revealing the important mechanical benefit of this interpenetrating nature. In addition, I will present a theoretical framework describing the complex mechanical response of living cells under such an interpenetrating network framework, including viscoelastic, nonlinear stiffening, microdamage, and healing behaviors. These works elucidate the coupling among interpenetrating cytoskeletal components, and the roles of this coupling in determining the mechanical response of eukaryotic cytoplasm. Through these works, we suggest that eukaryotic cell cytoplasm should be considered as an interpenetrating network.

## Bridging the Gap From Basic to Translational in Neurodegeneration Research

SG184
**Therapeutic Targeting of Nemo Like Kinase in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia.**
**S. Barmada**; University of Michigan, Ann Arbor, MI.

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are historically distinct neurodegenerative conditions that share a common pathological signature, consisting of mislocalization and aggregation of the RNA binding protein TDP43. Previous unbiased screens conducted in multiple fly and animal models identified nemo-like kinase (NLK) as a prominent genetic modifier of ALS/FTD phenotypes. However, the precise mechanism by which NLK mediates neurotoxicity is unknown. Here, we show that not only is NLK upregulated in affected neurons from ALS/FTD patients, but NLK overexpression also enhances TDP43 mislocalization in a kinase-dependent fashion. Subsequent studies demonstrated that NLK accumulation disrupts nucleocytoplasmic transport of TDP43 and other RNA binding proteins by disrupting Ran homeostasis via negative regulation of RanBP2/RanGAP1. These results suggest that NLK accumulation may precede and promote TDP43 mislocalization, thereby accelerating neurodegeneration in ALS/FTD. Consistent with this, knockdown of the NLK gene extends the survival of human neurons carrying ALS/FTD associated mutations. Together, these data highlight the central relevance of NLK to disease pathogenesis in ALS/FTD, and emphasize the potential of NLK-lowering therapies to slow or prevent neuron loss in these disorders.

SG185
**Developing Novel Therapeutics to Improve Autophagy and Lysosomal Function in Parkinson's Disease Patients.**
**T. Schwabe**; Nine Square Therapeutics, South San Francisco, CA

Autophagy and the proper functioning of lysosomes play a vital role in maintaining cellular health and preventing the harmful accumulation of protein aggregates, lipids or damaged organelles. Impairment in these pathways is associated with aging and can cause or increase the risk for neurodegenerative diseases. For example, Parkinson's disease, where genes responsible for lysosomal function, including GBA, LRRK2, VPS35, ATP13A2, and, most notably, Pink1 and Parkin, which serve as critical regulators of mitophagy, have been identified as contributors. Nevertheless, the development of drugs aimed at enhancing autophagy and optimizing lysosomal function in brain cells remains an area with limited success to date. Nine Square Therapeutics is developing small molecules to regulate the autophagy pathway by harnessing innovative chemical approaches. We are creating activators for the key lysosomal calcium channel Trpml1 to enhance initiation of autophagy, amplify downstream lysosomal activity and facilitate lysosomal fusion. Our aim is to remove aggregated proteins and lipids in dopaminergic neurons. In addition, we have developed a new class of Parkin activating compounds that are designed to increase the degradation of damaged mitochondria through mitophagy to improve cellular respiration. To validate the efficacy of our compounds, we employ cutting-edge automated image analysis and phenotypic clustering in cellular disease models, a strategy designed to increase our probability of success in future clinical trials.

SG186

**Dissecting and Disabling Lysosomal Mtorc1 Signaling in Neurodegeneration.**

**R. Zoncu**; University of California, Berkeley.

The lysosome plays a central role in cellular homeostasis through its participation in autophagy and its physical and functional association with the master growth regulator, mechanistic target of rapamycin complex 1 (mTORC1) kinase. mTORC1 integrates signals from nutrients, hormones and energy to control the balance between cellular growth and repair programs. Nutrients, including cholesterol, drive the localization of the master growth regulator, mechanistic Target of Rapamycin Complex 1 (mTORC1) to the lysosomal membrane, where mTORC1 regulates its downstream programs. Cholesterol sensing by mTORC1 occurs upstream of the heterodimeric Rag guanosine triphosphatases (GTPases) and is negatively regulated by the cholesterol exporter, Niemann-Pick C1 (NPC1) protein. In NPC1-deleted cells, mTORC1 becomes hyperactivated, leading to disruption of autophagy and mitochondrial function. These data implicate aberrant cholesterol-mTORC1 signaling as a candidate pathogenic driver in the neurodegenerative disease, Niemann-Pick type C (NPC), but the molecular mechanisms of mTORC1-dependent cellular dysfunction in NPC remain to be determined. I will present our recent effort to unravel lysosomal cholesterol sensing through a combination of organelle proteomics, bioinformatic analysis and functional assays. Moreover, I will discuss ongoing work leveraging functional genomics to identify new pathways for mTORC1 regulation and cellular quality control that may have implications for neuronal cell homeostasis and NPC pathogenesis.

SG187

**No Abstract Submitted**

**A. Abeliovich**; Leal Therapeutics, New York City, NY.

## Hacking Human Cellular Systems at Scale – Organogenesis & Cell Therapies

SG189

**Induced Pluripotent Stem Cell-derived Tissues For Cartilage Repair - Hacking Human Cellular Systems At Scale Organogenesis & Cell Therapies.**

**A. Craft**[1,2]; [1]Boston Children's Hospital, Boston, MA, [2]Harvard Medical School, Boston, MA.

Joint injuries and the associated progressive deterioration of the joint-lining articular cartilage and other connective tissues (commonly known as osteoarthritis) are highly prevalent across all demographics, affecting our ability to move our bodies and participate in activities of daily living without pain. Current therapies are inadequate. Here we will describe how we generate clinically relevant articular cartilage from iPSCs, in our efforts to establish a novel, low-risk, cost-effective, single-step treatment for sustained cartilage repair. Replacement of damaged tissue with functional cartilage will restore joint function long-term, providing significant benefits for patients, their families, the clinicians and surgeons who treat them, and the overall healthcare industry.

SG190

**From Stem Cells to Assembloids and Towards Building Circuits in Living Systems.**

**S. Pasca**; Stanford University, Stanford, CA.

A critical challenge in understanding the programs underlying development, assembly and disease of the human brain is the lack of direct access to intact, functioning human neural tissue for detailed investigation and manipulation. In my talk, I will first describe how my laboratory has been leveraging instructive signals to develop, starting from human pluripotent stem cells, self-organizing neural organoids resembling specific domains of the nervous system. I will next introduce a novel self-organizing cellular preparation named an assembloid that we created to model cell migration and circuit in the developing human nervous system. Lastly, I will illustrate how organoids and assembloids can be used to study development, evolution and to model genetic neuropsychiatric disease.

SG191

**Stem Cells in the Adult Human Retinal Pigment Epithelium and Their Therapeutic Potential.**

**S. Temple**; Neural Stem Cell Institute, Rensselaer, NY.

One in five people over age 75 in the US suffer from a blinding disorder, dry age-related macular degeneration (AMD). This is one of the most prevalent neurodegenerative diseases. In AMD, the retinal pigment epithelium (RPE), a pigmented layer of cells specialized to support the neural retina, degenerates. This in turn causes loss of central, high acuity vision. Patients with AMD lose the ability to read and recognize faces, and they lose independence, which significantly impairs their quality of life. Human stem cell technology has opened new therapeutic strategies to treat AMD by adding new stem cell-derived RPE cells. Several RPE cell-replacement strategies are being pursued, including using embryonic or induced pluripotent stem cells and, in our work, adult RPE stem cells (RPESCs). We showed that when adult human RPE cells are plated in culture they can be activated into a stem cell state capable of extensive cell division. These adult RPESCs are activated to proliferate with mitogens and can expand in culture to produce over a billion cells per single donor. Extensive preclinical tests in animals demonstrated promising safety and efficacy results following subretinal transplantation of adult RPESCs. Importantly, we discovered that a progenitor stage cell was most effective at preventing vision loss in a

rat model of retinal degeneration. These findings supported an investigational new drug application to the FDA for a cell replacement therapy for patients suffering from AMD. In 2022 we launched a Phase I/IIa clinical trial. Translating basic stem cell research towards the clinic presents challenges, including how to manufacture and define these complex cell products more efficiently and reliably. Deep characterization of the adult RPESC product has revealed significant differences from pluripotent stem cell-derived RPE cells, which also show promise in clinical trial. This comparison between RPE cell products will help us define cell characteristics that correlate with best patient outcomes.

SG192

**Precise Genetic Engineering Using the TFome™ Platform for Cellular Programming Technologies to Advance Therapeutic Application.**

**P. Khoshakhlagh**, A. Ng; GC Therapeutics, Cambridge, MA

Cellular reprogramming technologies hold incredible promise for regenerative medicine, yet challenges remain throughout the life-cycle of therapeutic product development. The TFome™ platform technology for cell fate programming aims to revolutionize the field by addressing critical issues from the discovery paradigm for identifying key programming factors to clinical translation and manufacturing. As process defines product for cell therapies, we embraced a process-centric discovery approach to identify key forward programming factors for converting pluripotent stem cells into desired cell states to enable highly efficient (up to 99% in the final population), streamlined (single-step, accelerated speed) and “plug-and-play” (single media, standardized unit operations) cell type conversion. By expanding and searching the TFome™ library representing over 1,700 transcription factor genes at the resolution of individual isoforms, at the single-cell level with omics-wide functionality readouts, we identified key cell fate regulators that by hacking cell differentiation machinery result into precise epigenetic programming in a cell-autonomous manner without the requirement for specialized cell-external factors. This enables the swapping of programming factor cocktails for different cell types in a common culturing framework without optimization of culturing conditions, thereby realizing a “plug-and-play” differentiation platform for scalable, high-quality cell manufacturing for programming any cell type. Retrospective analysis of potent forward programming factors suggest that global TFome™ exploration is essential, as computational and observation-based predictions missed key transcription factors. The TFome™ discovered key programming factors that shortcut cell culturing timeline and enabled expansion at the pluripotent stage, which resulted in no detectable karyotypic changes from cell engineering to forward programming into desired cell types. Altogether, this significantly improves the safety profile of the final product by enhancing genomic stability and decreasing pluripotent impurities which is a result of synthetic and precise epigenetic engineering. We envision the TFome™ as a true cell programming platform where strategic upfront investment in cell manufacturing for one cell type will generalize to subsequent programs to unlock the full potential of cellular programming technologies for therapeutic use.

## Subgroup: Beyond the Surface: Unraveling the Critical Role of Basement Membrane Specialization in Health and Disease

SG193

**Dissecting the role of basement membrane and extracellular cues in driving MET.**

S. Kan, **K. Campbell**; University of Sheffield, Sheffield, UNITED KINGDOM.

Epithelial cells show remarkable phenotypic plasticity, transitioning between epithelial states to mesenchymal states in a reversible manner. While the epithelial-to-mesenchymal transition (EMT) has been well characterised for its role in driving a more migratory state, the re-epithelization step, mesenchymal-to-epithelial transition (MET) is far less understood. We investigate this during the morphogenesis of the embryonic midgut epithelium in *Drosophila melanogaster (Drosophila)*, which relies on cells undergoing an MET after completing migration. We have shown that contact between midgut cells and the surrounding visceral mesoderm is absolutely required for MET to occur, and we have identified a critical role for specific basement membrane components in mediating these interactions. Secretion of the lamininW trimer, containing the laminin α1,2 chain, encoded by *wing blister (wb)*, by the mesoderm, is critical for the polarised secretion of the lamininA trimer, containing laminin α3,5 chain *(lanA)* by the endoderm. While *wb* appears sufficient for the polarisation of endoderm cells, *lanA* is also required for their efficient migration. Endoderm cells not only secrete LanA, but also express specific integrins, that bind to LanA, rather than wb. More recently, we have focused on understanding how adhesion to the underlying laminin network and the visceral mesoderm is transduced into the formation of a precisely shaped, apicobasal polarised epithelial cell. We found that while both the somatic and visceral mesoderm are capable of secreting wb and driving basal polarisation of integrins, this is not sufficient for the normal cell shape changes and polarisation of the apical plasma membrane that occurs during MET. Using a combination of scRNA-sequencing, genetics and high-resolution imaging approaches, we have identified and characterised a number of new players potentially mediating interactions between the visceral muscle and midgut cells in parallel to the integrin-laminin axis. Further investigation of these genes, will help reveal the mechanisms of contact-driven MET mediated by the visceral muscle.

SG194

**Piezo initiates transient production of collagen IV to repair damaged basement membranes.**

A. M. Stricker, **A. Page-McCaw**; Cell and Developmental Biology, Vanderbilt Medical School, Nashville, TN.

Basement membranes are sheets of extracellular matrix separating tissue layers and providing mechanical support. Their mechanical properties are determined largely by their most abundant protein, Collagen IV (Col4). Although basement membranes are repaired after damage, little is known about how. For example, since basement membrane is extracellular it is unknown how damage is detected, and since Col4 is long-lived it is unknown how its expression is regulated to avoid fibrosis. Using the basement membrane of the adult *Drosophila* midgut as a model, we show that repair is distinct from maintenance. In healthy conditions, midgut Col4 originates from the fat body, but after damage, a subpopulation of enteroblasts we term “matrix menders” transiently express Col4, and Col4 from these cells is required for repair. Activation of the mechanosensitive channel Piezo is required for matrix menders to upregulate Col4, and the signal to initiate repair is a reduction in basement

membrane stiffness. Our data suggests that mechanical sensitivity may be a general property of Col4-producing cells.

SG195

**An interplay of proteostasis, ECM-cell junctions and biomechanics ensures *C. elegans* circuit architecture.**

G. Rapti, F. Coraggio, **F. Caroti**, M. Bhushan, S. Roumeliotis, C. Bevilacqua, R. Prevedel; EMBL, Heidelberg, GERMANY.

The function of tissue relies on faithful assembly and resilient maintenance of their architecture across time and environmental change. In the nervous system, neurons and glia interact to sculpt circuits, and glial cell architecture defines functional connectivity. Yet, the mechanisms ensuring crosstalk of neural cells with non-neural cells and especially with extracellular matrix (ECM) and their environment, remain understudied. Mechanisms sculpting glial cell architecture remain particularly elusive. *C. elegans* is a powerful model for such studies: it offers defined tissue anatomy, connectivity, genetic tractability, live imaging, and glia largely dispensable for neuronal viability, allowing to study their biology *in vivo*. We dissect mechanisms ensuring integrity of astroglial cell shape and circuit architecture. We identify an interplay between proteostasis, ECM and mechanical properties of tissues and the environment, to sculpt glial cell and circuit architecture. We combine large-scale genetics with manipulation of genes, cells, and the environment, quantitative imaging of cellular, subcellular features, tissue material properties and ECM. In genetic screens, we isolated mutants with age-progressive, environment-dependent defects in glial cell architecture. Defective glial architecture also triggers shape alterations of neuron and synapses, revealing that glia ensure maintenance of circuit architecture across scales. A causal HSP70-chaperone malfunction in epithelia, underlies these defects and it integrates temperature, nutrition and mechanical cues to shape the density and distribution of basement membrane components. Abnormal ECM distribution in the mutants results in hypersensitivity to temperature and mechanical strain, causing changes in the ECM-cell attachments and subsequent loss of glial cell integrity. To dissect this interplay between biochemical, molecular and material properties, we performed biophysical measurements of non-invasive, elastography. Our analysis reveals that mutant animals suffer altered material properties. Further manipulations of genes and chemical drugs to modify either the ECM composition, the tissues properties or the animal's environment can recover the integrity of tissue architecture. Thus, a dynamic interplay of proteostasis, active ECM and tissue mechanics, ensures robust cell architecture and circuit integrity.

SG196

**Dysregulated Extracellular Matrix and Increased Stiffness in *LIS1*-Mutant Brain Organoids: Unraveling the Role of ECM in Lissencephaly Pathophysiology.**

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*LIS1* mutations are known to cause lissencephaly, a neurological disorder characterized by the absence of cerebral cortical convolutions. Here we show that brain organoids with *LIS1* mutations exhibit increased expression of extracellular matrix (ECM) proteins, which are less organized. The stiffness and steady-state stiffness of the mutant organoids increased. These changes are associated with dysregulated mRNA and microRNA. Short-term treatment of the mutant organoids, but not the control

ones, with the catalytic subunit of MMP9, which proteolytically cleaves ECM, reduced stiffness. The changes were associated with changes in gene expression. We generated a model incorporating the differences in the mutant's and control brain organoid composition and organization that matches and explains our findings. Our study provides insights into the importance of the composition and organization of the ECM during human brain development and the role of LIS1 in brain structure.

SG197

**A Dystroglycan-Laminin-Integrin axis controls cell basal geometry remodeling in the developing*Drosophila*retina.**

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Cell shape remodelling is a principal driver of epithelial tissue morphogenesis. While progress continues to be made in our understanding of the pathways that control the apical geometry of epithelial cells, we know comparatively little about those that control cell basal geometry. To examine this issue, we used the *Drosophila* ommatidium, which is the basic visual unit of the compound eye. The ommatidium is shaped as a hexagonal prism, and generating this three-dimensional structure requires ommatidial cells to adopt specific apical and basal polygonal geometries. Using this model system, we find that generating cell type-specific basal geometries starts with patterning of the basement membrane, whereby Laminin accumulates at discrete locations across the basal surface of the retina. We show that the Dystroglycan surface receptor is required for this localised Laminin accumulation. Moreover, our results reveal that localised accumulation of Laminin-Dystroglycan is required for polarization of Integrin adhesion in ommatidial cells. This underpins cell basal geometry remodelling by anchoring the basal surface of cells to the basement membrane at specific, discrete locations. We propose that patterning of a basement membrane by generating discrete Laminin domains can direct Integrin adhesion to induce cell polygonal geometry remodelling in epithelial morphogenesis.

SG198

**Modelling *COL4A1*-associated cerebral small vessel disease in zebrafish.**

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Cerebral small vessel disease (cSVD) is a major cause of vascular dementia and stroke. Human genetic studies have associated a network of extracellular matrix genes with the development of cSVD— the most significant being variants in COL*4A1,* which is required for the ubiquitous collagen IV-alpha 1,1,2 network in basement membranes. *COL4A1*-associated cSVD causes cerebral blood vessel tortuosity, spontaneous intracerebral haemorrhage (ICH), and white matter defects. There is no effective therapy and therefore a major unmet clinical need.

Zebrafish larvae are an effective model organism for studying brain injury due to their optical clarity and suitability for live imaging and since the molecular and morphological development of their blood brain barrier is comparable to humans, they are important for elucidating disease mechanisms. Despite high conservation of the *COL4A1* gene between species, its function has not yet been investigated in zebrafish in the context of cSVD. We therefore aimed to establish the potential of *col4a1* zebrafish crispants for modelling cSVD.

Microinjection of Cas9 and four targeting gRNAs in single cell embyros resulted in *col4a1* crispant zebrafish and we performed the knockdown in the transgenic *kdrl*-EGFP fluorescent reporter line to

facilitate vascular imaging. We used live-imaging with light-sheet microscopy to characterise neurovascular phenotypes in the *col4a1* crispant larvae compared to uninjected controls or larvae injected with Cas9 and a non-complementary gRNA. By three days post fertilisation *col4a1* crispants exhibited spontaneous ICH and neurovascular abnormalities that were not seen in control larvae. These phenotypes were rescued by microinjection of wild type human *COL4A1* mRNA. We observe dysregulation in *mmp9* expression in crispants and hypothesise this to play an important role in disease pathogenesis.
In summary, we have established a novel non-protected *in vivo* preclinical system for studying the loss of col4a1 function. We propose that zebrafish represent a powerful platform for studying the function of other extracellular matrix genes associated with cSVD and for a high-throughput therapy screening system.

SG199

**Fibulin-1 supports basement membrane stretching during ovulation in *C. elegans*.**

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All tissues are enwrapped and structurally supported by a thin, dense, and specialized extracellular matrix (ECM) known as the basement membrane (BM). A critical but understudied BM property is its ability to stretch to support mechanically active tissues such as muscles and vasculature. To elucidate the mechanism underlying BM plasticity, we are developing *C. elegans* ovulation as an *in vivo* experimental system to investigate how BMs dynamically expand and retract. During ovulation, the *C. elegans* spermathecal BM stretches ~2-fold in area to accommodate an oocyte for fertilization. Once fertilized, the oocyte moves into the uterus and the spermathecal BM rapidly shrinks. To map the spermathecal BM composition, a localization screen was performed using 58 conserved BM-associated proteins that have been endogenously tagged with mNeonGreen, including all core components, BM associated receptors and most known matricellular proteins, enzymes, and proteases. We discovered that the spermathecal BM consists of 13 BM proteins, which have all been associated with BM and tissue integrity in muscles and vasculature in worms and vertebrates. How these BM components stabilize BMs and yet promote flexibility to support dynamic tissue behavior remains unclear. To understand how BMs establish an optimal balance between BM stability and plasticity, we focused on the sole *C. elegans* fibulin, fibulin-1, which is enriched at the spermathecal BM. Notably, vertebrate fibulin-1 is required for BM and vasculature architecture, however, the underlying mechanism is unclear. Fibulin-1 depletion in adult animals led to disorganized core BM component type IV collagen, BM rupture and deformed spermatheca organs, suggesting that fibulin-1 might maintain the collagen network to promote BM and tissue integrity. Consistent with a specific role in BM stretching, defects in collagen organization, BM rupture and tissue shape increased with repeated ovulations. Strikingly, we also discovered that fibulin-1 is elevated 10-fold in the oocyte specifically during ovulation, suggesting that fibulin-1 is delivered to the spermathecal BM to reinforce the BM architecture during stretching. Together, our findings establish a new model to elucidate mechanisms underlying BM stretching and reveal a key role for fibulin-1 in maintaining BM integrity during tissue stretching.

## Subgroup: The Mechanoimmunology of Movement and Manipulation

SG200

**Mechanobiology of epithelial phagocytosis in the early vertebrate embryo.**

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Dynamic processes at the single cell level establish the structural and functional architecture of the organism during embryo development. This event is error-prone and can lead to aberrant and apoptotic cells in the earliest blastula and gastrula stages of development. We have recently shown that the embryonic surface epithelium in zebrafish and mouse, termed enveloping epithelial layer (EVL) and trophoblast, functions as an efficient phagocytic scavenger system in the early embryo (Hoijman et al. Nature 2021). Epithelial cells recognize and specifically clear dying cells that occur due to stochastic errors during embryogenesis. Here we will present molecular and biophysical mechanisms of epithelial clearance using 4D in vivo imaging and functional perturbation assays of phagocyte-target interactions and uptake dynamics. We found that epithelial cells flexibly utilize their actin machinery to build different types of actin-based protrusions while maintaining tissue and junctional integrity. Protrusion plasticity enables epithelial cells to cooperate mechanically based on cargo-load sharing for efficient tissue clearance. Our work further identified a novel role of the cell-cell interaction protein E-cadherin in regulating epithelial clearance. We found that E-cadherin establishes a coupling between myosin II-dependent cortical mechanics and cytoskeletal remodelling relevant for phagocytic uptake. Our work highlights that epithelia have a high morphodynamic cell and tissue plasticity that allows for functional multitasking and guarantees tissue homeostasis and robust embryo development.

SG201

**Mechanisms of Macrophage Attack in a Solid Tumor.**

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As one of the most common cellular components of solid tumors, macrophages are an attractive candidate for cancer immunotherapy. Successful therapies like the anti-Her2 monoclonal antibody are thought to induce cancer cell phagocytosis by activating the Fc Receptor in macrophages. Chimeric antigen receptors (CARs) combine an extracellular antibody fragment against a cancer cell marker and an intracellular Fc receptor domain to stimulate phagocytosis in macrophages. In *vivo* mouse experiments have demonstrated that CAR macrophage treatment reduces tumor burden and prolongs animal survival, motivating clinical trials of CAR macrophages. *Ex vivo* monolayer assays suggest that CAR macrophages phagocytose whole cancer cells. However, solid tumors have a complex microenvironment of cell-to-cell contacts and cell-to-extracellular matrix interactions that are absent in these studies. How macrophages overcome the high opposing strength of cell-to-cell contacts to pull an individual cell out of a tumor is not clear. To study how macrophages attack a solid tumor, I established an *ex vivo* tumoroid model. I genetically re-programmed bone marrow derived macrophages with a CAR to target Her2+ breast cancer cells. Tracking tumoroid size over two weeks, I found that CAR macrophages are sufficient to shrink or eliminate tumoroids. This represents a powerful new system for confocal imaging of macrophages attacking a 3D tumor model.
Using a combination of 2D and 3D assays, I have found that the majority of macrophages in a solid

tumor model nibble (e.g. trogocytose) rather than engulf whole cancer cells. of the cells that are phagocytosed, 38% are mitotic cancer cells (though only 5% of the overall population underwent division). This data led us to hypothesize that macrophages target biophysically vulnerable cells in solid tumors where cell-cell adhesions limit whole cell phagocytosis. We are currently testing individual biophysical parameters of the mitotic cancer cells that may make them susceptible to macrophage attack like changes in cell shape, size, or adhesion. While these experiments utilize CAR macrophages as a model, we suspect the characterization of antitumor activity will be applicable to antibody-based macrophage therapies too. This progress to better understand macrophage attack will suggest strategies to improve CAR efficiency or to determine how effective CAR macrophages will be in different tumor environments.

SG202

**Impact of tumor associated macrophages on extracellular matrix biophysical properties and associated immune cell infiltration.**

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The extracellular matrix constitutes the structural scaffold for organs and tissues and provides key physical and biochemical cues to cells. While the profound remodeling of the ECM during tumorigenesis has been long reported, its effect on anti-tumoral immune responses is still poorly understood. Using an interdisciplinary approach at the frontier of immunology, cell biology and biophysics, we investigated (1) how macrophages, key immune drivers of tumorigenesis, modulate the biophysical properties of the tumoral ECM and, (2) how those ECM changes impact on the anti-tumoral immunity. We here show that macrophage depletion at late stages of tumorigenesis induced a drastic remodeling of the tumor ECM architecture. We found that the collagen capsula was thickened, disorganized and softer, as assessed by atomic force microscopy. In addition, fiber-rich areas were more frequently observed within the tumor core upon macrophage depletion. Both ECM modifications correlated with a massive T lymphocyte infiltration and were associated to decreased tumor growth. Analysis of the mechanisms underlying these ECM changes by combination of single-cell RNA sequencing and MIIC (Multivariate Information-based Inductive Causation algorithms) pointed to an essential role of the collagen 3/collagen 1 ratio and upstream transcription factor AEBP1. Our study brings fundamental knowledge on how remodeling of the tumor ECM defines its recognition and elimination by the immune system, contributing to the emergence of the nascent field of immunophysics.

SG203

**Focal adhesions are essential for T cell migration in confinement.**

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Immune cells need to migrate rapidly and efficiently to sites of inflammation site. As they do so they are exposed to different complex environments composed of various extracellular matrix (ECM) components, architectures, and density. T cells have been, up to now, categorized as using integrin-independent amoeboid migration modes. Previous in vivo studies, however, demonstrated that blocking

integrins resulted in impaired T cell migration. The suggested necessity of integrin-dependent adhesion during T cell migration challenges the mesenchymal vs amoeboid model and suggests a continuum rather than binary categories. We hypothesize that CD4+ T cells uses integrin-mediated adhesion to navigate complex tissues.
Using primary T cells, we find that their migration depends on ECM and geometry, and specifically that confinement induces rapid and robust migration, and that ECM is required to move in confined spaces. When in the presence of ECM proteins, we find that T cells make focal adhesions similar to mesenchymal cells although at a smaller scale. These focal adhesions include integrin clusters and adaptor proteins such as vinculin and talin. These adhesion sites also coincide with regions of traction stress as measured using Traction Force Microscopy. We find that contractile traction stresses are exerted on both upper and lower surfaces, and that the cell pushes the ECM out of its way as it migrates through tight environments. Finally, we find that as T cells migrate through confined spaces, they tend to preferentially follow the path left by other cells and will move faster along previously established paths.
Together these data suggest that CD4+ T cells require integrin-mediated adhesion, which provides new insights into the regulation of immune cell migration and offers potential novel therapeutic pathways. This work also demonstrates that the distinction between mesenchymal and amoeboid migration needs to be revisited.

SG204

**Tropomyosin and integrin engagement cooperate to promote actomyosin forces required for robust target cell killing.**

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Efficient target cell killing by CD8+ T cells is thought to require integrin-dependent adhesion to the target cell and the exertion of force by the T cell on the target cell membrane (Basu et al, Cell 2016; Wang et al, Nat. Comm 2022). T cells create a contractile actomyosin network in the medial, pSMAC portion of their immune synapse (IS) through the combined action of the formin mDia1 and the motor protein myosin 2A (Murugesan et al., JCB 2016). TIRF-SIM imaging shows that this network is composed of concentric actin arcs that are decorated with bipolar filaments of myosin 2A, and that co-localize with the adhesive ring driving T cell: target cell interaction through binding of the T cell integrin LFA-1 with ICAM-1 on the target cell surface. Tropomyosins are actin-binding proteins that associate with linear, formin-generated filaments, where they serve to prevent cofilin-dependent filament severing and to promote actomyosin force generation by recruiting and activating type 2 myosins (Hardeman et al, Sem Cell Dev Biol 2020). Here we show that mouse CD8+ T cells express tropomyosins 3.1 and 3.2 (Tpm3.1/3.2), that Tpm3.1/3.2 colocalize with the pSMAC actomyosin arcs, and that abrogating Tpm3.1/3.2 function using a either small molecule inhibitor (ATM3507) or CRISPR-mediated gene deletion attenuates the recruitment of myosin 2A to the IS and disrupts the concentric organization of the pSMAC actin arcs. Conversely, ligating LFA-1 with ICAM-1 dramatically increases the amount of myosin 2A recruited to the synapse and the concentric organization of the pSMAC actin arcs. Together, these results argue that Tpm3.1/3.2 and LFA-1 ligation cooperate to promote actomyosin-dependent force generation at the T cell synapse. Consistent with the idea that the exertion of force by the T cell on the target cell membrane promotes target cell killing, tropomyosin inhibition reduces the ability of effector CD8+ T cells to kill antigen-pulsed target cells *in vitro*, despite having no obvious effect on pTyr signaling. We conclude, therefore, that the pSMAC actomyosin arcs are responsible for the forces

exerted on the target cell that promote target cell lysis. Our results also add to the growing appreciation for the role that integrin ligation plays in promoting the function of both T cells (Lotscher et al., Cell 2022) and B cells (Wang et al., ELife 2022).

SG205

**Phosphatidylserine-mediated phagocytosis involves filopodial wedging and coreceptors TREM2, CD14 and integrin $\alpha_M\beta_2$.**

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Macrophages eliminate a wide array of threats in tissues, ranging from antibody (Ab)-coated bacteria to phosphatidylserine (PS)-exposing apoptotic cells. They do so through phagocytosis, which has emerged as a therapeutic target for a range of diseases. Our ability to precisely modulate this process is limited by our incomplete understanding of how macrophages recognize, engulf, and respond to distinct prey. Here, we present a thorough investigation of the differences in dynamics and regulation between two phagocytic pathways: an immunostimulatory form of phagocytosis triggered by Ab-opsonized targets and an immunosuppressive form triggered by PS-exposing targets. We employed deformable microparticles (dMPs), designed to have similar size and rigidity as apoptotic cells, to study phagocytic dynamics in J774 macrophages. PS-mediated engulfment is characterized by thin F-actin rich protrusions forming around the target in early cup formation. On soft particles, and when faced with apoptotic cells, these thin protrusions extend micrometers into the target, acting as wedges that disrupt target integrity. This presents a novel mechanism of piecewise engulfment (trogocytosis) that operates inside-out. Late PS- cups appear sunken into the cytoplasm before cup closure. Together, these features make for dynamics that dramatically differ from the outgrowing, constricting, F-actin rich pseudopods formed during Ab-mediated phagocytosis. To understand the molecular basis of these differences, we used dMPs in a comparative genome-wide screen for regulators of PS-and Ab-mediated phagocytosis. This approach both identified a core of shared regulators common to these uptake pathways, as well as many target-specific regulators of phagocytosis. Surprisingly, although many PS-receptors have previously been implicated in efferocytosis, our screens identified CD14, integrin $\alpha_M\beta_2$ and neurodegenerative disease-associated TREM2 as critical co-receptors in PS-mediated phagocytosis. High-resolution immunofluorescence confirms the accumulation of these receptors in PS-cups, each with a unique distribution and with remarkable clustering of TREM2 at the rim of sunken cups. The importance of CD14, a GPI-anchored protein, is illustrated by 24 hits in the GPI-anchoring pathway specifically being required for PS-phagocytosis. These are likely all attributed to CD14, since enzymatic removal of other GPI-anchored proteins does not affect phagocytic efficiency. Together, these findings reveal novel dynamics and molecular players involved in PS-mediated efferocytosis.

SG206

**Plasma membrane abundance controls myeloid cell phagocytosis and migration.**

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Professional phagocytes, such as neutrophils and macrophages, mediate the clearance of pathogens, cellular debris, and apoptotic corpses. To function properly, phagocytes must decide what to eat, how

much to eat, and when to move following eating. Here, we show that plasma membrane abundance is a key arbiter of these physiological decisions. Neutrophils and macrophages lacking the heterotrimeric G-protein subunit $G\beta_4$ exhibited dramatic expansion of their plasma membranes, accompanied by a marked reduction in plasma membrane tension. This biophysical phenotype was associated with profound increases in both the rate and amplitude of phagocytosis. In $G\beta_4$ knock-out mice, enhanced phagocytosis by myeloid cells conferred resistance to lung infection by the fungal pathogen *Aspergillus fumigatus*. $G\beta_4$ deficient neutrophils also lost the ability to arrest following cargo uptake, indicative of dysregulated crosstalk between phagocytosis and motility. Lipidomic analysis linked the loss of $G\beta_4$ to increased levels of sphingolipids, and pharmacological inhibition of sphingolipid synthesis reversed both the plasma membrane expansion and the hyperphagy characteristic of the $G\beta_4$ knock-out phenotype. Taken together, these results reveal a previously unappreciated role for G-protein signaling in constraining myeloid phagocytosis, and they also identify plasma membrane abundance as a biophysical master regulator of immune effector function.

## Subgroup: #Meiosis4Eva: Frontiers in Meiosis and Germ Cell Biology

SG207

**Diffusion within the synaptonemal complex directs the localization of crossover patterning proteins.**

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Sexually reproducing organisms use meiosis to recombine their genomes and partition chromosomes into haploid gametes. A key step in this process is the creation of a tightly-regulated number of physical interchanges, or crossovers, between parental chromosomes. Notably, meiosis in most organisms exhibits what is known as “crossover interference,” where crossovers are spaced apart farther than would be expected by chance. One factor implicated in crossover regulation is the synaptonemal complex (SC), which constitutes a liquid-like interface between chromosomes. While SC subunits and SC-localized signaling proteins have been shown to redistribute during meiosis, the nature of these dynamics and their relevance to proposed mechanisms of crossover interference remain an area of active research.

For a new perspective on this question, we developed a method to track single fluorescently-labeled proteins in live meiotic nuclei within the *C. elegans* gonad. By optimizing an imaging approach using the innate dark states of synthetic dyes, we achieved seconds-long single-molecule trajectories at ~25 nm precision. with this method, we found that both subunits of the SC scaffold (SYP-3) and pro-crossover signaling proteins (ZHP-3) exhibited a mix of static binding and Brownian diffusion along the length of the SC. While SYP-3 molecules underwent only relatively short diffusive excursions, ZHP-3 molecules often diffused freely for >10 s at a time, leading to an effective diffusion coefficient an order of magnitude larger than for SYP-3. Both proteins exhibited a small reduction in diffusivity from early to late pachytene, but remained largely diffusive, ruling out loss of SC fluidity as a mechanism of crossover interference. However, we did find that the trajectories of ZHP-3 molecules exhibited extended binding at designated crossover intermediates. At nanoscopic scale, ZHP-3 foci at these intermediates appeared to be partially separate from the SC, further suggesting a long-lived “capture” state. Numerical simulations confirmed that a diffusion and capture mechanism for ZHP-3 localization could reach steady-state within the biological timescale of pachytene progression. In sum, our results indicate that the SC

forms a conduit for rapidly diffusing signals that is sufficient to enable crossover interference over the length of meiotic chromosomes.

SG208

**Multiple factors contribute to meiotic crossover suppression on chromosome 4 in *Drosophila melanogaster*.**

**N. Crown**, J. Terry, A. Siamphone; Biology, Case Western Reserve University, Cleveland, OH.

Meiotic crossovers (COs) are completely suppressed on chromosome 4 in *Drosophila melanogaster*, but the underlying mechanism has been debated for over 100 years. One model suggests that because chromosome 4 is small, the entire chromosome is subject to the centromere effect. The other prevailing model is that the high heterochromatin content of chromosome 4 prevents COs from occurring. We will present data showing that there are multiple contributing factors to suppressing COs on chromosome 4 and that these models are not mutually exclusive. We find that when genome-wide CO patterning is disrupted by the presence of a heterozygous inversion (the interchromosomal effect), COs occur on chromosome 4 at an average rate of 0.74 cM. Additionally, we find that when heterochromatin structure is disrupted specifically on chromosome 4, COs also occur. We will also present data on the genetic interactions between these two contributing factors.

SG209

**The conserved ATPase PCH-2 prevents early double strand breaks from becoming crossovers.**

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Meiotic crossovers generate genetic diversity and promote accurate chromosome segregation during gametogenesis. Our research focuses on understanding how the evolutionarily ancient AAA ATPase, PCH-2, regulates meiotic recombination to ensure the correct number and distribution of crossovers to accomplish both of these goals. In *C. elegans*, loss of *pch-2* results in a decrease in the number of crossovers and persistence of PCH-2 on meiotic chromosomes leads to extra crossovers, consistent with a role in promoting crossover formation. However, *pch-2* mutants also show an altered distribution of crossovers, suggesting a role in limiting crossovers. Given that defects in meiotic recombination are a universal phenotype of *pch-2* mutants in diverse model organisms, these opposing roles in controlling crossover formation may represent its conserved meiotic role. To better understand these two opposing roles in recombination, we took advantage of situations in which double strand breaks (DSBs) are limiting (*dsb-2* mutants). DSB-2 is required for the appropriate number of DSBs. The reduction of DSBs in the *dsb-2* mutant results in a loss of crossovers, producing defects in chromosome segregation. In contrast, *dsb-2;pch-2* double mutants exhibit an increase in crossovers and a reduction in chromosome missegregation. The number of DSBs does not increase in *dsb-2;pch-2* double mutants, allowing us to draw two conclusions: 1) there are enough DSBs in *dsb-2* mutants to support crossover formation, and 2) PCH-2 specifically inhibits these DSBs from becoming crossovers. To test whether this role in inhibiting crossovers was a general or regulated phenomenon, we irradiated worms to introduce extra DSBs at different stages of meiotic prophase and evaluated crossover formation. These experiments revealed that PCH-2's role in regulating crossover formation is cell cycle regulated: PCH-2 specifically inhibits crossover formation when DSBs are introduced in early meiotic prophase. We are testing this model further, as well as identifying which proteins PCH-2 remodels to control the fate of these early DSBs. Our work will reveal unexpected connections between when DSBs happen, what role they may play in meiosis, and how PCH-2 controls this process to affect the number and distribution of crossovers.

SG210

**Heterochromatin component HP2 is required for meiotic satellite DNA transcription and male fertility in *Drosophila melanogaster*.**

**T. Kumon**, J. M. Fingerhut, R. Y. Li, Y. M. Yamashita; Whitehead Institute, Cambridge, MA.

Satellite DNA sequences are actively transcribed during the long meiotic prophase of spermatocytes in *Drosophila melanogaster*. Satellite DNA transcription is also reported in the mouse and human spermatogenesis. However, mechanisms or functions of the male meiotic satellite DNA transcription remain unknown. We found that a component of heterochromatin, HP2, is required for the expression of satellite DNA in *Drosophila melanogaster* spermatocytes in a sequence-specific manner. Spermatocyte-specific RNAi of HP2 reduces fertility. Reduction in fertility is likely caused by DNA damage in spermatids and defective protamine incorporation. Interestingly, the Y chromosome appears to be more susceptible to DNA damage upon HP2 RNAi, leading to biased demise of Y chromosome-containing spermatids and thus to biased sex ratio (more females) in the offspring. Upon HP2 RNAi, spermatocytes accumulate histone modifications (H3K9me3) associated with heterochromatin, and fail to incorporate protamine properly. Taken together, our work provides insights into a potential function of satellite DNA transcription.

SG211

**A Piezo-dependent checkpoint at the nuclear envelope is important for oocyte quality control in *C. elegans*.**

**C. Liu**, A. F. Dernburg; University of California, Berkeley, Berkeley, CA.

Successful sexual reproduction relies on robust quality control during meiosis. The formation of the synaptonemal complex between homologous chromosomes (synapsis) is crucial for accurate segregation of homologous chromosomes and genetic recombination. When homologous chromosomes fail to synapse, a “synapsis checkpoint” is activated to remove defective gametes via apoptosis. How meiotic cells detect and respond to synapsis defects remains unclear. In *C. elegans*, specialized chromosome sites known as pairing centers interact with the nuclear envelope during early prophase. The Polo-like kinase PLK-2 is recruited to these sites; it phosphorylates the nuclear lamina to facilitate chromosome movement, pairing, and synapsis. Synapsis defects result in persistence of PLK-2 at Pairing Centers. Using a new chemically inducible proximity system, we found that the presence of PLK-2 at the pairing centers during late meiotic prophase is sufficient to induce apoptosis. Tethering of PLK-2 to the X chromosome pairing center results in prolonged phosphorylation and weakening of the nuclear lamina. This in turn leads to dramatic deformation of the nuclear envelope. Unexpectedly, we find that a mechanosensitive Piezo1/PEZO-1 channel localizes to the nuclear envelope and is required to promote apoptosis in response to synapsis defects. In response to asynapsis, PEZO-1 at the NE redistributes into clusters where dynein and LINC complexes are also present and known to transduce microtubule-based force to oocyte NE. Loss-of-function mutation in the LINC component SUN-1 abolishes synapsis checkpoint activation, further supporting a role of LINC-mediated mechanotransduction during synapsis checkpoint response. Intriguingly, in response to asynapsis, PEZO-1 redistribution at the NE requires PCH-2, an AAA-ATPase known to be required for synapsis checkpoint. Together, our findings reveal that cytoskeletal forces and mechanosensitive ion channels at the nuclear envelope monitor nuclear events and promote quality control during gamete production.

SG212

**Emerging mechanisms of chromosome segregation in mammalian eggs.**

**B. Mogessie**, K. Scheffler; Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT.

Chromosome segregation in female meiosis is driven by cooperative action of the microtubule and actin cytoskeletons. Female reproductive aging is accompanied by chromosome segregation errors in oocytes that are broadly associated with infertility and genetic disorders. We recently discovered unique, actin-based chromosome segregation mechanisms that limit oocyte aneuploidy (Mogessie and Schuh, 2017; Dunkley and Mogessie, 2023). In this study, we have investigated the function of actin-microtubule associated proteins in meiotic spindle assembly and chromosome segregation. By combining powerful protein degradation tools with quantitative 3D tracking of spindle morphology and chromosome movement, we reveal key proteins required for initial microtubule assembly in prometaphase, as well as spindle bipolarization and accurate chromosome segregation in meiosis I. By acutely removing candidate proteins from mouse eggs arrested in metaphase of meiosis II, we provide new evidence for actin motor proteins that crucially maintain spindle architecture and chromosomal alignment. We are currently investigating how actin motors promote meiotic spindle assembly and chromosome segregation, and whether female reproductive aging-related decline in this new function predisposes oocytes to detrimental aneuploidies.

SG213

**The Role of Meiotic Factors in Ploidy Dynamics.**

**M. Bravo Núñez**, A. W. Murray; Molecular and Cellular Biology, Harvard University, Cambridge, MA.

Errors during chromosome segregation can lead to cells with the wrong number of chromosomes, a condition known as aneuploidy. Aneuploidy is a driving force in cancer progression and has been linked to the rapid emergence of drug resistance in fungal pathogens. For these reasons, it is important to understand the etiology of aneuploidy and the fitness advantage that aneuploidy may confer under environmental perturbations. Inappropriate expression of meiotic genes is a potential cause of aneuploidy as the normal function of these genes is to alter the ploidy of cells in order to generate haploid gametes (e.g., sperm or egg). Using the budding yeast *Saccharomyces cerevisiae*, we tested this idea by creating a library of meiotic genes (under the control of an inducible promoter), expressed them in mitotically-dividing cells, and determined their contribution to genome instability and their ability to adapt to exogenous stresses. Our work shows that genes involved in altering centromere behavior during meiosis can increase the levels of aneuploidy and faster adapt to constant DNA replication stress. This work illustrates how meiotic genes may contribute to mitotic segregation errors and drive ploidy changes outside of gametogenesis. In addition, it showcases examples in which generating aneuploidies may be advantageous.

SG214

**Hybrid incompatibility in cohesin protection leads to egg aneuploidy and female sterility.**

**T. Akera**, W. El Yakoubi; NIH, National Heart, Lung, and Blood Institute, Bethesda, MD.

Hybrid incompatibility is the genetic cause of postzygotic reproductive isolation, contributing to speciation. Such incompatibilities arise when the genomes of two different populations take distinct evolutionary trajectories, resulting in incompatible genomic interactions in F1 hybrids that lead to their

lethality or sterility. Here we found that female hybrids between *Mus musculus domesticus* and *Mus spicilegus* are sterile due to non-disjunction of homologous chromosomes in oocyte meiosis I, producing aneuploid eggs. Rec8 cohesin was present all along the non-disjoining chromosomes in the meiosis II egg, suggesting that Separase was not able to cleave the cohesin to separate homologous chromosomes in anaphase I. Interestingly, our preliminary results suggest that the Separase activity was similar between control *domesticus* oocytes and hybrid oocytes. Instead, the major cohesin protector in mouse oocytes, Sgo2, mis-localized to the chromosome axis in hybrid oocytes in contrast to its typical enrichment at peri-centromeres in parental species. This ectopic Sgo2 localization can over-protect cohesin rings on the chromosome, explaining why cohesin remained uncleaved on the chromosome despite the normal Separase activity. Consistent with this idea, Sgo2 RNAi in hybrid oocytes canceled the cohesin over-protection and chromosomes fell apart in anaphase I. Altogether, this work showed that cohesin mis-regulations in meiosis can create a reproductive isolating barrier in mice.

SG215

**PLK-1 suppresses ectopic microtubule nucleation in *C. elegans* oocytes to ensure faithful meiosis.**

**J. G. Narula**, S. M. Wignall; Molecular Biosciences, Northwestern University, Evanston, IL.

Sexual reproduction relies on meiosis, a specialized cell division program that produces haploid gametes. Female gametes (oocytes) in most organisms lack centrosomes, yet the mechanisms by which acentrosomal spindles are formed and stabilized are poorly understood; we use *C. elegans* as a model to investigate these mechanisms. Polo-like kinases are critical regulators of mitotic chromosome segregation and spindle formation across species. In *C. elegans*, polo-like kinase 1 (PLK-1) plays an essential role in centrosome maturation during mitosis and is required for nuclear envelope breakdown in oocytes. A recent study reported spindle defects upon PLK-1 inhibition in oocytes (1); however, how PLK-1 contributes to spindle assembly and whether it also promotes spindle stability are not known. To address these questions, we utilized rapid auxin-inducible degradation to deplete PLK-1 from oocytes either prior to spindle assembly or after bipolar spindle formation. Depletion of PLK-1 prior to spindle assembly resulted in a failure of nuclear envelope breakdown, consistent with previous findings. Further, we discovered that removal of PLK-1 from fully formed spindles disrupted their organization, indicating that PLK-1 is required to maintain spindle integrity. Interestingly, in both of these depletion conditions we often observed the formation of ectopic microtubule asters, both near the oocyte chromosomes and near the sperm DNA. This was notable because in *C. elegans*, the oocyte is fertilized before the meiotic divisions and the sperm centrioles are held inactive until Meiosis II is complete. We therefore hypothesized that the asters near the sperm could be a result of premature centrosome activation. Consistent with this hypothesis, we found that PLK-1 localized to sperm centrioles in wild-type oocytes, placing it in a location where it could suppress centrosome activation. Moreover, PCM components were prematurely recruited to sperm centrioles during the meiotic divisions in PLK-1-depleted oocytes, concomitant with ectopic aster formation. These findings therefore reveal a surprising new role for PLK-1 in preventing PCM recruitment in oocytes, opposite to its canonical function in mitosis. Taken together, our work identifies PLK-1 as a key player in *C. elegans* meiosis, playing multiple essential roles that promote faithful meiosis.1. Taylor et.al. (2023), *eLife*

SG216

**Cohesin and Chromosome Segregation in Oocytes: a Goldilocks scenario.**

Y. Yun, **N. Hunter**, K. Ikami, D. Do; University of California, Davis, Davis, CA.

Chromosome segregation is compromised in eggs from women of both young and advanced maternal ages. Deficient sister-chromatid cohesion is an underlying cause of segregation errors in eggs from older females but the cause of missegregation in the eggs of young women is unknown. We show that oocytes from juvenile mice show severe chromosome lagging during anaphase I, leading to high levels of nondisjunction or even complete failure of the first meiotic division. These defects can be rescued by weakening cohesion, or by enhancing its resolution during anaphase I. By contrast, in oocytes from adult mice, cohesion is demonstrably weaker and lagging and nondisjunction are rare. Thus, opposite to situation in older females, excessive cohesion impedes segregation in oocytes of young females. We infer that sexual maturation is associated with weakening of cohesion to optimize oocyte chromosome segregation. This model can explain the very high rates of nondisjunction reported in the eggs of young women.

SG217

**Meiosis II Spindle Disassembly Requires Two Distinct Pathways.**

**X. Mucelli**[1], B. C. Seitz[1], M. Majano[1,2], Z. Wallis[1,3], A. C. Dodge[1,2], C. Carmona[1,2], M. Durant[1], S. Maynard[1], L. S. Huang[1]; [1]Biology, University of Massachusetts Boston, Boston, MA, [2]Biology, Brandeis University, Waltham, MA, [3]Biology, Boston College, Boston, MA.

At the end of meiosis II, *Saccharomyces cerevisiae* yeast cells must disassemble the meiosis II spindle and undergo cytokinesis; these two events must be coordinated. In budding yeast, meiosis II cytokinesis occurs by prospore membrane closure; the prospore membrane is a newly synthesized membrane that grows around each of the haploid nuclei for cellularization of the developing spore within the mother cell. Previous studies demonstrate that *SPS1*, which encodes a STE20-family GCKIII kinase that acts downstream of the Hippo-like kinase encoded by *CDC15*, and *AMA1*, which encodes a meiosis specific activator of the Anaphase Promoting Complex, are required for both spindle disassembly and cytokinesis in meiosis II. To better understand the regulation of spindle disassembly and cytokinesis in meiosis II, we have explored the roles of *SPS1* and *AMA1* in exit from meiosis II using biochemistry and microscopy. We find that the prospore membrane closure defects of both *sps1Δ* and *ama1Δ* cells do not result from the failure of spindle disassembly, indicating that these two processes are regulated independently of one another by *SPS1* and *AMA1*. We also find that *sps1Δ* and *ama1Δ* cells exhibit distinct defects in spindle disassembly. By examining known microtubule-associated proteins important for spindle disassembly in mitosis, we find *AMA1* is required for the removal of Ase1 and Cin8 from the meiosis II spindle, while *SPS1* is required for proper removal of Bim1. Our data indicate that the two pathways represented by *SPS1* and *AMA1* are required for different aspects of meiosis II spindle disassembly. Furthermore, the regulation of proteins important for spindle disassembly are regulated similarly in mitosis and meiosis, despite the use of meiosis-specific regulatory proteins.

SG218

**The Mechanism of Head-Tail Connection in Sperm Formation.**

**D. Buglak**, K. Holmes, B. Galletta, N. Rusan; National Heart, Lung, and Blood Institute, NIH, Bethesda, MD.

During spermiogenesis, spermatids undergo dramatic morphological changes to develop into mature sperm. The final architecture of the sperm requires the lateral attachment of the axoneme (sperm tail) to the needle shaped nucleus (sperm head). Secure attachment is mediated by the head-tail coupling apparatus (HTCA) and its failure results in male sterility due to sperm decapitation. How the HTCA forms and remodels over time is not well understood. Here, we describe a four stage process of HTCA development: 1. Search and capture of the nucleus by the centriole, 2. Centriole attachment to the nucleus, 3. Centriole invagination into the nucleus, and 4. Centriole lateralization. Using structured illumination microscopy, we imaged late stage spermatids to better understand HTCA remodeling. We identified two unique structures at the HTCA during late spermiogenesis: The “cap” and “shelf”. We found that the cap is associated with centriole invagination into the nucleus, while the shelf is associated with centriole lateralization. Two important HTCA components, the testis-specific SUN-domain protein Spag4 and the gravitaxis protein Yuri Gagarin, both localize to the cap and shelf. Furthermore, we found that the cap and shelf are closely associated with two additional sperm centriole structures, the proximal centriole-like (PCL) and centriole adjunct (CA). Mutant analysis led to a model whereby the PCL acts as an anchor during centriole invagination into the nucleus and serves as a template for centriole/axoneme lateralization. Thus, we identify two unique structures in the sperm HTCA and novel roles for the PCL and CA during late stage spermiogenesis that are important in male fertility.

## Subgroup: Biological Size Control and Scaling

SG219

**A fitness landscape instability governs the morphological diversity of tip-growing cells.**

**E. Rojas**; New York University, New York City, NY.

Cellular morphology affects many aspects of cellular and organismal physiology. This makes it challenging to understand the evolutionary basis for specific morphologies since the various facets of cellular physiology may exert competing selective pressures on this trait. The influence of these pressures, moreover, will depend on the mechanisms of cellular morphogenesis. To address this problem, we combined experimental cell biology with mechanics-based theory to analyze the morphological diversity of tip-growing cells from across the tree of life. We discovered that an instability in the convergent mechanism of ``inflationary'' growth shared by these cells leads directly to a bifurcation in their fitness landscape, which imposes a strong global constraint on their morphologies. Additionally, we found that co-selection for cell size and elongation rate explains variation among observable morphologies. This analysis rationalizes the morphology - and provides quantitative insight into the ecology - of an enormous diversity of important fungal, plant, protistan, and bacterial systems. More broadly, our study illustrates a paradigm for how evolutionary dynamics emerge from physical and developmental constraints to sculpt biological form.

SG220

**Global protein-turnover quantification in *Escherichia coli* reveals cytoplasmic recycling under nitrogen limitation.**

**A. Johnson**, M. Gupta, E. Cruz, E. Costa, R. Guest, T. J. Silhavy, N. S. Wingreen, Z. Gitai, M. Wühr; Princeton University, Princeton, NJ.

Protein turnover is critical for proteostasis, but turnover quantification is challenging, and even in well-studied *E. coli*, proteome-wide measurements remain scarce. Here, we quantify the degradation rates of ~3.2k *E. coli* proteins under 12 conditions by combining heavy isotope labeling with complement reporter ion quantification and find that cytoplasmic proteins are recycled when nitrogen is limited. We use knockout experiments to assign substrates to the known cytoplasmic ATP-dependent proteases. Surprisingly, none of these proteases are responsible for the observed cytoplasmic protein degradation in nitrogen limitation, suggesting that a major proteolysis pathway in *E. coli* remains to be discovered. Lastly, we show that protein degradation rates are generally independent of cell division rates. Thus, we introduce broadly applicable technology for protein turnover measurements and provide a rich resource for protein half-lives and protease substrates in *E. coli*, complementary to genomics data, that will allow researchers to decipher the control of proteostasis.

SG221

**Cell size determines proteome content in mammalian, yeast, and bacterial cells.**

**M. C. Lanz**[1], J. E. Elias[2], J. M. Skotheim[1]; [1]Stanford University, Stanford, CA, [2]CZ Biohub, Stanford, CA.

Cell size is tightly controlled in both human tissues and single-celled organisms, and aberrant cell size is often associated with pathologies like cancer and aging. Despite the prominence of size regulation in cell biology, it remains unclear how differences in cell size impact cell physiology. To address this, we measured how the cell's proteome changes with increasing cell size in organisms across the tree of life. We found that, in all cases, increasing cell size drives widespread changes in the concentrations of individual proteins. In eukaryotes, cell size-dependent changes to the proteome are highly conserved and best predicted by a protein's subcellular localization. Size-dependent changes to the proteome are not observed when an increase in cell size is accompanied by an increase in ploidy, suggesting that ploidy-to-cell size ratio, rather than cell size *per se*, determines proteome content. Finally, the proteome changes that coincide with decreasing genome concentration are reminiscent of the starvation response. This observation indicates that extrinsic (nutrient levels, growth factors) and intrinsic (genome concentration) determinants of the cellular growth rate may remodel the proteome in similar ways. Overall, our findings indicate that cell physiology is significantly impacted by cell size and provide a rationale for why cells tightly regulate their division cycle to maintain a desired target volume.

SG222

**Beyond G1/S regulation: how cell size homeostasis is tightly controlled throughout the cell cycle?.**

**X. Liu**[1], M. Kirschner[1], J. Yan[2]; [1]Harvard Medical School, Boston, MA, [2]Stanford University, Stanford, CA.

Proliferating cells develop mechanisms to counteract stochastic noise in order to stabilize the cell mass distribution in a population. It is widely believed that cell size is controlled by the size-dependent timing of the G1/S transition. However, a model based only on such a size checkpoint cannot explain why cell lines with deficient G1/S control are able to maintain a very stable cell mass distribution and how cell mass variation is controlled throughout the S and G2 phases of the cell cycle. To answer such questions,

we applied a recently developed form of computationally enhanced Quantitative Phase Microscopy (ceQPM), which provides much-improved accuracy of cell mass measurement for individual cells. ceQPM allows us to investigate the factors contributing to cell mass homeostasis. We find that cell mass homeostasis is robustly maintained, despite disruptions of the normal G1/S transition or cell growth rate. Cell mass control is exerted throughout the cell cycle, where the coefficient of variation in cell mass for the population declines well before the G1/S transition and throughout the cell cycle in both transformed and non-transformed cells. Furthermore, the detailed response of cell growth rate to cell mass differs in different cell types. This process of size-dependent growth rate modulation occurs through both mTORC1-dependent and mTORC1-independent processes, which are independently regulated. The reduction of mass accumulation rate, slightly below that of exponential growth, is used to effectively reduce cell mass variation in the population. Both size-dependent cell cycle regulation and size-dependent growth rate modulation contribute to cell mass homeostasis in a compensatory manner. These findings expose new principles in cell size control for normal and pathological proliferating cells, as well as terminally differentiated cells. Further, they suggest new avenues for the discovery of the underlying molecular mechanisms.

SG223

**A hardwired link couples cell size to DNA content independent of gene dosage.**

M. Estrada, **G. Neurohr**; ETH Zurich, Zurich, SWITZERLAND.

Cell size and DNA content strongly correlate with each other, both within and across species and alterations in the DNA:cytoplasm ratio have important physiological consequences during development and cell senescence. Despite this widely conserved correlation and the apparent functional importance of maintaining the DNA:cytoplasm ratio constant, how cell size and DNA content are coupled is poorly understood. Studying this question has been challenging because changes in DNA content are typically associated with gene copy number changes, which often dominate the phenotypes of cells carrying extra DNA.

To overcome this challenge, we have developed orthogonal systems to conditionally and reversibly accumulate up to more than one genome equivalent of non-coding and coding DNA in budding yeast cells. Interestingly, cell size adapts to increasing DNA content even if there are no functional genes encoded on the extra DNA, demonstrating that size is coupled to DNA content independent of gene dosage.

In budding yeast, cell size is set at the G1/S transition through a network of cell cycle regulators. While accumulation of non-coding DNA prolongs the duration of G1-phase, the DNA to size coupling occurs independent of the described G1/S control network, suggesting that there is a more fundamental link between cell size and DNA content. Interestingly, human DNA is being transcribed in yeast cells even though it does not encode any functional genes. When we re-direct a large fraction of the general transcription machinery to ectopic DNA using a strong inducible transcription system, this also results in a corresponding cell size increase. Our preliminary data therefore support a model in which cell size is coupled to DNA content through titration of the general transcription machinery against the genome. Such a model involving the core growth machinery could explain the widely conserved coupling of cell size and DNA content.

SG224

**Euploidy for the mitochondrial genome - How is it sensed and maintained?.**

**S. P. Rath**, V. K. Mootha; Molecular Biology, Massachusetts General Hospital, Boston, MA.

The human mitochondrial genome (mtDNA) varies >1000-fold in copy number across tissues, and each cell operates at an intrinsic setpoint, which we call “mtDNA euploidy”. How dividing cells sense and maintain mtDNA euploidy remains mechanistically unknown. To elucidate this, we leverage a phenomenon where cells naturally recover from an induced mtDNA depletion and re-establish their initial mtDNA copy number. First, we define the reversible proteomic, metabolic, and bioenergetic cellular states induced by mtDNA loss. Second, using chemical-genetic perturbations, we discover two mechanisms that make mtDNA recovery possible: (1) nuclear-encoded solutions support mtDNA replication independently of mtDNA-encoded proteins and (2) mtDNA depletion slows cell proliferation which prevents further depletion of mtDNA copies by dilution to daughter cells. Proliferation improves with mtDNA recovery and serves as the built-in brake on mtDNA copy number. Finally, we provide genetic evidence that the latent mitochondrial parameter coupling cell proliferation to mtDNA euploidy is the proton motive force. Our molecular insight into how cells sense and cope with mtDNA loss could inspire treatment for mtDNA depletion syndrome and reveal a cellular logic that is fundamental for the maintenance of organellar genomes.

SG225

**A liquid-to-solid transition of the pericentriolar material scaffold limits centrosome size.**

**M. Amato**; UT Southwestern Medical Center, Dallas, TX.

The centrosome is composed of a pair of barrel-shaped centrioles that organize a supramolecular proteinaceous scaffold known as the pericentriolar material (PCM) that organizes microtubule asters. PCM accumulation is consistent within and between cells, yet the mechanism of PCM scaling remains poorly understood. PCM assembly has been best understood by two competing models which have different implications for PCM sizing. In the first model, diffusion-limited accretion of soluble PCM components assemble into a solid lattice that radiates from the centrioles. In this model, the size of the PCM is set by the kinase activity of the mitotic kinase PLK-1, which decays radially from the centrioles. In the second model, the PCM initially forms through liquid-liquid phase separation and then solidifies into a gel-like scaffold (liquid-to-solid transition). In this model, the size of the PCM is set by the kinetic arrest of polymeric PCM components which terminates further growth. Here we use a combination of in vitro reconstitution, microrheology, and live-cell imaging to differentiate between these two models to better understand PCM sizing in C. elegans embryos. We show evidence of an initial liquid-like state of the PCM in vivo that then transitions to a static state as a function of the cell cycle. We have reconstituted this transition in vitro, demonstrating a long-lived liquid behavior of reconstituted PCM droplets that undergo a phosphorylation-induced liquid-to-solid transition. Hyperphosphorylation of the PCM scaffold protein SPD-5 results in smaller centrosomes in vivo and in vitro, consistent with model #2. Our results support a mechanism by which the PCM undergoes a phosphorylation-induced liquid-to-solid transition that limits its size.

SG226

**Probing Cell Cycle Commitment at the Single-Molecule Level in Budding Yeast.**

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Cell growth and division must be tightly coordinated to maintain a homeostatic size at the population level. In budding yeast, the daughter cell keeps growing during G1 phase until reaching a critical cell size, at which point the cell starts budding. The commitment to cell division is called Start. Despite substantial information about the machinery that controls Start, the mechanisms that couple the cell size with G1/S transition are still obscure. We hypothesize that a change in the dynamics of a critical regulator sets the time of Start. To test this, we set up an *in vivo* single-molecule fluorescence microscopy technique in budding yeast to characterize the binding kinetic at chromatin of Whi5 and SBF during G1 phase. Combined with an estimate of the protein copy number, we found that the number of Whi5 bound on chromatin decreases during G1 phase while the number of chromatin-bound SBF increases. mRNA-FISH results confirm that G1 cyclin Cln2's transcription probability increases with SBF chromatin-bound number. Whi5 overexpression results in a drop of SBF binding on chromatin, suggesting that Whi5 represses SBF by preventing its binding to chromatin. Our single particle tracking data also suggest that SBF has a longer binding time on chromatin in late daughter cells. We propose that it is the relative amount of Whi5 and SBF bound to chromatin, instead of the concentration of Whi5 or SBF alone, that plays the critical role in deciding the time of Start.

SG227

**Experimentally Evolving Cell Size Homeostasis in Budding Yeast.**

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Cell size is a fundamental determinant of cellular physiology that varies greatly across organisms and cell types, ranging from micrometers in bacteria to millimeters in oocytes. Despite this diversity, each cell type maintains its characteristic size and volume through a process known as cell size homeostasis. Nonetheless, how size homeostasis regulation evolves to give rise to the wide range of cell sizes observed in nature remains unclear. To understand the mechanisms governing cell size regulation and their impact on intracellular scaling patterns, we studied how evolution can alter cell size homeostasis using the model organism *Saccharomyces cerevisiae*. We developed an experimental evolution strategy in which cells are selected based on their small or large size relative to the rest of the population. Preliminary results reveal that the mechanisms governing cell size homeostasis are dynamic, leading to both increases and reductions in cell size over 1000 generations. These alterations in cell size influence cell cycle progression, growth dynamics and sedimentation behavior. By unraveling the genetic basis of the altered cell size, we aim to understand key molecular mechanisms governing the evolution of cell size homeostasis and their impact on cellular physiology. Overall, our experimental approach is a promising method to explore the physical and evolutionary limits for cell size in microorganisms, ultimately advancing our understanding of size homeostasis across different species and cell types.

SG228

**Direct quantification of sinking velocities and their cell size-dependencies across unicellular algae.**

**T. P. Miettinen**[1], A. L. Gomez[2], Y. Wu[1], W. Wu[1], T. R. Usherwood[1], B. R. K. Roller[3], M. F. Polz[3], S. R. Manalis[1]; [1]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, [2]Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA, [3]Centre for Microbiology and Environmental Systems Science, University of Vienna, Vienna, AUSTRIA.

Eukaryotic phytoplankton, also known as algae, form the basis of marine food webs and are responsible for most of marine carbon sequestration when their dead biomass sinks to the ocean floor. In living algae, cell sinking defines the cells' access to light at the ocean surface and nutrients in deeper ocean layers. Thus, detailed knowledge about cell sinking is needed for understanding algae physiology, marine ecosystems, and global carbon cycles. The gravitational sinking velocity of algae is dependent on the size and composition of the cells, but typical measurements of cell sinking cannot decouple gravitational sinking from cell motility. Here we combine measurements of cell mass and volume to directly quantify gravitational cell sinking velocities according to Stokes' law in diverse clades of unicellular marine microalgae. We find that species vary radically in sinking velocities, and we reveal that the cell size-dependency of cell sinking velocities both within and between species follow ~2/3 power law scaling. However, only ~50% of the cell-to-cell sinking velocity variability within a species is accounted for by the variation in cell volumes, as cell densities are more variable than in classical model organisms. Furthermore, we observe examples of motile dinoflagellate and green algal species where nutrient limitation causes cells to increase their gravitational sinking velocity. Surprisingly, these sinking velocity changes under nutrient limitation are driven by increased cell mass, while cell volume remained approximately constant. Overall, our work exemplifies how cell mass and volume are key parameters for algae physiology, and how algae can display unexpected cell size regulation upon nutrient limitations. More broadly, our results and approach for quantifying sinking velocities can support future models of marine ecosystems and carbon fluxes in the oceans.

## Subgroup: Building Tissues across Scales: From Micro to Macro

SG229

**Reconstituting mouse embryonic development *ex utero*.**

**A. Aguilera Castrejon**[1], J. Hanna[2]; [1]4D Cellular Physiology, HHMI Janelia, Ashburn, VA, [2]Molecular Genetics, Weizmann Institute of Science, Rehovot, ISRAEL.

Understanding the processes leading to the formation of tissues and organs represents a fundamental question in developmental biology. After implantation, mammalian embryos initiate the process of gastrulation, in which the pluripotent epiblast transits from a symmetrical ball of cells into an advanced embryo with a defined body plan. Yet, the small size and intrauterine confinement of the developing embryos limits the study of post-implantation embryogenesis, due to the inability to observe and manipulate living embryos at these stages. In this work, we present an extended system for *ex utero* culture of mouse post-implanted embryos, enabling proper development of embryos from pre-gastrulation (E5.5) until the last day of organogenesis (E12.5). Besides, we present an *in vitro* implantation culture able to support growth of totipotent zygotes from after fertilization until organ formation stages (E9.5) entirely *in vitro*. These culture systems allow the introduction of a variety of embryonic perturbations and micro-manipulations, which can be followed *ex utero* in living embryos.

Further, we adapted the *ex utero* platform for culturing stem cell-embryo models, which complete gastrulation and develop until early organogenesis when cultured *ex utero*. These stem-cell-derived embryos resemble E8.5 natural embryos and contain both embryonic and extraembryonic compartments originated from naïve pluripotent stem cells as the sole stem cell source. The ability to continuously culture natural and stem cell-assembled mouse embryos opens new possibilities for understanding mammalian embryogenesis by allowing experimentation in living embryos during stages that have remained hidden inside the maternal uterus, and paves the way for implementing analogous culture methods in non-human primates and human embryos.

SG230

**Extracellular matrix microenvironment collaborates with cell mechanics to guide early embryonic cell fate selection.**

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Embryogenesis is a self-governed process of sequential cell fate transitions controlled by the interplay of morphogenesis and cell fate transitions, leading to the emergence of tissue shapes and patterns. The gene regulatory networks governing early embryonic fate transitions are relatively well understood, but how they are coordinated with cell and tissue shape transformations remain unclear. This project aims to understand the mechanisms of this coordination by addressing two fundamental open questions: 1) How do individual embryonic cells sense their position and couple this to fate selection? 2) What mechanisms relay the information of location to the transcriptional machinery? By combining various in vitro models of human gastrulation with quantitative imaging and sequencing, we show that the biochemical composition of the extracellular matrix impacts early lineage decisions. The signals from the microenvironment are relayed by the contractile actomyosin cytoskeleton to impact the mechanical deformation of the nucleus. Further, we observe that transient nuclear deformation directly causes changes in the transcriptional potential of cells with long lasting effects on epigenetic landscape. Together, this study establishes a link between physical forces and biochemical signalling for coordination of mammalian embryogenesis.

SG231

**Zones of mitotic activity during Left-Right Organizer development drive asymmetric cilia organization during lumen formation.**

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The coordination of cell division, cilia formation, and lumen formation are essential processes that need to occur during the assembly of cyst or tube-like cellular structures. To study these processes, we took advantage of the external zebrafish embryo development that allows for observation and manipulation of a conserved ciliated tissue with a fluid filled lumen called the Left-Right Organizer (LRO). While events downstream of cilia-driven flow have received attention, little is known about how the LRO is formed. What is known is that LRO development occurs in approximately 5 stages: a migratory stage, mesenchymal epithelial transition (MET), apical clustering (where LRO cells start arranging in 1 or more

rosette-like structures), lumen formation, and lumen expansion. Previous studies characterized that during lumen expansion KV cells undergo significant shape changes that are associated with a 2-fold increase in the number of cilia towards the anterior end of the LRO (near notochord) compared to the posterior. Here we test if zones of LRO cell proliferation during development contributes to the asymmetry in cilia towards the anterior end. We find that most mitotic events occur before lumen opening and at the anterior end of the LRO closest to the notochord where post-mitotic cells reside. If we disrupt these division events through genetic manipulation of key mitotic components (Pericentrin-null lines) or laser ablation of mitotic cells, we find that the LRO has less cells, smaller lumen, disorganization of ZO-1, and cilia/cells are positioned symmetrically around the LRO. Since there is normally 2x greater cilia towards the anterior side of the LRO compared to the posterior, our findings suggest that delaying/inhibiting KV cells mitotic entry/exit results in a decrease in the number of KV cells that can organize in the anterior. Based on these findings, we propose a model where spatially regulated mitotic events dictate downstream biophysical, cellular, and developmental processes that are essential for the full functionality of the LRO. This study sheds light on the intricate mechanisms governing the assembly of cellular structures and provides insights into the potential factors influencing cilia distribution in the LRO.

SG232

**Branching morphogenesis of holothurian ossicles in multicellular, active, syncytial confinement.**

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Biomineralization is ubiquitous in both unicellular and multicellular living systems and has remained elusive due to the limitations in the simultaneous understanding of both physicochemical and biomolecular processes. Out of all the scales, biomineralization in complex multicellular eukaryotes involves collaboration between multiple cells to create mineral structures much larger than the individual participating cells. The principles and processes governing these structures' shape and size regulation remain largely undiscovered. Specifically, in holothurians (sea cucumbers), biomineralization occurs in the form of discrete ~100 μm length scale distributed skeletal structures called ossicles that diversify in shapes not only across holothurian species but even within an individual animal. To understand the fundamental processes enabling cellular cooperation at multi-cell length scales during the growth of ossicles, we performed direct live imaging of ossicle growth in Parastichopus parvimensis juveniles enabled by their almost transparent epithelial tissues. We establish that these structures grow from individual crystalline seeds (~1-2$ μm) inside a multi-cell syncytial complex with the biomineralized phase completely wrapped with a membrane-bound cytoplasmic sheath. We demonstrate that the initial seed transforms into a fully formed multi-holed structure through 4 key steps - symmetry breaking in the seed, tip extension, tip splitting or budding, and the merging of two tips. Through large-scale statistics on micro-CT data, we probe the robustness of the processes described. Through geometrical analysis, we highlight unique geometrical features depicting finiteness.

Throughout the growth, we find that cytoskeletal and cytoplasmic activity restricted to the surface of the biomineralized phase rather than the motility of participating cell bodies regulate the material transport and directional growth of the structure. Using abstract models of constrained growth of self-closing active branching networks, we highlight the hidden universality in the growth process of ossicles. By observing distinct developmental niches, we demonstrate differential symmetry breaking and cell-cluster dispersion as the structure grows, which acts as an additional layer of control over the underlying

growth process. The system thus serves as a unique playground merging aspects of non-equilibrium growth processes and classical branching morphogenesis in living systems.

SG233

**Membrane Remodeling by Macropinocytosis Regulates Tension Distribution and Tissue Crowding during Epithelial Morphogenesis.**

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Morphogenesis involves the extensive reshaping of developing epithelia. In addition to whole tissue changes, there are also local cell rearrangements including cell division, extrusion, and intercalation. Precise regulation of forces within the tissue is essential during these dynamic processes. In this study, we propose that apical membrane remodeling by macropinocytosis plays an important role in governing these forces. Through observation of *Xenopus* embryo epidermis, we identified large macropinocytosis events covering approximately 25% of the total apical cell surface. These events internalize significant amounts of the plasma membrane thus reducing the cell's apical surface by approximately 10%. Macropinocytosis events were triggered by a decrease in membrane tension or inhibition of the membrane tension receptor Piezo1. Using inference models, we found that membrane internalization by macropinocytosis led to a local increase in junctional tension. Inhibiting macropinocytosis in the embryo resulted in a disorganized epithelium, ultimately leading to embryo death. This suggests that macropinocytosis can serve as a tension regulator, it maintains proper tension distribution during epithelial formation by locally increasing tension in response to tension decrease. Additionally, we observed that macropinocytosis occurs preferentially around multiciliated cells (MCCs), a cell type that extrudes from the epithelium during development in a tissue crowding-dependent manner. Inhibition of macropinocytosis accelerated the extrusion of MCCs, suggesting that apical area size reduction by macropinocytosis locally regulates crowding around MCCs, ensuring precise timing of their extrusion during development. In conclusion, our study highlights a novel role for macropinocytosis in membrane remodeling, proposing that it plays a central role in the organization of the apical surface in developing epithelial tissues. The massive internalization of apical membrane leads to a dual effect, increasing membrane tension while reducing the cell's apical area. This local regulation of tension and crowding allows macropinocytosis to regulate force distribution at the tissue-scale during epithelial morphogenesis. The proper force distribution during epithelial morphogenesis in turn ensures accurate tissue shaping and functional organ development.

SG234

**Tissue structure and mechanics vary along the apical-basal axis of the *Drosophila* germband epithelium.**

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Epithelial tissues undergo extensive structural changes as they remodel during morphogenesis through changes in cell shape and movement. Precise quantification of cell morphologies and rearrangements has uncovered a link between cell shapes and tissue mechanics, revealing that the mechanical properties of epithelial tissues are regulated in space and time to promote tissue flow and remodeling. In the context of the *Drosophila* germband epithelium, much of this work has inferred tissue mechanical behavior from cell shapes at the apical side of the tissue at the level of adherens junctions. However, germband cells are not exclusively prismatic in shape; rather, cell shape and cell neighbor relationships can differ widely along the apical-basal axis, and relatively little is known about how these variations

contribute to tissue mechanics and morphogenesis. Here, we use confocal fluorescence microscopy to image the *Drosophila* germband epithelium along its apical-basal axis as the tissue undergoes convergent extension. We segment cells in 3D, track them over time, and analyze cell shapes and cell rearrangements at different z-positions along the apical-basal axis. We find that the average cell shape index is highest at the apical side and decreases toward the basal side of the tissue, suggesting enhanced apical tissue fluidity, and this difference along the apical-basal axis increases during germband extension. As a readout for cell rearrangements, which involve intermediate states where 4+ cells meet at a vertex, we analyzed vertex coordination number in the tissue. We find that average vertex coordination is highest at the apical side of the tissue and decreases towards the basal side, suggesting differences in cell rearrangement events along the apical-basal axis, consistent with the variations in tissue fluidity. In conclusion, our findings suggest distinct mechanical environments along the apical-basal axis of the tissue, which are likely to influence the progression of cell rearrangements and tissue flow during convergent extension. The approaches and findings in this work are likely to be critical to understanding the mechanics of a wide variety of morphogenetic processes during development and disease.

SG235

**How the gut got its spots: Patterning and folding of intestinal villi by active mesenchymal dewetting.**

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Tissue folding generates structural motifs critical to organ function. In the intestine, bending of a flat epithelium into a periodic pattern of folds gives rise to villi, the numerous finger-like protrusions that are essential for nutrient absorption. However, the molecular and mechanical mechanisms driving the initiation and morphogenesis of villi remain a matter of debate. Here, we identify an active mechanical mechanism that simultaneously patterns and folds intestinal villi in mice. We find that PDGFRA+ subepithelial mesenchymal cells generate myosin II-dependent forces sufficient to generate patterned curvature in neighboring tissue interfaces. At the cell-level, this occurs through a process dependent upon matrix metalloproteinase-mediated tissue fluidization and altered cell-ECM adhesion. By combining computational models with in vivo experiments, we reveal these cellular features manifest at the tissue-level as differences in interfacial tensions that promote mesenchymal aggregation and interface bending through a process analogous to the active de-wetting of a thin liquid film.

SG236

**Fate, form, and the organizing role of the supracellular nexus.**

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During vertebrate organogenesis, functional units emerge at a scale much larger than their cellular and molecular constituents. Recently, we have explored how such long-range order robustly emerges during symmetry breaking in the embryonic skin, where follicle units emerge synchronously across the largest organ in the body. Our previous work refuted the prevailing chemical Turning conception for periodic pattern formation in the avian skin. Specifically, we showed localized gene expression does not precede morphological changes, thereby excluding a molecular blueprint mechanism. Instead, we found that contractile forces generated by dermal mesenchymal cells were sufficient to initiate follicle pattern. This

work provided experimental evidence for the regulatory nature of mesenchymal mechanics in a large developing vertebrate organ. In our current study, we articulate a precise mechanism for how contractile mesenchymal cells self-organize to generate higher order tissue pattern. This understanding was made possible through the development of a novel ex vivo platform where we reconstitute the process of skin symmetry breaking. We paired novel experimental measurements with concepts and theory from soft matter physics. Our findings give us mechanistic insight into how the cell biology of living systems creates emergent supracellular dynamics that possess a strong regulatory nature rivaling that of regulation more commonly studied at the genetic level.

## Subgroup: Cellular and Molecular Mechanisms of Transformation, Dormancy, and Drug Resistance in Cancer

SG237

**Epithelial tissue architecture acts as a barrier to cancer initiation.**

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Although 40% of people will be diagnosed with cancer in their lifetime, given the trillions of cells that each person possesses, tumour formation is relatively rare. Over 85% of all cancers are carcinoma, which form in epithelial tissues. A hallmark of carcinoma is loss of polarized epithelial tissue architecture, whereby epithelial structures, like ducts or alveoli, lose organization and become multilayered. Up to 80% of cells in a phenotypically normal adult epithelial tissue can possess oncogenic mutations. It remains poorly understood how tissue disorganization occurs during carcinoma initiation, and more intriguingly, what protective mechanisms epithelial tissues possess to prevent cancer initiation upon oncogene expression. To address this, we cultured 3D epithelial organoids expressing doxycycline-inducible oncogenes frequently expressed across diverse cancer types ($KRAS^{G12V}$, ERBB2, MYC). While all oncogenes tested caused an increase in proliferation, these oncogenes displayed variable dose-dependent abilities to disrupt tissue architecture. KRAS was the most efficient oncogene tested. High levels of KRAS expression caused lumen collapse, while low levels caused tissue multilayering. ERBB2 and MYC expression predominantly caused tissue multilayering. Oncogene expression induced prior to the establishment of apical-basal polarity and lumen formation increased the severity of tissue disorganization. Interestingly, live-cell imaging of KRAS-expressing cells revealed the formation and expansion of multiple actin-rich puncta, suggesting an impairment in the establishment of polarity and/or a single lumen. Conversely, ERBB2 and MYC expression, which showed less severe tissue disorganization, caused minimal polarity disruption or multi-lumen formation. This highlights the protective nature of polarized epithelial tissue architecture. This was further demonstrated through live cell imaging, where control cells dividing in an incorrect orientation rearranged to maintain a single layer. KRAS expression increased tissue multilayering through both randomization of division orientation and less efficient correction of the misplaced daughter cells. Our results reveal that oncogene expression alone is often insufficient to cause tumour formation. Loss of normal tissue architecture is necessary to create a permissive environment for tissue disorganization and tumour initiation.

SG238

**Unraveling alterations in the pre-neoplastic breast microenvironment of BRCA1/2 mutation carriers using spatial transcriptomics.**

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Breast cancer is the most common cancer in females, affecting one in every eight women and accounting for the majority of cancer-related deaths in women worldwide. Germline mutations in the *BRCA1* and *BRCA2* genes are significant risk factors for specific subtypes of breast cancer. *BRCA1* mutations are associated with basal-like breast cancers, whereas *BRCA2* mutations are associated with luminal-like disease. There are currently few chemoprevention strategies available for *BRCA1/2* mutation carriers, and irreversible prophylactic mastectomy is the primary option. Designing chemo-preventive strategies requires an in-depth understanding of the physiological processes underlying tumor initiation. Here, we employ spatial transcriptomics to investigate defects in mammary epithelial cell differentiation accompanied by distinct microenvironmental alterations in preneoplastic breast tissues from *BRCA1/2* mutation carriers and normal breast tissues from non-carrier controls. We uncovered spatially defined receptor-ligand interactions in these tissues for the investigation of autocrine and paracrine signaling. We discovered that β1-integrin-mediated autocrine signaling in *BRCA2*-deficient mammary epithelial cells differs from *BRCA1*-deficient mammary epithelial cells. In addition, we found that the epithelial-to-stromal paracrine signaling in the breast tissues of *BRCA1/2* mutation carriers is greater than in control tissues. More integrin-ligand pairs were differentially correlated in *BRCA1/2*-mutant breast tissues than non-carrier breast tissues with more integrin receptor-expressing stromal cells. These results reveal alterations in the communication between mammary epithelial cells and the microenvironment in *BRCA1* and *BRCA2* mutation carriers, laying the foundation for designing innovative breast cancer chemo-prevention strategies for high-risk patients.

SG239

**Disrupting Translesion Synthesis (TLS) activity enhances cell death and prevents tumor growth.**

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DNA-damaging chemotherapy agents such as cisplatin have been a first line approach in cancer treatment for decades; however, their long-term success is often reduced by intrinsic and acquired drug resistance. Although the mechanisms causing drug resistance are quite distinct, they are directly connected to mutagenic translesion synthesis (TLS). The TLS pathway promotes DNA damage tolerance by supporting both replication opposite to a lesion and inaccurate single strand gap filling. Inhibiting TLS reduces cisplatin resistance and secondary tumor formation. Therefore, targeting TLS is a promising strategy for improving chemotherapy. We recently discovered a small molecule, named c#3*, that directly binds to the central TLS protein - MAD2L2, and inhibits TLS activity. Using a mouse model, we demonstrated that combined treatment with cisplatin and c#3 significantly prevented tumor growth. Moreover, c#3 sensitized various cancer cell-lines to cisplatin treatment, co-treatment caused significant elevation in DNA damage levels, explaining the sensitization effect. Using diverse biochemical methods, we demonstrated that c#3 directly binds to MAD2L2 and prevents the formation of the TLS complex in

cancer cells. Importantly, the drug is not cytotoxic when administered alone. Therefore, c#3's activity underlines its potential as a lead compound for developing novel TLS inhibitors for improving chemotherapy treatment reducing the appearance of metastasis and alleviating patients side effect.
*C#3 is under PCT Patent Application No. PCT/IL2022/051173

SG240

**CAFs contractility stimulate cancer cell resistance to therapy.**

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Cancer-associated fibroblasts (CAFs) are a prevalent cell type within the tumor stroma, possessing both pro and anti-tumorigenic functions; they promote tumor growth, invasion, metastasis, and also influence the functions of other stromal cells within the tumor microenvironment. Remarkably, rather than being dispersed randomly within the tumor microenvironment, CAFs envelop tumor nests, forming a capsule. The question of whether this CAF capsule protects cancer cells from therapeutic treatments remains unanswered. To determine if the CAF capsule offers drug resistance, we developed a 3D co-culture model, integrating tumor spheroids into a collagen-I gel containing human rectal cancer CAFs at densities typical of rectal tumors. In this setting, CAFs aligned around the tumor spheres, mimicking their organization in vivo. The alignment of CAFs depended on their contractile properties and tumor growth. Subsequently, we exposed these co-cultures to 5-FU, a standard chemotherapy drug for colorectal cancers, and evaluated their resistance. The cancer cells, when encapsulated by CAFs, exhibited an increased survival compared to those cultured in collagen gels devoid of CAFs. Additionally, our investigations revealed that CAFs do not protect cancer cells through secreted molecules nor do they act as a metabolic shield for 5-FU. Rather, it is the physical existence of the CAF capsule that confers drug resistance. Considering the CAF capsule also inhibited tumor growth, we sought to discern if CAFs contribute directly to resistance or indirectly by suppressing proliferation, thereby hindering the effect of drugs targeting cell division. By imposing physical constraint on cancer spheroids using high molecular weight dextran, we could restrict their growth without affecting their resistance to cancer. CAFs are highly contractile cells that can organize into supracellular contractile units. By incorporating polyacrylamide beads of specified stiffness into the CAF-collagen I capsule, we observed their ability to exert compression. Likewise, by depleting myosin II in CAFs, we discovered that CAFs can compress cancer spheroids in a contractility-dependent manner. Finally, we investigated whether compression of cancer cells by CAFs stimulate cancer resistance. We found that depletion of Myosin II in CAFs partially restored tumor sensitivity to 5-FU in a manner independent of secreted molecules. In conclusion, our findings indicate that CAFs protect cancer cells from chemotherapy by exerting compression via actomyosin contractility. We are currently investigating the underlaying mechanism.

SG241

**Inhibition of autocrine HGF maturation overcomes cetuximab resistance in colorectal cancer.**

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Although amplifications and mutations in receptor tyrosine kinases (RTKs) act as bona fide oncogenes, in most cancers, RTKs maintain moderate expression and remain wild-type. Consequently, cognate ligands

play a critical role in many facets of tumorigenesis, including resistance to RTK targeted therapies. We previously showed that activation of wild-type RTKs MET and RON led to resistance to the EGFR-directed therapeutic antibody, cetuximab in colorectal cancer (CRC). Herein, we show that MET and RON ligands, HGF and HGFL, respectively, are synthesized as inactive precursors and require cleavage by proteases (HGFA, Matriptase, and Hepsin) to be biologically active. We generated and tested several HGF/HGFL protease inhibitors and showed that inhibition of ligand maturation can overcome both *de novo* and acquired modes of cetuximab resistance. Overexpression of human HGF in cetuximab-sensitive CRC cells, was necessary and sufficient to induce cetuximab resistance and loss of polarity, an EMT-like phenotype. Moreover, HGF-induced cetuximab resistance could be overcome by the downstream MET inhibitor, crizotinib, and by upstream protease inhibitors. In addition, an endogenous inhibitor of HGF/HGFL proteases, HAI-1, was downregulated in CRC and exogenous addition of recombinant HAI-1 overcame cetuximab resistance in CC-HGF cells. HAI-1 genomic methylation was also associated with poor prognosis in TCGA CRC datasets and HAI-1 expression correlated with cetuximab response in a panel of cancer cell lines. Thus, we describe an autocrine HAI-1/HGF/MET axis that is dependent on ligand delivery and processing by their endogenous proteases and inhibitors, identifying previously unrecognized vulnerabilities that can be exploited therapeutically.

SG242

**Profilin-1 is a novel regulator of immunosuppression and a target for immunotherapy in renal cancer.**

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Clear cell renal cell carcinoma (ccRCC), the most prevalent form of kidney cancer, is associated with a highly vascularized tumor microenvironment (TME) resulting from loss of Von-Hippel Lindau (VHL) gene. Overexpression of actin-binding protein profilin1 (Pfn1) correlates with advanced disease features and adverse clinical outcome in ccRCC patients. However, unlike many other types of human cancer, dysregulation of Pfn1 expression occurs predominantly in tumor-associated vascular endothelial cells (EC) rather than in tumor cells. In this study, we combined *in vivo* strategies involving EC-specific gene deletion and overexpression of Pfn1 to demonstrate a role of endothelial Pfn1 in accelerating tumor progression in orthotopic mouse models of RCC regardless of the VHL expression status of cancer cells. Endothelial Pfn1's action promotes tumor infiltration by macrophages while limiting the recruitment of effector CD8+ T cells in the TME, findings that are further corroborated by immune deconvolution of transcriptome data (bulk and single-cell RNAseq) and multiplexed quantitative immunohistochemistry analyses of clinical samples of human ccRCC. We established endothelial Pfn1's ability to promote expression of immunomodulatory cytokines/chemokines known to induce immunosuppressive phenotype in macrophages, and conversely, suppress production of pro-inflammatory T-cell recruiting cytokines, and further linked to negative regulation of interferon signaling. To explore Pfn1 as a potential target for immunotherapy, we generated novel Pfn1-peptide based dendritic cell (DC) vaccines that are capable of eliciting T cell response and retarding the growth of RCC tumors without induction of immune-related adverse events *in vivo*. In conclusion, this study demonstrates for the first time a direct causal relationship between vascular endothelial Pfn1 dysregulation and an immunosuppressive TME supportive of RCC disease progression with underlying mechanism of action identified. Our study also

provides evidence that Pfn1 may be targeted by DC-based vaccines in a safe and therapeutically effective manner in the RCC setting.

SG243

**Inflammasome signaling crosstalk during cutaneous skin cancer development.**

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The epidermal epithelial barrier is essential for animal survival. Epithelial barrier integrity relies on resident stem cells that ensure the rapid replacement of lost or damaged tissue. Despite their critical regenerative capacity, however, the proliferative and plastic nature of stem cells could pose tumorigenic consequences. Notably, the most common human tumors are skin stem cell-derived cutaneous carcinomas thought to primarily develop in response to DNA damage. Our previous work revealed that genotoxic damage to normal skin promotes epithelial stem cell proliferation and plasticity, characteristics associated with tumorigenesis. This response was driven by fibroblast-specific activation of an innate immune signaling module called the NLRP3 inflammasome, which promotes IL-1β secretion and subsequent binding to cell surface IL-1 receptors. Nevertheless, the roles of fibroblasts and inflammasomes in cutaneous skin cancers remain largely unexplored. In this study, we applied human skin specimens, transgenic mouse models, and a novel intravital imaging approach to reveal key differences in cancer-associated fibroblast (CAF) dynamics in skin tumors compared to normal tissue. Additionally, we discovered significant intratumoral heterogeneity whereby juxtaposed subpopulations of cutaneous skin cancer epithelia and CAFs exhibit inflammasome and IL-1 receptor signaling activation, suggesting a tumor-promoting feed-forward loop. We also noted changes in inflammasome component localization upon cell-cell contact disruption, signifying that inflammasome activity may be altered during epithelial-to-mesenchymal transitions and tumor invasion. Furthermore, we observed skin cancer-specific stem cell fate misspecification, which was also apparent in hyperproliferative pathological skin conditions like psoriasis but absent during the proliferative process of embryonic skin development. Coupled with previous work, these findings suggest activation of a pre-CAF-like state in damaged fibroblasts as well as the involvement of inflammasome/IL-1 feed-forward signaling in driving stem cell fate misspecification and cutaneous skin cancer development.

SG244

**Midkine inhibits dormancy of melanoma disseminated cancer cells.**

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Melanoma patients remain asymptomatic for an extended period of time after the successful removal of the primary tumors. However, many patients experience disease recurrence and it is believed that the reactivation of disseminated cancer cells (DCCs) is responsible for the formation of deadly metastases. DCCs remain in a non-proliferative state defined as dormancy resisting conventional therapy, but may also reactivate and give rise to relapse. We report here that inhibition of the secreted cytokine Midkine (MDK) induces dormancy in melanoma DCCs by upregulating the NR2F1-dependent dormancy program. Depletion of MDK increases the p-p38$^{high}$/p-ERK$^{low}$ ratio, which induces NR2F1 and its targets SOX9, p27

and RARβ. Activation of NR2F1 and its targets with a specific agonist prevented proliferation of $MDK^{HIGH}$ melanoma cell lines and patient-derived organoids when cultured in 3D Matrigel. This effect was synergized after MDK inhibition. Thus, the combination of an NR2F1 agonist and MDK inhibitor may be a potential therapeutic alternative to induce an NR2F1-dependent dormancy program that may extend the dormancy phase of melanoma DCCs, thus preventing metastasis formation.

SG245

**A mechanistic model of cancer cells micronuclei collapse.**

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Chromosome-containing micronuclei are a hallmark of aggressive cancer cells. Micronuclei frequently undergo irreversible collapse during interphase exposing their enclosed chromatin to the cytosol, catalyzing chromosomal rearrangements, heritable epigenetic abnormalities, and inflammatory signaling. Thus, micronuclear collapse initiates a cascade of processes resulting in distant metastasis, poor prognosis and therapy resistance. Yet the mechanisms that govern micronuclear integrity are poorly understood. For the first time we demonstrate that mitochondria-derived reactive oxygen species (ROS) drive micronuclear rupture by promoting a noncanonical function of the membrane repair ESCRT-III complex scaffolding protein, CHMP7. By using advanced super-resolution microscopy techniques, proteomics, genetic manipulations and mutagenesis and in vitro biochemistry assays, we demonstrate that ROS inhibit CHMP7 micronuclear export while reducing its interaction with ESCRT-III. Instead, ROS-induced cysteine oxidation promotes CHMP7 oligomerization and its binding to the inner nuclear membrane associated protein, LEMD2, thereby disrupting micronuclei integrity by physically pulling LEMD2 and the associated lamina from the membrane. Finally, we show that ROS-dependent CHMP7-LEMD2 interaction explains the prevalence of micronuclear rupture and inflammatory signaling under hypoxic conditions. Moreover, analyzing two different cohorts of patients samples, we observed that human tumors characterized by hypoxia have a significantly increased prevalence of ruptured micronuclei, providing a mechanistic link between tumor hypoxia and downstream processes that drive cancer progression.

SG246

**Macrophage-driven arginine depletion causes pancreatic tumors to become lipid auxotrophic.**

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Pancreatic ductal adenocarcinoma (PDAC) tumors are composed of diverse stromal cell types in addition to cancer epithelial cells. One way these cell populations affect tumor biology is by competing with cancer epithelial cells for nutrients. Our group recently found that macrophages—the most abundant immune cell in PDAC tumors—outcompete cancer cells for arginine in the tumor microenvironment. Understanding how PDAC cancer cells adapt to arginine-restricted conditions may lead to novel metabolic therapeutics and illuminate new roles that arginine deprivation plays in influencing cellular biology and metabolism. To interrogate how PDAC cells adapt to deprivation of exogenous arginine, our group designed a novel cell culture medium that faithfully recapitulates the nutrient availability in PDAC, termed tumor interstitial fluid medium (TIFM). By analyzing the transcriptional profile of PDAC cells grown in TIFM, I found that nutrients in PDAC perturb the activity of SREBP transcription factors. These transcription factors facilitate transcription of fatty acid and sterol synthesis machinery in response to lipid starvation. Surprisingly, I found that PDAC cells cultured in TIFM are unable to activate SREBP

transcription factors even under lipid deprivation, indicating that TIFM nutrients override the ability of SREBP to sense and maintain cellular lipid homeostasis. Systemic analysis of TIFM nutrients revealed that arginine deprivation is responsible for inhibiting SREBP activation and subsequent fatty acid synthesis in PDAC. Consistent with these observations, I found that arginine deprived PDAC cells rely on upregulated fatty acid uptake to grow. Thus, this work demonstrates that macrophage-mediated arginine starvation silences SREBP in PDAC cancer cells, revealing arginine depletion as a novel and critical regulator of SREBP activity. This work also reveals that PDAC cancer cells are lipid auxotrophic due to arginine starvation in the tumor microenvironment, presenting a novel therapeutic target for PDAC patients.

## Subgroup: Cryo-EM and Cryo-ET in the Discovery of Cellular Mechanisms

SG247

**Cortactin Stabilizes Actin Branches by Bridging Activated Arp2/3 to Its Nucleated Actin Filament.**

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Regulation of the assembly and turnover of branched actin filament networks nucleated by the Arp2/3 complex is essential during many cellular processes including cell migration and membrane trafficking. Branch formation requires activation of the Arp2/3 complex by Class 1 nucleation promoting factors (NPFs) such as WAVE and N-WASP. Cortactin is a Class 2 NPF that alone is a weak activator of the Arp2/3 complex, but synergizes with Class 1 NPFs to stimulate efficient Arp2/3 mediated actin branch formation. In addition, once formed cortactin stabilizes Arp2/3 mediated actin branches. Cortactin is therefore important in many cellular processes such as epithelial integrity and intracellular trafficking as well as in a range of pathologies including bacterial infection and cancer metastasis. However, despite its functional importance, the precise mode of action of cortactin and its mechanism of synergy with Class 1 NPFs remains unknown. To fill this knowledge gap, we have determined the structure of cortactin-stabilized Arp2/3 actin branches using cryo-electron microscopy (cryo-EM) and single particle reconstruction. We have uncovered that cortactin connects the activated Arp2/3 complex and the newly nucleated daughter actin filament. This unexpected finding has important implications for branch stability and turnover and is contrary to previous proposals that cortactin binds to the mother filament. We find that cortactin preferentially binds to activated Arp3, stabilising its nascent F-actin-like interface with the first actin subunit of the new filament. The central repeats of cortactin extend along successive daughter filament subunits thereby providing additional stabilization. Our data now resolve the long-standing mystery of how cortactin binds F-actin as well as how cortactin targets and stabilizes Arp2/3-mediated branches. Furthermore, our data provide a structural explanation for the previously enigmatic synergy of cortactin with Class 1 NPFs in stimulating actin branch formation and subsequent stabilization.

SG248

**Cryo-Electron Microscopy - A method to bridge scales in biological systems.**

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Cryo electron microscopy (cryo-EM), is an advanced structural biology technique that can bridge the resolution between light microscopy and observations at molecular level. Importantly, the recent technological developments for EM pushed the targeted resolution limit to atomic level, opening tremendous opportunities for the visualization and analysis of biological assemblies. My laboratory employs a wide spectrum of imaging techniques to understand molecular actions in various cellular contexts. This talk introduces our new pipeline of structural cell biology, we will discuss recent molecular findings on focal adhesion, the control hub at the cell periphery for decision-making processes and neuronal regeneration. Using *in situ* cellular cryo-electron tomography (cryo-ET) and single-particle cryo-EM (SPA), we aim to understand the molecular actions of the remodelling of cellular structures and machineries during critical events of neuronal morphogenesis and regeneration.

SG249

**The Tubulin Nano-Code: a protofilament-specific pattern of tubulin post-translational modifications regulates ciliary beating mechanics.**

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Control of ciliary beating is crucial for motility and signaling in eukaryotic cells and requires spatially restricted interactions between axonemal proteins and specific protofilaments within the ciliary microtubules. How these interactions are regulated remains poorly understood, but increasing evidence indicates that tubulin post-translational modifications (tPTMs) are required for proper ciliary motility. The Tubulin Code refers to the concept that tPTMs can modulate the function of individual microtubules in cells. Here we use a combination of immuno-cryo-electron tomography, expansion microscopy and mutant analysis to show that, in motile cilia, tubulin glycylation and polyglutamylation form mutually exclusive protofilament-specific nano-patterns at sub-microtubular scale. We show that these two nano-patterns are consistent with the distributions of axonemal dyneins and nexin-dynein regulatory complexes, respectively, and are required for their regulation during ciliary beating. Our discovery of a tubulin nano-code in cilia highlights the need of higher-resolution studies also in other cellular compartments to understand the molecular role of tPTMs.

SG250

**Integrated Model of the Vertebrate Augmin Complex.**

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Accurate segregation of chromosomes is required to maintain genome integrity during cell division. In eukaryotic cells, this feat is accomplished by the microtubule-based spindle. To build a spindle rapidly and with high fidelity, cells take advantage of branching microtubule nucleation, which rapidly amplifies microtubules during cell division. Branching microtubule nucleation relies on two large protein complexes, the microtubule nucleator γ-TuRC and its recruitment factor, the hetero-octameric augmin complex. However, lack of structure information about the augmin complex—an especially challenging

target for structural studies—has hindered our understanding of how branching occurs. Here, we report the structure of the vertebrate augmin complex, synthesizing information gained from cryo-electron microscopy, advanced protein structural prediction, and the visualization of fused bulky tags via negative stain electron microscopy. This structure has revealed that the augmin complex contains a previously unidentified, yet highly conserved, microtubule binding site that we hypothesize plays a role in establishing branch angle. In addition, evolutionary analysis of divergent augmin complexes revealed that the structure of the complex is highly conserved across eukaryotes, supporting broad conservation of branching mechanism across eukaryotes. In summary, determining the structure of the augmin complex—the "missing link" in branching microtubule nucleation—provides insight towards the structural determinants of the branch site and, thus, the mechanism of branching microtubule nucleation itself.

SG251

**Studying protective barrier formation and the molecular architecture of epidermal cell layers in natively preserved skin using cryo-electron tomography.**

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The skin is the largest organ of the human body. It fulfils vital tasks, including protection against physical and chemical influences, thermoregulation, sensory functions and endocrine and exocrine processes. Skin is organized in three different layers, the epidermis, dermis and hypodermis, all three of which have a different anatomy and function. The epidermis itself is also composed of several layers divided from the inside to the outside into the stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and finally the stratum corneum. These layers illustrate the various stages of keratinocyte differentiation, which is the process by which the basal layer proliferates and produces new cells that differentiate towards the skin's surface. Between these different layers an insulating barrier composed of lipids sheets maintains the integrity and protective function of the skin, supported by a dense meshwork of keratin filaments. The precise molecular architecture of intracellular components within these different skin layers, and how for example the intermediate filament cytoskeleton contributes to cell shape and resilience is not understood. This is because the majority of studies on skin architecture/morphology used mainly histology and conventional electron microscopy methods. These methods compromise structure details, due to the use of chemical fixatives and metal contrasting agents, among other compounds. Here, we have used cryo-focused ion beam scanning electron microscopy (FIB-SEM) lift-out milling and cryo-electron tomography to obtain insights into the ultrastructural organization of different epidermal layers in natively preserved mouse skin tissue. Our cryo-electron tomograms provide the first high-resolution 3D insights into the in-situ architecture of the stratum corneum and stratum granulosum, revealing details such as the exocytosis of lipid lamellar bodies to form the protective barrier, or how keratin filaments form distinct architectural assemblies in the outermost layers of the skin. In summary, our structural data of skin layers provide a 4D snapshot of temporal (as defined by layer differentiation) and spatial organization of skin formation and extend the structural atlas of how the components in the different epidermal layers provide strength and protective function of the skin.

SG252

***In situ* cryo electron tomography enables localization-dependent structural studies: Finding Symmetric Nuclear Pore Complexes.**

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Nuclear pore complexes (NPCs) are located at fusion points of the inner and outer nuclear membrane and facilitate nucleocytoplasmic transport. Numerous copies of over 30 distinct protein species assemble to form a nuclear pore complex. The NPC's scaffold structure is symmetric across the nuclear envelope. However, distinct proteins involved in compartment-specific functions, such as mRNA export and genome organization, are exclusively associated with either the nuclear or cytoplasmic side. It remains unclear how such compositional asymmetry is established despite both sides exhibiting identical binding interfaces. Here, we propose that the cell uses the surrounding cellular milieu as a cue for the correct allocation of components specific to the two sides.

Our model would predict the existence of symmetric NPCs when exposed to the same environment on both sides. To test this hypothesis, we investigated NPC-like structures outside the nuclear envelope using fluorescence-targeted FIB milling, cryo-electron tomography (cryoET), subtomogram averaging, and template matching.

Investigating NPCs in nucleoplasmic membrane sheets in an *S. cerevisiae* mutant strain revealed the complete absence of cytoplasm-specific densities when both faces are exposed to the nucleoplasm. In *Drosophila*, NPCs in cytoplasmic membrane sheets natively exist in oocytes and early embryos. However, these stages are not amenable to standard cryoET sample preparation. We engineered a *Drosophila* line that exhibits cytoplasmic membrane sheets containing NPCs in other cell types by strengthening interactions between Nup358 monomers, a protein whose condensate formation is necessary for cytoplasmic NPC formation. CryoET investigation of these NPCs in isolated cells from modified *Drosophila* ovaries revealed cytoplasm-specific peripheral components on both sides.

We conclude that the NPC's asymmetry across the nuclear envelope is determined by the difference between the nuclear and cytoplasmic environment. For lower resolution investigations in a native system, refer to the abstract presented by B. Hampoelz.

The existence of different subpopulations dependent on the cellular milieu highlights the importance of *in situ* structural biology to discover context-dependent regulation of macromolecular complex composition.

SG253

**A helical fulcrum in eIF2B coordinates allosteric regulation of stress signaling.**

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The Integrated Stress Response (ISR) enables cells to survive a variety of acute stresses, but chronic activation of the ISR underlies age-related diseases. ISR signaling down-regulates translation and activates expression of stress-responsive factors that promote return to homeostasis and is initiated by inhibition of the decameric guanine nucleotide exchange factor eIF2B. Conformational and assembly transitions regulate eIF2B activity, but the allosteric mechanisms controlling these dynamic transitions and mediating the therapeutic effects of the small-molecule ISR inhibitor ISRIB are unknown. Using cryo-

EM and hydrogen deuterium exchange-mass spectrometry, we identified a central alpha-helix whose orientation allosterically coordinates eIF2B conformation and assembly. Biochemical and signaling assays show that this "Switch-Helix" controls eIF2B activity and signaling in cells. In sum, the Switch-Helix acts as a fulcrum of eIF2B conformational regulation and is a highly conserved actuator of ISR signal transduction. This work uncovers a novel allosteric mechanism and unlocks new therapeutic possibilities for ISR-linked diseases.

SG254
**Structural Analysis of Filament Assembly by Yeast Asparagine Synthetase.**
**S. J. Calise**, J. Molfino, J. M. Kollman; University of Washington, Seattle, WA.

In the budding yeast *S. cerevisiae*, nutrient starvation drives the assembly of over 20 different metabolic enzymes into micron-scale filaments. Cytoplasmic acidification is thought to be the trigger for assembly of these filaments, as has been shown with the pH-dependent polymerization of the CTP synthase Ura7. Asparagine synthetase is a critical enzyme for maintaining amino acid homeostasis, catalyzing the ATP-dependent conversion of aspartate and glutamine to asparagine and glutamate. The yeast paralogs Asn1 and Asn2 co-assemble into filaments in stationary phase cells, but Asn2 requires the presence of Asn1 for polymerization. Asn1 filaments also associate with Ura7 filaments, suggesting possible crosstalk between amino acid and nucleotide synthesis pathways. Previous studies identified mutations that either disrupt or promote Asn1 filament assembly in cells, but the function and structural basis of polymerization remain unclear. Here, we use electron microscopy (EM) to investigate mechanisms of *in vitro* filament assembly by purified Asn1. By negative stain EM, we found that Asn1 assembles into polymers that laterally associate into ordered filamentous bundles at pH 6. We then solved a cryo-EM structure of the Asn1 filament bound by glutamine and the reaction intermediate aspartyl-AMP, which provides insight into assembly of the substrate-bound filament. The polymer is composed of repeating dimers with C2 symmetry and contains two assembly interfaces. Neither interface resembles the human or *E. coli* crystallographic dimer interfaces, suggesting a unique mechanism of regulation for yeast Asn1. One interface contains a key salt bridge between His334 and Glu505 on opposing monomers, similar to an essential histidine at the assembly interface of Ura7. Thus, histidine protonation acting as a pH sensor may be a broad mechanism for pH-dependent filament assembly. We are currently designing new mutations to disrupt or promote *in vitro* Asn1 polymer formation for further structural and enzymatic analysis, with the future goal of determining the function of Asn1 filament assembly in living cells.

## Subgroup: Lipids in Organelle Form, Function, and Communication

SG255
**Membrane editing and proximity labeling reveals a dynamic network regulating phosphatidic acid metabolism.**
**R. Tei**[1], J. Baskin[2]; [1]Stanford University, Palo Alto, CA, [2]Cornell University, Ithaca, NY.

Cellular membranes contain numerous lipid species, which regulate diverse cellular processes via spatially defined lipid-protein interactome. One such lipid is phosphatidic acid, which is an important signaling molecule as well as a central intermediate in phospholipid biosynthesis. However, efforts to understand the biological functions of individual lipids have been stymied by a lack of approaches for controlled modulation of membrane composition in situ. Here, we present a strategy and application of editing phospholipids, the most abundant lipids in biological membranes, to generate phosphatidic acid

and other phospholipids in a spatiotemporally defined manner. Our membrane editor is based on a bacterial phospholipase D (PLD), which exchanges phospholipid head groups through hydrolysis or transphosphatidylation of phosphatidylcholine with water or exogenous alcohols. Exploiting activity-dependent directed enzyme evolution in mammalian cells, we have developed and structurally characterized a family of 'superPLDs' with up to a 100-fold enhancement in intracellular activity. Using this superPLD to rapidly produce phosphatidic acid on selective membranes, we have found that phosphatidic acid levels were subject to a strong homeostatic pressure in cells, likely enabled by rapid trafficking and metabolism of excess phosphatidic acid molecules to reroute them to other phospholipid biosynthesis pathways. To understand mechanisms underlying this homeostasis, we have applied superPLD to "feed" phosphatidic acid to individual organelle membranes and TurboID-based proximity labeling followed by shotgun proteomics to "fish" out proteins that are enriched on the same membranes. This Feeding-and-Fishing strategy has identified several known and potentially novel phosphatidic acid interactors, metabolizing proteins, and transporters that were both enriched on and depleted from phosphatidic acid-fed membranes. Because of the ability of our superPLD-based membrane editor to efficiently generate phosphatidic acid and other natural and unnatural phospholipids on membranes of interest, we envision its wide applications to identify lipid-protein networks involved in lipid metabolism, transport, and signaling.

SG256

**An inner nuclear membrane-sensing screen reveals binding preferences in amphipathic helices for the nuclear envelope.**

**S. Lee**, S. Bahmanyar; Yale University, New Haven, CT.

Emerging evidence suggests that the inner nuclear membrane has unique membrane properties driven by its lipid composition that determine its protein identity. We recently discovered that the resident nuclear envelope protein Sun2 contains a membrane sensing amphipathic helix that associates with the inner nuclear membrane and is regulated by lipid metabolism. Amphipathic helices are peripheral membrane binding regions with preferences for distinct membrane properties (e.g. degree of saturation or curvature) depending on their amino acid compositions. Whether other nuclear envelope-associated proteins contain membrane sensing amphipathic helices with a preference for the unique properties of the inner nuclear membrane is not known. Here, we performed computational data mining for peripheral membrane binding amphipathic helices in hundreds of known and previously uncharacterized nuclear envelope-associated proteins followed by live imaging-based screening. We identified organelle membrane-bound amphipathic helices from >30 nuclear envelope-associated proteins. Further assessment of selected amphipathic helices targeted to the nucleus revealed varied affinities in their association to the inner nuclear membrane. Comparison of the amino acid sequences of membrane targeted and non-targeted amphipathic helices revealed patterns in compositions correlated with their preferences for association to the inner nuclear membrane. Mutation in specific residues switched binding affinities in favor of inner nuclear membrane association demonstrating the potential for an 'inner nuclear membrane-sensing code.' Future work will assess how amino acids in these amphipathic helices recognize membrane lipid compositions at a molecular level to inform on the general biophysical properties of the nuclear envelope. In sum, screening of a wide range and diversity of amphipathic helices shows membrane binding preferences for known and uncharacterized nuclear envelope proteins and has the potential to reveal the biophysical parameters that define the inner nuclear membrane.

SG257

**Membranes in balance: An ERAD-independent role for derlins in regulating sphingolipid metabolism.**

**S. Neal**; School of Biological Sciences, University of California San Diego, La Jolla, CA.

Nearly one-third of nascent proteins are initially targeted to the endoplasmic reticulum (ER), where they are correctly folded and assembled before being delivered to their final cellular destinations. To prevent the accumulation of misfolded membrane proteins, ER-associated degradation (ERAD) removes these client proteins from the ER membrane to the cytosol in a process known as retrotranslocation. Our previous work demonstrated that rhomboid pseudoprotease Dfm1 is involved in the retrotranslocation of ubiquitinated membrane integral ERAD substrates. Herein, we found that Dfm1 associates with the SPOTS complex, which is composed of serine palmitoyltransferase (SPT) enzymes and accessory components that are critical for catalyzing the first rate-limiting step of the sphingolipid biosynthesis pathway. Furthermore, Dfm1 employs an ERAD-independent role for facilitating the ER export and endosome- and Golgi-associated degradation (EGAD) of Orm2, which is a major antagonist of SPT activity. Given that the accumulation of human Orm2 homologs, ORMDLs, is associated with various pathologies, our study serves as a molecular foothold for understanding how dysregulation of sphingolipid metabolism leads to various diseases.

SG258

**Regulation of cellular cholesterol distribution via non-vesicular lipid transport at ER-Golgi contact sites.**

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The distribution of cellular cholesterol between various organelles and membranes is precisely controlled, and its dysregulation is associated with a number of diseases, including neurodegenerative disorders. Regulated transport of cellular cholesterol is essential for maintaining its proper distribution, yet the underlying mechanisms remain incompletely understood. While the regulatory network that controls cholesterol production and uptake is located in the endoplasmic reticulum (ER), cholesterol is more enriched in other cellular compartments with highest enrichment in the plasma membrane (PM). Therefore, the ER needs to sense the levels of cholesterol in various other cellular membranes and adjusts its production and uptake. We previously showed that the evolutionarily conserved and ER-anchored lipid transfer proteins (LTPs), namely the GRAMD1s/Asters (GRAMD1a/1b/1c), contribute to this process by transporting excessive cholesterol from the PM to the ER. Here, we show that a set of two other evolutionarily conserved LTPs, namely ORP9 and OSBP, functionally cooperate with GRAMD1s to control non-vesicular cholesterol transport at points of contact between the ER and the trans-Golgi network (TGN), thereby maintaining cellular cholesterol distribution. Using a novel cholesterol biosensor based on the cholesterol sensing element (GRAM domain) of GRAMD1b, we conducted a screen and identified ORP9 as a critical regulator of cholesterol distribution. ORP9 localizes to the TGN via interaction of its tandem α-helices with ORP10 and ORP11. ORP9 extracts PI4P from the TGN to prevent overaccumulation of PI4P in the Golgi and contributes to the suppression of OSBP-mediated PI4P-driven cholesterol transport from the ER to the Golgi. By contrast, the GRAMD1s transport excess cholesterol from the Golgi to the ER, thereby preventing the build-up of cholesterol in the Golgi. Cells lacking ORP9 show cholesterol accumulation at the Golgi, which is further enhanced by additional depletion of GRAMD1s with major accumulation of cholesterol in the PM. This is accompanied by chronic activation

of SREBP-2, the master transcription factor that senses the levels of cholesterol in the ER and regulates cholesterol production and uptake. Our findings reveal the critical importance of regulated lipid transport at ER-Golgi contacts mediated by ORP9, OSBP, and GRAMD1s for maintaining cellular cholesterol distribution and homeostasis.

SG259

**Asymmetric distribution of phospholipids and cholesterol results in unique plasma membrane properties.**

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The plasma membrane is the interface between a cell and its environment and is therefore responsible for a myriad of parallel processing tasks that must be tightly regulated to avoid aberrant signaling. To achieve this functional complexity, mammalian cells produce hundreds of lipid species, nearly all of which are asymmetrically distributed between the two membrane leaflets. Using quantitative lipidomics we determined the asymmetric distribution of all phospholipids in human erythrocyte plasma membranes. In addition to defining the asymmetric lipidomes of the two PM leaflets, we discovered that the cytoplasmic leaflet contains 50% more phospholipids than the exoplasmic leaflet. This finding invalidates the long-standing assumption that the two leaflets of a lipid bilayer should contain equal numbers of phospholipids. We show that this imbalance of phospholipids in the plasma membrane is enabled by the large abundance of cholesterol (~40%) in the plasma membrane, which can rapidly flip between leaflets to buffer stresses. Through computational and experimental approaches, we find that the combination of the preference of cholesterol for the more saturated exoplasmic lipids and the overabundance of lipids in the cytoplasmic leaflet yields a dramatic enrichment of cholesterol in the exoplasmic leaflet. Furthermore, we show that this asymmetric distribution of phospholipids and cholesterol results in unique plasma membrane properties, namely low permeability, fast cholesterol diffusion, and more cytoplasmic hydrophobic defects which enables lipidated protein interactions with the cytoplasmic leaflet. Our observations of these previously overlooked aspects of membrane asymmetry represents an evolution of existing models of plasma membrane structure and physiology.

SG260

**Endocytic machinery are primed at neuronal synapses through enrichment of PI4P and PS at endocytic zones.**

**B. Goulden**, Y. Imoto; Johns Hopkins, Baltimore, MD.

Ultrafast endocytosis can retrieve membrane and synaptic vesicle protein within 50 ms after vesicle fusion. This recently discovered form of endocytosis utilizes the splice variant of dynamin 1 (Dyn1xA) and a BAR domain containing protein Syndapin 1. Both Dyn1xA and Syndapin 1 are primed at endocytic zones in synapses to accelerate the kinetics needed for ultrafast endocytosis. However, it is currently unclear how these proteins are targeted to the distinct membrane regions where ultrafast endocytosis occurs. Here we show that plasma membrane lipids, phosphatidylinositol-4-phosphate (PI4P) and phosphatidylserine (PS), are enriched at endocytic zones and mediate recruitment of Dyn1xA and Syndapin 1. Using genetically encoded lipid biosensors and super-resolution imaging with STED microscopy, we analyzed nano-scale organization of membrane lipids. We demonstrate that PI4P and PS are colocalized with Dyn1xA at endocytic zones, which is devoid of other major membrane signaling lipids. To maintain the endocytic zones PI4P abundance, we have shown that Synaptojanin1 localizes at

endocytic zones and dephosphorylates the 5-phosphate of another membrane phosphoinositide PI(4,5)P2 to produce PI4P. The dephosphorylation of PI(4,5)P2 to PI4P is essential for generating the curvature needed for endocytosis. In addition, we have shown that PS can localize membrane bending and fission proteins such as Syndapin 1 using STED imaging and lipid binding mutants of Syndapin 1. STED imaging and time-resolved electron microscopy experiments demonstrate that Dyn1xA does not require its lipid-protein binding domain for localization and function during endocytosis. Instead, Dyn1xA localization at endocytic zones depends on binding to Syndapin 1 which recognizes PS through its membrane binding BAR domain. Together, these results indicate a unique lipid nano domain is formed at the endocytic zone and its formation is essential for the accumulation of endocytic proteins, allowing for the accelerated kinetics that are needed for synaptic endocytosis.

SG261

**Understanding the role of cellular lipid homeostasis in lysosomal membrane repair processes.**

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Lysosomal dysfunction is linked to a growing number of diseases and age-related neurodegenerative disorders. Increased lysosomal membrane permeabilization is a common characteristic of lysosomal diseases, including Niemann-Pick C (NPC) disorder. NPC1-KO cells have increased lysosomal cholesterol levels and defects in lysosomal proteolytic capacity. Since lysosomes harbor degradative enzymes, lysosomal lesions are rapidly resolved by the ESCRT machinery. Recently, ER-lysosome lipid-transport machinery has also been described in mediating lysosomal membrane repair. How ESCRT-dependent membrane repair pathways and cellular lipid transport and homeostasis pathways are interrelated, however, is still poorly characterized. To identify novel targets involved in lysosome repair, we conducted a genome-wide CRISPRi screen in cells lacking NPC1 (compared to control cells) treated with the lysosomotropic agent, LLOMe, since NPC1-KO cells are more sensitive to LLOMe treatment. We built a bioinformatic pipeline to combine our CRISPRi screen data with CRISPR co-essentiality datasets and protein-protein interaction datasets to pinpoint previously under-characterized gene hits from our screen to known biological pathways. We identified a cluster containing cellular sterol regulatory genes, including hits from our screen such as SCAP and MYLIP, and other lesser characterized proteins. We found that down-regulating these hits, in the background of lysosomal cholesterol load (via NPC1-KO), is associated with decreased growth rate and increased sensitivity to LLOMe treatment. Furthermore, we observed dysregulated cellular cholesterol localization upon knockdown of these hits in both control and NPC1-KO backgrounds and decreased recruitment of the ESCRT complex to the lysosomal membrane upon LLOMe challenge specifically in NPC1-KO cells, suggesting that this pathway may regulate lysosomal lipid compositions in a way that make these membranes less conducive to damage repair. Altogether, by leveraging cellular models of lysosomal lipid storage disorder in combination with a genome-wide CRISPRi screen, we found novel putative regulators of lysosomal lipid homeostasis that may be implicated in membrane repair processes.

SG262

**Mitochondria on a knife's edge: cristae formation is a mechanical buckling event controlled by the inner membrane lipidome.**

**K. Venkatraman**, C. T. Lee, P. Rangamani, I. Budin; University of California, San Diego, La Jolla, CA.

Cristae are high curvature structures crucial for ATP synthesis that appear as inward folds of the inner mitochondrial membrane (IMM). While cristae-shaping proteins have been defined, analogous mechanisms for lipids have yet to be elucidated. Here we combine experimental lipidome dissection with multi-scale modeling to investigate how lipid interactions govern IMM morphology and ATP generation. When tuning phospholipid (PL) saturation in engineered yeast strains, we observed a surprising breakpoint in cristae structure and respiratory capacity driven by loss of ATP synthase dimerization at cristae ridges. This breakpoint shifted the IMM from a high curvature state to flat, onion-like morphologies. At intermediate levels of PL saturation, induced either genetically or by microaerobic growth, we found that spontaneous curvature provided by negatively curved lipids such as cardiolipin (CL), were required for cristae assembly independently of ATP synthase dimerization. We integrated our results into a continuum model which synergized the contributions of lipid and protein-derived curvatures in forming cristae membranes. The model highlights a snapthrough instability, which could drive IMM collapse upon small changes in lipid composition. We also observed a suprising role for CL remodelling in shaping cristae structure under microaerobic, but not aerobic growth conditions. These results demonstrate how the formation of highly curved cristae membranes is dependent on the mechanical contributions of multiple lipid species, which then dictate mitochondrial function.

SG263

**Peroxisomes contact with lipid droplets to promote their growth.**

**R. Hua**, J. Togo, J. Phillips, M. S. Choe, H. Sung, P. K. Kim; SickKids, Toronto, ON, CANADA.

Lipid droplets (LDs), the neural lipid storage organelles, play a central role in the maintenance of energy homeostasis of the cell. It responds to cellular energy needs by storing (i.e., lipogenesis) or releasing (i.e., lipolysis) lipids. Originating from the endoplasmic reticulum (ER), LDs can interact with most other cellular organelles via membrane contact sites, including peroxisomes, an organelle that is also involved in lipid homeostasis. LD-peroxisome contact formation has been demonstrated to facilitate fatty acid transfer from LD to peroxisomes during lipolysis. However, whether peroxisomes also play a role in LD biogenesis is unclear. Here, we report a novel membrane contact site between LD and peroxisomes during LD biogenesis, which is mediated by the peroxisome-ER contact site protein ACBD5. First, the depletion of peroxisomes delays LD growth in multiple cell types, including the 3T3-L1 preadipocytes, implying an important role of peroxisomes in LD biogenesis. Second, peroxisomes form contacts with LDs during LD growth, which is dependent on ACBD5. In line with this, LD growth is delayed in the ACBD5-deficient cells. Furthermore, preadipocytes isolated from our preadipocyte-specific peroxisome knockout (PDGFR-α-Cre;Pex16$^{fl/fl}$) mice exhibit defects in adipocyte differentiation in the *in vitro* differentiation assay. Collectively, our study supports the role of peroxisomes in LD biogenesis and adipocyte development.

SG264

**Metabolic re-wiring in a 'fat-less' *Drosophila* reveals novel triglyceride:carbohydrate crosstalk in growth and development.**

**R. Ugrankar-Banerjee**, S. Tran, W. M. Henne; Cell Biology, UT Southwestern Medical Center, Dallas, TX.

Obesity and lipodystrophy lie on opposite ends of the adiposity spectrum, yet both conditions predispose individuals to dyslipidemia, cardiovascular disease, and type 2 diabetes (T2D). Similarly, dysregulation in energy storage as either fat (triglyceride, TG) or carbohydrates (glycogen), contributes to metabolic diseases. A major knowledge gap is how animals sense and balance TG and glycogen storage, and how metabolic pathways communicate to ensure organismal homeostasis. The high evolutionary conservation between *Drosophila* and humans, and the ability to generate obese and lean flies by targeting conserved pathways, makes *Drosophila* a highly enabling model to interrogate metabolic dysregulation. The central metabolic organ in *Drosophila* is the fat body (FB), analogous to the mammalian adipose tissue and liver, and the primary TG depot. Here, we find that the bulk of FB TG is maintained by the key enzyme fatty acid synthase 1 (FASN1), which catalyzes the *de novo* synthesis of fatty acids from malonyl-CoA. Notably, fat tissue in FB-specific $FASN1^{RNAi}$ larvae is fully developed with normal-sized adipocytes but has ~90% reduced TG stores. Strikingly, we find that FASN1-deficient larvae are healthy and complete their developmental cycle despite lacking the lipid biomass critical for metamorphosis. This is achieved through metabolic re-wiring that drastically elevates FB glycogen storage (~20 fold) and sugar utilization. Consistent with this, metabolic profiling uncovers Glycogen Synthase (GlyS) and glycogen catabolic enzyme Target-of-brain-insulin (Tobi) as uniquely required proteins in FASN1-deficient *Drosophila*. FASN1-deficient animals also mobilize excess glycogen during metamorphosis compared to controls, underscoring increased carbohydrate utilization in the absence of TG. $FASN1^{RNAi}$ adults are viable but lack detectable TG stores, and females exhibit potent infertility due to defects in ovarian differentiation and oocyte development. We also find that FASN1-depleted flies are sensitive to nutrient starvation, and exhibit shortened median lifespan. Collectively, we uncover metabolic fat:carbohydrate remodeling in a 'fat-less' *Drosophila* model that actively adapt their developmental program in the absence of TG by re-wiring carbohydrate metabolism and storage to complete development.

## Subgroup: Subcellular Localization of RNA and Translation

SG265

**Imaging translation of single normal and aberrant mRNAs in live cells.**

**B. Wu**, N. Livingston, M. J. Latallo, S. Wang, D. Dong, S. Sun; Johns Hopkins School of Medicine, Baltimore, MD.

Translation is the fundamental biological process that converts the genetic information from mRNAs into functional proteins. Dysregulation of translation leads to cancers and neurodegenerative diseases. We have previously developed Single Molecule Imaging of Nascent Peptides (SINAPS) to assess translation of individual mRNAs in live cells. This approach allows direct measurement of initiation and elongation dynamics in cells. We have applied the method to study canonical as well as aberrant translation process, which reveals rich spatial and temporal dynamics of translation process. We developed a new strategy to track single mRNAs and their translation dynamics for hours. We observe that translation is not a constitutive process but instead cycles between active and inactive states or "bursts". We used genetic and pharmacological interventions to perturb various aspects of translation

bursting, and built a unified model of translation output. Furthermore, we applied the method to study the spatiotemporal dynamics of pathological repeat RNA. Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are currently incurable diseases caused by motor neuron degeneration. A hexanucleotide repeat expansion in the first intron of *C9ORF72* gene is the most common genetic cause of both ALS and FTD. RNA containing repeats can be exported from nucleus and translated via repeat associated non-AUG (RAN) translation to generate different toxic poly dipeptide repeats (DPR). However, it remains unclear how the intron-localized repeats are exported and translated in the cytoplasm, and how different frames are differentially expressed. We applied SINAPS and single RNA tracking to study translation of repeat containing RNAs in live cells. Our study revealed an uncharacterized disease-causing RNA species mediated by repeat expansion and demonstrates the importance of RNA spatial localization, and the translation heterogeneity to understand the disease etiology and treatment.

SG266

**Subcellular localization and function of mRNA in intact tissue.**

**D. Mason**, S. Mili; Laboratory of Cellular and Molecular Biology, National Cancer Institute, NIH, Bethesda, MD.

Compartmentalization of mRNAs and proteins is necessary for many cellular functions. Subcellular localization of specific proteins has systemic effects on tissue homeostasis. Whether mRNA localization is also important for tissue homeostasis is unknown. To address this question, we first visualized the distribution of several localized mRNAs in normal tissues. We found that mRNAs that localize to mesenchymal cell protrusions *in vitro* are abundantly expressed and basally localized in the epithelium of mouse kidney, intestine, skin, and tongue *in vivo*. This effect was most pronounced in the basal keratinocytes of mouse tongue and skin, which we confirmed to be evolutionary conserved in primate tongue and human skin. Interestingly, we found that mRNAs localize to cytoplasmic structures that resemble cellular protrusions which interdigitate with the basement membrane. We also found significant size variability in mRNA foci which suggests mRNA clustering *in vivo*. Large foci were commonly found at keratinocyte attachment points to vertical fibers extending from the dermis through the basement membrane. Basal mRNA localization did not correlate with keratinocyte proliferation status but did depend on a combination of cell differentiation or detachment from the basement membrane. Differentiating basal cells detaching from the basement membrane had prominently localized mRNAs, but once they fully integrated into the spinous layer of the skin or tongue, mRNA localization and abundance were greatly reduced. Together, this suggests cell-basement membrane connections play an important role in regulating mRNA localization. To determine the functional importance of localized mRNAs we injected antisense oligonucleotides (ASOs) targeting 3'UTR elements important for basal localization. ASOs specifically reduced basal mRNA localization 2 days after injection. This short-term treatment reduced cell proliferation, albeit moderately. Together these results suggest that subcellular mRNA localization can influence cell physiology in intact tongue epithelium and provide an important step towards understanding the roles of localized mRNAs in intact tissues.

SG267

**Imaging the lifetime of a single mRNA molecule.**

**W. Li**, L. Degroux, R. Singer; Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY.

Imaging and tracking individual mRNA elucidates gene expression at single-molecule resolution. However, the duration of molecular tracking is limited to a few seconds because tracking requires a high imaging acquisition frequency, which, in turn, leads to photobleaching. This limitation prevents us from comprehensively imaging longer events, such as the life cycle of an mRNA (tens of minutes in yeast). Here, we solved this challenge by devising a sparse labeling method that controls the number of mRNA molecules being fluorescent. In budding yeast, we methodically fine-tuned the system so that certain cells contained only one fluorescent mRNA molecule, which could be unambiguously tracked even in regions with a high mRNA concentration. Using this method, we achieved long-term single-particle tracking of mRNA and prolonged the tracking duration from 4 seconds to 20 minutes—a 300-fold increase. This advancement enables us to capture molecular events at time scales ranging from milliseconds to tens of minutes, holding great promise in revealing profound insights into the intricate processes of gene regulation.

SG268

**Nucleolar dynamics are determined by the ordered assembly of the ribosome.**

**J. Sheu-Gruttadauria**[1], X. Yan[2], N. Stuurman[1], S. N. Floor[1], R. D. Vale[3]; [1]University of California, San Francisco, San Francisco, CA, [2]Stanford University, Palo Alto, CA, [3]Janelia Research Campus, Ashburn, VA.

The nucleolus is the most prominent nuclear body in the cell and is responsible for organizing the fundamental process of ribosome biogenesis. Recent work suggests that the nucleolus forms through biomolecular condensation, giving rise to uniquely dynamic material properties. These dynamic features are thought to be essential for function, as defects in nucleolar assembly and morphology are hallmarks of cancers, neurodegeneration, and aging. However, the principles that govern dynamics within the nucleolus and their impact on ribosome biogenesis remain poorly defined. Here, we describe a platform for systematically identifying factors that regulate macromolecular dynamics within the nucleolus using fully-automated, high-throughput fluorescence recovery after photobleaching (FRAP), which we call HT-FRAP. Using this platform, we screened 400 RNA metabolism genes for their impact on dynamics of the nucleolus. We find that nucleolar dynamics are sensitive to stalls in discrete ribosome biogenesis steps and define nucleolar biophysical signatures for stages of ribosome assembly. Further characterizing nucleolar composition of these signatures reveals that nucleolar dynamics are determined by the relative levels of preribosome assembly intermediates. Accumulation of early precursors leads to rigid phenotypes, while accumulation of late intermediates leads to increased dynamics. Changes in dynamics correlate with the thermodynamic stability of interactions between preribosome intermediates and nucleolar scaffolds. These results suggest that nucleolar dynamics are tuned by the ordered assembly of the ribosome. In addition, our screening approach identifies novel connections between ribosome biogenesis and mRNA splicing and export, uncovering potential mechanisms for integration across RNA processing pathways. Together, this work defines mechanistic ties between the biophysical features of the nucleolus and its molecular function, while establishing a toolbox for understanding how molecular dynamics impact function across other biomolecular condensates.

SG269

**Multifunctionality in a tissue-sized single cell in the placenta.**

**M. Keenen**, V. Farmer, A. Gladfelter; Duke University, Durham, NC.

During human pregnancy the interface between the maternal and fetal vasculature is the syncytiotrophoblast (STB): a tissue-sized single cell with billions of nuclei. This giant cell is formed via cell-cell fusion with a proliferative population of cytotrophoblast (CTB) cells and is responsible for nutrient and oxygen transport, performs essential metabolic functions, and is the primary producer of hormones during pregnancy. How does a single cell accomplish these diverse cellular tasks? in fact, the STB appears to spatially organize its cytoplasm to accomplish distinct functions locally. For instance, hormones are produced in cytoplasmic regions that exhibit high rates of translation and secretion. In contrast, areas adjacent to the fetal vasculature have a less dense cytosol to facilitate diffusion and express vasculature growth factors. How are these specialized functional zones constructed in a continuous cytoplasm? **We hypothesize STB nuclei establish these territories by expressing different sets of genes that act locally. To** test the hypothesis that STB nuclei have different gene expression programs, we apply single nucleus RNA sequencing analysis of primary placenta tissue. Fluorescence in situ hybridization (FISH) was then used to spatially map nuclear subtypes onto primary placenta tissue and organoids derived from the same placenta tissue. We find the STB nuclei cluster into multiple distinct gene expression subtypes. One is an undifferentiated population that mirrors a transition state in gene expression between CTB cells and the STB. We identified two nuclear types that specialize in hormone and metabolic functions while one is enriched in angiogenic signaling molecules. RNA FISH demonstrates these nuclear subtypes are spatially distributed in the STB cytoplasm in primary tissue and organoids. Our work demonstrates that multiple nuclear subtypes exist in the STB of term placenta. This suggests local variation in cytoplasmic function could be accomplished via transcriptional specialization of STB nuclei. Future work visualizing target genes in organoids through space and time will enable testing if transcriptional specialization is necessary and sufficient to achieve functional cytoplasmic zones in the STB.

SG270

**Co-translational sorting enables a single mRNA to generate polysomes with different localizations and fates.**

**E. Bertrand**, S. Salloum, X. Pichon; CNRS and Montpellier University, Montpellier, FRANCE.

Many proteins participate in biochemically distinct complexes that have unrelated functions. A well-known example is beta-catenin, which is a multi-functional protein playing essential roles in tissue homeostasis and cancer. It either bridges E-cadherin to the cytoskeleton or activates transcription in response to Wnt signaling. Plasma membrane beta-catenin is stable whereas in absence of Wnt signals, the cytoplasmic one is degraded by the destruction complex, composed of APC, Axin and GSK3. Here, we show that this complex associates with beta-catenin polysomes via the nascent protein chains. This occurs on single beta-catenin mRNAs and on RNA foci termed translation factories. APC promotes these co-translational interactions and they are important to facilitate beta-catenin binding by the destruction complex. Remarkably, E-cadherin also binds beta-catenin co-translationally, and a single beta-catenin mRNA localizes with either APC in the cytosol or E-cadherin at the plasma membrane. Thus, co-translational interactions sort beta-catenin mRNAs into distinct polysomes that spatially segregate in the cell and synthesize proteins with different functions. Genome-wide data suggest that other mRNAs use

co-translational polysome sorting, providing a simple mechanism to regulate the fates of multi-functional proteins.

SG271

**Drosophila germ granules activate the translation of localized mRNA.**

**R. Chen**[1], W. Stainier[2], J. Dufourt[3], M. Lagha[3], R. Lehmann[1]; [1]Whitehead Institute, Cambridge, MA, [2]The Francis Crick Institute, London, UNITED KINGDOM, [3]University of Montpellier, Montpellier, FRANCE.

Biomolecular condensates organize various biochemical processes at the subcellular level. Ribonucleoprotein (RNP) granules, a prevalent type of condensate that governs post-transcriptional regulation, are generally described as storage depots for translationally repressed mRNA. Whether RNP granules can also activate the translation of localized mRNA remains unclear. Here, using a single-molecule imaging method, SunTag, we demonstrate that Drosophila germ granules, the RNP granules for germ cell formation, are the sites of active translation for localized *nanos* mRNA while unlocalized *nanos* mRNA is translationally repressed. We find that *nanos* translation preferentially takes place on the germ granule surface, with the 3'UTR embedded inside granules. Through quantitative imaging, we show that germ granules activate *nanos* translation mainly by antagonizing the translational repressor Smaug, instead of boosting the rate of steady-state translational initiation and elongation. Mechanistically, germ granule protein Oskar allows *nanos* translation by controlling the selective localization of Smaug into germ granules. Together, our findings demonstrate that RNP granules can function as compartments for translational activation of localized mRNA by counteracting translational repressors to achieve spatial control of protein synthesis.

SG272

**Mitochondrial protein heterogeneity stems from the stochastic nature of co-translational protein targeting in cell senescence.**

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A decline in mitochondrial function is a hallmark of aging and many neurodegenerative diseases. It has been proposed that changes in mitochondrial morphology, including fragmentation of the tubular mitochondrial network, can lead to mitochondrial dysfunction, yet the mechanism of this loss of function is unclear. Most proteins contained within mitochondria are nuclear-encoded and must be properly targeted to the mitochondria. Here we report that sustained mRNA localization and co-translational protein import leads to heterogeneous protein distribution across fragmented mitochondria. We find that age-induced mitochondrial fragmentation drives an exponential increase in protein expression noise across fragments. Using a translational kinetic and molecular diffusion model, we find that protein expression noise is explained by the nature of stochastic compartmentalization and that the co-translational protein import is the main contributor to the increased heterogeneity. We observed that cells reduce heterogeneity of protein distribution primary through mitochondrial fission-fusion reaction and not through the mitophagy pathway. Furthermore, we could reduce heterogeneous protein distribution by inhibiting co-translational protein targeting. This research lays the framework for

a better understanding of the detrimental impact of mitochondrial fragmentation on the physiology of cells in aging and disease.

SG273

**FMRP Granules Locally Control Mitochondrial Fission in Neurons.**

**A. Fenton**, R. Peng, C. Bond, S. Hugelier, M. Lakadamyali, Y. Chang, E. Holzbaur, T. Jongens; University of Pennsylvania, Philadelphia, PA.

Mitochondrial health and function are maintained in neurons by network remodeling and local translation, which allow for rapid responses to changing cellular demands. Yet, it is not understood how neurons orchestrate the timing and positioning of translation or how local translation is coupled with mitochondrial dynamics to maintain network integrity. The Fragile X Messenger Ribonucleoprotein Protein (FMRP) is a critical regulator of translation in neurons whose absence causes Fragile X Syndrome, a severe neurodevelopmental disorder. Loss of FMRP disrupts mitochondrial health in neurons, resulting in a fragmented network with impaired metabolic function. However, the mechanism by which FMRP supports mitochondrial homeostasis in neurons is not known. Here, we use DNA-PAINT super-resolution microscopy and live-cell confocal microscopy to demonstrate close contacts between FMRP granules and mitochondria in mammalian neurons. FMRP preferentially clusters at sub-organellar regions of mitochondria: The ends and the midzone. End contacts are dynamic and allow for long-distance co-transport of FMRP with mitochondria. Midzone-associated FMRP marks sites of mitochondrial fission, where the FMRP granule stays associated with the mitochondrial end following fission. Endosomes are known to associate with sites of mitochondrial fission and to deliver RNA granules to mitochondria in neurons. We demonstrate that endosomes contact FMRP granules and contribute to their positioning at mitochondrial ends and fission sites. Using a combination of live-imaging, RNA *in situ* hybridization, immunocytochemistry, and cryo-electron tomography, we demonstrate that mitochondria-associated FMRP granules are ribosome-rich sites of protein synthesis, which contain mRNAs for nuclear-encoded proteins that drive mitochondrial function and dynamics. Further, mitochondrial fission at FMRP granules is dependent on protein synthesis and facilitated by local translation of Mitochondrial Fission Factor within these granules. These findings reveal a role for FMRP in the control of mitochondrial fission and suggest that FMRP granules serve as platforms to selectively regulate the dynamics of individual mitochondria in neurons.

SG274

**Stress induces the localization of chaperone mRNAs to sustain neuronal protein homeostasis.**

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Maintaining protein homeostasis over the neuronal architecture (neuronal projections, connections, and soma) is fundamental for neuronal functionality. Protein misfolding and aggregation are associated with multiple neurodegenerative diseases. A logical therapeutic strategy, supported by considerable data from experimental models, is to boost chaperoning capacity by inducing heat shock proteins (HSPs). HSPs are molecular chaperones in charge of protein folding and homeostasis. Cells have the remarkable capacity to tailor the expression of HSPs to the extent of protein damage. The precise regulation of HSP expression is required to safeguard protein homeostasis and ensure cell functionality upon proteotoxic damage. Using single-molecule fluorescence microscopy techniques, we have discovered that beyond the activation of HSP transcription, neurons regulate the subcellular distribution and localized translation of HSP mRNAs in response to stress. mRNA localization to neuronal projections emerges as a

novel mechanism to regulate the local pool of HSPs and sustain proteostasis at neuronal processes and connections.

SG275

**Lysosome-coupled mRNA transport maintains axonal homeostasis.**

**S. Ghosh**[1], R. De Pace[1], V. H. Ryan[2], M. Sohn[3], M. Jarnik[1], P. Rezvan Sangsari[4], N. Y. Morgan[4], R. K. Dale[3], M. E. Ward[2], J. S. Bonifacino[1]; [1]Neurosciences and Cellular and Structural Division, NICHD, NIH, Bethesda, MD, [2]Neurogenetics Branch, NINDS, NIH, Bethesda, MD, [3]Bioinformatics and Scientific Programming Core, NICHD, NIH, Bethesda, MD, [4]Biomedical Engineering and Physical Science Shared Resource, NIBIB, NIH, Bethesda, MD.

Lysosomes were classically defined as cytoplasmic organelles that function to degrade biomacromolecules in the endomembrane system of eukaryotic cells. More recently, lysosomes were found to play multifaceted roles in nutrient sensing, regulation of gene expression, plasma membrane repair, immunity and cholesterol transport. Previous work showed that these functions are influenced by the positioning and motility of lysosomes within the cytoplasm. In particular, we discovered a lysosome-associated, hetero-octameric complex named BORC that sequentially recruits the small GTPase ARL8 and kinesin motors for anterograde movement along microtubules. Interference with this machinery causes redistribution of lysosomes towards the cell center in non-neuronal cells and depletion of lysosomes from the axon in neurons. Based on recent work showing that RNA granules hitchhike on lysosomes for transport into the axon, we examined the effect of knocking out BORC subunits on axonal mRNA transport in human iPSC-derived neurons grown in microfluidic devices. We observed that BORC KO caused a dramatic depletion of many mRNAs encoding components of mitochondria and ribosomes. The depleted axonal mRNAs were common with those involved in pathways of neurodegeneration in Parkinson's, Alzheimer's, Huntington's and prion diseases, as well as amyotrophic lateral sclerosis (ALS). A puromycin-proximity ligation assay (Puro-PLA) revealed decreased synthesis of mitochondrial and ribosomal proteins in the axon of BORC-KO neurons. Moreover, immunoblot analyses of BORC-KO axons showed reduced levels of components of the mitochondrial electron transport chain (ETC) and the mitochondrial contact site and cristae organizing system (MICOS). In addition, we observed that axonal mitochondria were smaller, had disorganized cristae and reduced membrane potential, and were targeted for mitophagy in BORC-KO axons. Finally, we found that BORC-KO axons developed swellings filled with mitochondria, autophagosomes and Tau aggregates, and eventually degenerated. These findings thus demonstrated a critical role of lysosome-coupled mRNA transport into the axon for the maintenance of mitochondrial homeostasis. Failure of this mechanism could explain the pathogenesis of neurodevelopmental disorders caused by mutations in BORC subunits, and, more generally, of neurodegenerative disorders characterized by defective lysosomal transport and mitochondrial function.

## Cell Biology of Neurodegeneration and Aging

SG276

**Intestinal Stem Cell Aging: Local and Systemic Effects.**

**H. Jasper**; Genentech Inc., South San Francisco, CA.

In aging and diseased tissues, regeneration andregenerative therapies are limited by stem cell dysfunction and unfavorabletissue environments. We study stem cells and tissue repair in barrier

epitheliaand the retina of *Drosophila* and miceto explore the causes and consequences of age-related regenerative dysfunction.These studies have led to the discovery of interventions targeting age-relatedinflammation, stem cell proliferation, stem cell metabolism, innate immuneresponses, and the commensal microbiota as strategies to enhance regenerationand extend lifespan. I will discuss these strategies and provide perspectivesfor the development of targeted interventions to improve tissue function in theelderly. We have recently discovered an impact of intestinalstem cell aging and epithelial dysfunction on olfactory perception. Infection-induced aversion against enteropathogensis a conserved sickness behavior that can promote host survival. *The* etiology of this behavior remainspoorly understood, but studies in *Drosophila*have linked olfactory and gustatory perception to avoidance behaviors againsttoxic microbes. Whether and how enteric infections directly influence sensoryperception to induce or modulate such behaviors remains unknown. We have shownthat enteropathogen infection in *Drosophila*can modulate olfaction through metabolic reprogramming of ensheathing glia (EG)of the antennal lobe (AL). Infection-induced Unpaired cytokine expression inthe intestine activates JAK/STAT signaling in EG, inducing the expression ofglial monocarboxylate transporters (MCTs) and the apolipoprotein *glial lazarillo* (*Glaz*), and thus impacting glia/neuron metabolic coupling in the AL.This modulates olfactory discrimination, promoting avoidance of bacteria-lacedfood and increasing animal survival. While transient in young animals,gut-induced metabolic reprogramming of EG becomes constitutive in old animalsdue to age-related intestinal inflammation, contributing to an age-relateddecline in olfactory discrimination. Our findings have identified adaptiveglial metabolic reprogramming by gut-derived cytokines as a mechanism causinglasting changes in a sensory system in the aging animal.

SG277

**Unlocking the Potential of TREM2: VG-3927 as a Novel Therapeutic for Alzheimer's Disease.**

**B. Dejanovic**; Vigil Neuroscience, Watertown, MA.

TREM2, a lipid-sensing receptor highly expressed bymicroglial sub-populations within neuropathological microenvironments, isimplicated as a key regulator of Alzheimer's disease (AD) risk due to theenrichment of loss-of-function variants in AD patients. We present preclinicaldata on VG-3927, the first small molecule TREM2 agonist in clinical development.VG-3927 exhibits highly potent and selective TREM2 agonism with favorablefunctional profiles and brain penetration. Mechanistically, VG-3927 induces aunique TREM2-DAP12 complex formation in microglia. Using a human iPSC-derivedneuron-microglia-astrocyte tri-culture model, we demonstrate that VG-3927promotes anti-inflammatory microglia activation and suppressesneurodegeneration-associated biomarkers. Furthermore, we utilized a humanizedTREM2 amyloidosis mouse model (hTREM2-5xFAD) to determine the impact of VG-3927on AD-associated neuropathological endpoints. Extended oral dosing of VG-3927reduced amyloid burden and levels of aggregated APOE, highlighting its impacton multiple AD risk factors. VG-3927 represents the first small molecule TREM2agonist potentially capable of favorably modulating human microglia activationand imparting a broadly neuroprotective profile across preclinical modelsystems. Ongoing clinical studies aim to further investigate its potential asan AD disease-modifying therapeutic.

SG278
**Blood Drivers of Neurodegeneration: From Mechanisms to Therapies.**
**K. Akassoglou**; Gladstone Institute.
No Abstract Submitted.

## Subgroup: Bioengineering 3D Human in Vitro Tissue Models for Therapeutic Discovery

SG280
**Utility of a 3D Engineered Skeletal Muscle Organoid System to Assess Exon Skipping, Dystrophin Protein Restoration and Functional Improvement in a Human DMD Cell Model.**
**N. L. Estrella**, S. L. Blake, M. Fredericks, A. M. Bonilla, C. M. Brennan, M. Streeter, A. N. Hicks, K. J. Kamer, M. Kheirabadi, M. Dhanabal, Z. Qian, M. Girgenrath; Entrada Therapeutics, Boston, MA.

Robust *in vitro* models of skeletal muscle tissue are critical to understanding disease mechanism, identifying novel therapeutic targets, and throughout the preclinical development process. However, culturing muscle cells is challenging as mature myotubes typically detach from their substrate within one week of initiating the differentiation regimen when cultured in a two-dimensional (2D) matrix. To circumvent these challenges, 3D-engineered skeletal muscle tissues (EMTs) which improve cytoarchitectural maturation and extend culture longevity *in vitro* are being developed, thereby enabling long term studies with clinically relevant models to support the drug discovery process. We have previously demonstrated efficacious exon skipping and dystrophin restoration in Duchenne muscular dystrophy (DMD) preclinical models using a novel ASO delivery system composed of Entrada's proprietary Endosomal Escape Vehicle (EEV$^{TM}$) construct conjugated to a phosphorodiamidate morpholino oligomer (PMO) in a 2D culture system. Here, we developed a 3D-engineered skeletal muscle organoid system using muscle cells derived from a DMD patient to assess the functional delivery of an EEV-PMO construct targeting human *DMD* to promote exon skipping, dystrophin protein restoration, and functional improvement.
Initial proof of concept studies in EMTs derived from a DMD patient cell line demonstrated robust exon skipping and dystrophin protein restoration following exposure to the EEV-PMO construct. Dystrophin protein was observed 7 days post-test article washout, detectable for at least 6-weeks after treatment, and localized correctly at the sarcolemma. Treated EMTs showed improvement in muscle contractile forces at baseline and resistance to fatigue when compared to the untreated group.
These results demonstrate the ability of the EEV platform to efficiently deliver exon skipping oligonucleotides to 3D-engineered skeletal muscle tissues in patient-relevant models of DMD. Further, the 3D organoid system allowed us to demonstrate EEV-PMO construct efficacy and long-term duration of effect, underscoring the utility of a 3D culture system for the drug development process.

SG281
**Using 3D engineered cardiac and skeletal muscle tissues for disease modeling and drug discovery.**
A. S. T. Smith[1], **D. L. Mack**[2], J. Dupont[3], S. Luttrell[4], P. Barrett[5], C. Mandrycky[6], N. Geisse[4], M. Regnier[6]; [1]Physiology and Biophysics, University of Washington, Seattle, WA, [2]Rehabilitation Medicine, Bioengineering, Biophysics, University of Washington, Seattle, WA, [3]Translational Research in Gene Therapies, University of Nantes, Nantes, FRANCE, [4]Curi Bio, Inc., Seattle, WA, [5]Rehabilitation Medicine, University of Washington, Seattle, WA, [6]Bioengineering, University of Washington, Seattle, WA.

Stem cell-derived engineered muscle tissues enable the characterization of the contractile properties of human cardiac and skeletal muscle from a developmental perspective. Tissues carrying disease-causing mutations can be subjected to repeated measures to track the initiation and progression of pathology and to determine the timing and extent of functional recovery after treatment. Our team created the Magetometric Analyzer for eNgineered Tissue ARRAY (MANTARRAY) platform for disease modeling and drug discovery. We modeled the cardiomyopathy and skeletal muscle defects of Duchenne muscular dystrophy (DMD) to study the early consequences of dystrophin deficiency during heart and skeletal muscle development. Mutations in the *DMD* gene lead to a severe X-linked neuromuscular disorder which manifests itself as young boys acquire motor functions. DMD is diagnosed after 2 to 4 years, but the absence of dystrophin has an impact before symptoms appear in patients, which poses a serious challenge in current standards of care. Our dystrophic engineered heart tissues (EHTs) and engineered muscle tissues (EMTs) showed force deficits, relaxation delays, elevated resting calcium, blunted calcium transients and impaired mitochondrial function. In addition, we used single-cell transcriptomic profiling to characterize the myogenic trajectory and to track the deleterious molecular cascade downstream of dystrophin deficiency. Our data showed that DMD cells bifurcate to an alternative branch when they reach the somite differentiation stage driven by marked gene dysregulations in cell junction families, in relation to the dynamics of cell state transitions observed during somitogenesis. This work points towards an early function of dystrophin during embryonic development, which should be considered in future therapeutic strategies for DMD. Viral vector transduction of EHTs and EMTs with AAVMyo1-CK8e-microDys5 corrected the force deficits and calcium dysregulation in a dose-dependent manner and lasted for several weeks. Overall our data serve as proof of concept that Mantarray is a powerful tool for profiling complex genetic disorders including muscular dystrophies, congenital myopathies and metabolic myopathies. Combining 3D structural and functional analyses at the tissue level with the latest transcriptomic and proteomic methods will transform our ability to identify the molecular drivers of pathology and to target therapeutics faster and cheaper than animal studies.

SG282

**Human muscle cells and systems to enable drug discovery.**

**C. A. Hinckley**[1], I. K. Sunyovski[2], H. L. Heusey[1], A. R. Palla[1]; [1]Biogen, Cambridge, MA, [2]Biogen, at time of studies, Cambridge, MA.

Human cellular models of disease are emerging as powerful tools in drug discovery, supporting a detailed understanding candidate therapies and their interactions with human targets in a disease relevant cellular context. Despite this promise, traditional cell culture methods often fail to support readouts of key aspects of cellular biology such as muscle contraction. To overcome these limitations, we have established an hiPSC derived three-dimensional skeletal muscle culture system which supports the interpretation molecular and biochemical changes in the context of muscle contractile function.When embedded in fibrin hydrogel hiPSC derived skeletal myoblasts differentiate into contractile muscle fibers over the course of 7-10 days. These engineered muscle tissues (EMTs) remain viable through at least 4 weeks, without the use of non-muscle support cells. In contrast to cardiac EMTs, skeletal muscle is minimally spontaneously active but respond to electrical stimulation with twitches and tetanic contractions in a frequency dependent manner. We also find that the contractile properties of EMTs mature over the course of 2-3 weeks. Ongoing work will identify functional deficits in disease mutation bearing EMTs compared to healthy controls.

SG283

**Engineering Functional Microtissues to Model Cardiac, Skeletal, and Uterine Muscle Disorders.**

**M. L. McCain**; Alfred E. Mann Department of Biomedical Engineering, University of Southern California, Los Angeles, CA.

Human disorders are caused by a diverse variety of genetic and/or acquired factors that manifest as dysfunction across molecular, cellular, and/or multi-cellular levels. Thus, there is a need for human disease models that enable: (1) robust control over cell and tissue architecture; (2) systematic manipulation of genetic and environmental factors; and (3) multi-modal readouts of gene expression, cell and tissue structure, and cell and tissue physiology. Our group uses a variety of microfabrication techniques, such as hydrogel micropatterning and 3-D printing, to engineer 2- and 3-dimensional microscale models of cardiac, skeletal, and uterine muscle tissues. We are also combining surface micropatterning approaches with synthetic biology (i.e., synthetic Notch receptors) as a new approach for engineering tissues with spatial control over cell lineages. We also develop assays to quantify the key structural and physiological properties of tissues, such as electrophysiology and contractility. We are implementing these systems to model and understand disorders with diverse etiologies that afflict cardiac, skeletal, and uterine muscle tissues, such as myocardial infarction, amyotrophic lateral sclerosis, and pre-term labor, respectively. These functional microtissues enable new insights into the pathophysiology of human muscle disorders and can serve as medium-throughput platforms for drug development and screening.

SG284

**High-Throughput Cardiomyocyte Morphological Profiling and 3D Functional Tissue Engineering Unravel Insights into Cardiac Biology.**

**A. Chopra**[1], R. Pei[1], P. Ellinor[2]; [1]Bayer U.S. LLC, Cambridge, MA, [2]Broad Institute, Cambridge, MA.

Induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) have emerged as valuable tools for modeling various cardiovascular diseases, including dilated cardiomyopathy (DCM). Nevertheless, existing methodologies face limitations in scalability. In this study, we introduce a novel approach called "cardiomyocyte morphological profiling," a high-throughput imaging-based cellular assay designed to investigate disease-associated genes in iPS-CMs. Leveraging this technique, we are able to distinguish subtle similarities and differences in cellular perturbations on a large scale. Additionally, we integrated our morphological profiling results with 3D engineered artificial muscle to gain functional insights into the structural findings. This comprehensive platform was applied to a 37-gene CRISPR perturbation knockout screen, featuring candidate genes identified from a contractile genome-wide association study (GWAS). We identified YWHAE as a significant gene, and its knockout exhibited a similar morphological profile to that of a known DCM disease-causing gene, titin. To validate these findings functionally, we employed 3D engineered heart tissues. Our study demonstrates the power of combining morphological profiling and 3D functional engineered tissues to unveil insights into functional genomics on a large scale, ultimately enhancing our comprehension of genes influencing cardiac biology. This innovative platform holds significant promise for exploring novel biology, validating genes of interest, and identifying crucial biological mechanisms for potential therapeutic applications in heart failure research.

SG285
**Muscle Makers.**
**M. Lewis**; Loughborough University, Loughborough, UNITED KINGDOM.

Muscle Makers - Recreating human skeletal muscle requires solving multiple technological and intellectual questions. It is a tissue that is mechanically active and which requires mechanical stimulation. It is a tissue that is electrically active and which requires electrical stimulation. It is a tissue that has a highly specialized core cell type and which requires interactions of multiple cell types. It is a tissue that is capable of gain and that is capable of loss. It is a tissue that can regenerate and can rehabilitate. Using multiple technologies in a cross-disciplinary way, we have been heavily involved in tissue engineering and regenerative medicine since its beginnings where we take an iterative approach so the "feed-forward" and "feedback" with in vivo systems is vital to inform as to where our bioengineered systems fit in. Our activity is also multi-disciplinary with physiologists, biomechanists, mechanical, electrical, chemical engineers, (bio)chemists and clinicians adding to our expertise in molecular cell biology. Current work is split broadly into three overlapping areas which are exercise medicine (EM), exercise science (ES) and MSK tissue engineering (MSK TE). We are looking forward to contributing to this sub-group gathering!

## Subgroup: Liquid Condensates in Neurons and Glia

SG286
**Membrane-associated Periodic Skeleton Proteins Phase Separate during Axonal Development.**
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A tightly regulated and highly dynamic cytoskeleton is fundamental for the development of neurites to establish a functional neuronal network. This is especially true of the membrane-associated periodic skeleton (MPS), a structure consisting of periodic rings of actin and actin-binding proteins interleaved with spectrin tetramers which spans most of the length of mature axons and both confers structural stability to the axon and acts as a scaffold for signaling complexes. While some work has been done to investigate the development of the MPS, our data suggests that the current model of its formation, subunit incorporation progressing from the soma, is incomplete. In cultured hippocampal mouse neurons we have observed that early in neuronal development, distinct patches of adducins and spectrins appear in the distal axon which lack the characteristic periodicity of mature MPS. These axonal patches resembled phase-separated structures seen in many other biological processes but only recently associated with cytoskeletal proteins. Indeed, overexpression of full-length αII-spectrin or fragments of βII-spectrin or α-adducin in non-neuronal cell lines produced rounded inclusions considerably brighter than cytoplasmic signal. These spectrin inclusions demonstrated preferential internal mixing over exchange with the external milieu as measured by MOCHA-FRAP, which is a key feature of liquid-like biomolecular condensates. Expression of protein fragments identified in this non-neuronal cell screen in primary mouse hippocampal neurons impaired the assembly of periodic MPS, supporting a functional role of phase separation in periodic skeleton assembly. Using these data we have created a Condensation-Assembly-Coalescence model of MPS development. We hypothesize that at early stages of axon development, spectrins and adducins form liquid-like condensates which subsequently recruit additional MPS components and begin to assemble periodic structures. These small patches of assembled MPS then extend longitudinally and coalesce into the mature axon-spanning

structure. An intriguing feature of this model is that the condensates of adducin and spectrin would provide both a mechanism by which MPS components could be concentrated prior to assembly into the highly ordered lattice, as well as structural flexibility to allow for developmental processes such as axon guidance, branching and synaptogenesis.

SG287

**Single-molecule imaging reveals synaptic vesicle confinement by liquid-liquid phase separation.**

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Brain functioning critically relies on neuronal communication that mainly occurs by chemical signaling at the specialized contacts known as synapses. At synapses, messenger molecules are packed into synaptic vesicles (SVs), which are secreted upon the arrival of an action potential. Indeed, loss of SVs and synaptic deficits are associated number of neurodegenerative diseases. Hundreds of SVs accumulate at each synaptic bouton. Despite being held together, SVs are highly mobile, so that they can be recruited to the plasma membrane for their rapid release during neuronal activity. However, how such confinement of SVs corroborates with their motility remains unclear. To bridge this gap, we employ ultrafast single-molecule tracking (SMT) in the reconstituted system of native SVs and in living neurons. SVs and synapsin 1, the most highly abundant synaptic protein, form condensates with liquid-like properties. In these condensates, synapsin 1 movement is slowed in both at short (i.e., 60-nm) and long (i.e., several hundred-nm) ranges indicating that the SV-synapsin 1 interaction raises the overall packing of the condensate. Furthermore, two-color SMT and super-resolution imaging in living axons demonstrate that synapsin 1 drives the accumulation of SVs in boutons. Even the short intrinsically-disordered fragment of synapsin 1 was sufficient to restore the native SV motility pattern in synapsin triple knock-out animals. Thus, synapsin-driven condensation is sufficient to guarantee reliable confinement and motility of SVs, allowing for the formation of mesoscale domains of SVs at synapses and functional neurotransmission.

SG288

**The tubulin-polymerization-promoting protein (TPPP) forms condensates and aggregates in Multiple Systems Atrophy.**

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The tubulin-polymerization-promoting protein (TPPP) is an oligodendrocyte-specific microtubule-associated protein that facilitates the nucleation of microtubules. In oligodendrocytes, TPPP localizes to Golgi outposts that serve as microtubule nucleation centers at branch points of highly arborized processes that myelinate axons (Fu et al. 2019). New findings highlight that in cases of Multiple System Atrophy (MSA), TPPP aggregates are present both within myelin sheaths and the cell body. Remarkably, these aggregates seem to form independently of alpha-synuclein (aSyn). TPPP is highly intrinsically disordered, and forms condensates at physiological concentrations in vitro. Moreover, the protein can

form larger aggregates like sheets and fibrils. Remarkably, such recombinant TPPP aggregates are toxic to primary oligodendrocytes. We postulate that the propensity of TPPP for condensation is integral to its fundamental function—microtubule nucleation. On the downside, the ability to self-interact likely produces aberrant condensed states of TPPP, such as aggregates and fibrils in instances of MSA.

SG289

**Ataxin-2 polyglutamine expansions sequester TDP-43 within aberrant ribonucleoprotein condensates, impair mRNA transport along the axon and suppress local translation.**

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Amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease of motor neurons, is characterized by mislocalization and aggregation of TDP-43, an essential RNA/DNA-binding protein with multiple roles in post-transcriptional RNA processing, including splicing and suppression of cryptic exons, mRNA localization and translation. TDP-43 undergoes liquid-liquid phase separation *in vitro*, and forms rounded puncta within the nucleus and cytoplasm of cells, including in the axon, consistent with de-mixed distribution. Missense mutations in TDP-43 and other RNA-binding proteins have been linked to familial and sporadic ALS, and we have previously shown that ALS-linked mutations perturb the dynamic, liquid-like properties and transport of TDP-43 positive ribonucleoprotein (RNP) condensates along the axon. Recent studies have identified genetic interactions between TDP-43 and Ataxin-2, an RNA-binding protein 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 cellular and 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/Ataxin-2 interactions within RNP condensates, disrupt RNP condensate dynamics and function, and increase the propensity for pathologic transformation of TDP-43. Here, we used live-cell confocal imaging, photobleaching and single molecule translation reporters to study the localization, transport dynamics and mRNA regulatory functions of TDP-43/Ataxin-2 RNP condensates in rodent primary cortical neurons. We show that Ataxin-2 polyQ expansions aberrantly sequester TDP-43 within RNP condensates, and disrupt both its motility along the axon and liquid-like properties. Using the SunTag reporter assay, we show that Ataxin-2 polyQ expansions suppress translation across multiple subcellular compartments. Furthermore, we observe markedly impaired local translation specifically within RNP condensates positive for polyQ expanded forms of Ataxin-2; i.e., actively translating mRNA within Ataxin-2 Q30 or Q39 positive condensates is reduced compared to local translation within Ataxin-2 Q22 positive condensates. Taken together, our data suggest that Ataxin-2 governs motility and translation of neuronal RNP condensates and that Ataxin-2 polyQ expansions fundamentally perturb spatial localization of mRNA and suppress local translation. Overall, our results support a model in which Ataxin-2 polyQ expansions disrupt stability, localization, and/or translation of critical axonal and cytoskeletal mRNAs, particularly important for motor neuron integrity.

SG290

**Phosphorylation of HSP27 regulates the fluidity of p62/SQSTM1 phase condensates in autophagy.**

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Maintenance of intact lysosomes is central to cell viability. Upon injury, lysosomes are rapidly repaired or targeted for degradation. Damaged lysosomes are degraded via selective autophagy, a process by

which autophagosomes isolate specific cellular cargo that is marked by binding of selective autophagy adaptor proteins. The adaptor p62/SQSTM1 has a well-established role in several forms of selective autophagy; recently, we identified that p62 is also required for the clearance of injured lysosomes (lysophagy). We found that p62 promotes lysophagy by forming liquid-like condensates at damaged lysosomes, while loss of p62 inhibited lysosomal turnover. Further, we found that p62 condensates are tuned by the heat shock protein HSP27. Depletion of HSP27 decreased the liquidity of p62 condensates following lysosomal damage, observing a decreased rate of fluorescence recovery of GFP-labeled p62 after photo-bleaching and an increased accumulation of insoluble p62. Live-imaging experiments revealed the recruitment of HSP27 to p62-positive damaged lysosomes, which was lost in p62 knockout cells. Next, we investigated the molecular trigger regulating the p62-HSP27 interaction. We found that lysosomal permeabilization induced HSP27 phosphorylation at three serine residues, and mutating these sites blocked HSP27 recruitment to injured lysosomes. HSP27 is a target of the kinase MK2, following its activation by p38 MAPK. Inhibition or depletion of either MK2 or p38 MAPK inhibited HSP27 phosphorylation and thus lysophagy, identifying HSP27 phosphorylation as a critical regulatory step in this pathway. We next asked whether HSP27 maintains p62 condensates in other contexts. To test this hypothesis, we induced proteotoxic stress via proteasome inhibition, and observed activation of both p38 MAPK and MK2, leading in turn to the phosphorylation of HSP27. Mutation of the three regulatory phospho-sites in HSP27 blocked recruitment of the protein to p62-positive protein aggregates. This failure to recruit HSP27 reduced the liquidity of p62 condensates surrounding protein aggregates induced by proteasome inhibition, mirroring the effects induced by inhibition of HSP27 phosphorylation on damaged lysosomes. Thus, our work identifies a conserved mechanism regulating p62-dependent autophagy, in which a p38 MAPK-MK2 axis promotes p62-dependent autophagy via phosphorylation of HSP27.

SG291

**The E3 ubiquitin ligase TRIM9 regulates actin dynamics and synapse formation.**

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In neurons, actin-rich filopodia are critical at many stages of 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. Previously, we have demonstrated the E3 ubiquitin ligase TRIM9 localizes to growth cone filopodia and regulates axon pathfinding downstream of the guidance cue netrin. *Trim9*$^{-/-}$ mice have overt spatial learning memory deficits, yet the role of TRIM9 in synapse formation and maintenance is unknown. Here we show TRIM9 is enriched in the post-synaptic density following differential centrifugation, suggesting a role for TRIM9 in dendritic spines. We find Netrin-1 dependent increases in dendritic spine number, synapse maturation, and neuronal firing in vitro, and all these responses are abrogated in *Trim9*$^{-/-}$ neurons. Furthermore, neurons over-expressing TRIM9 show defects in spine maturation but not dendritic spine number. In vivo, we demonstrate that loss of *Trim9* alters the proteome of the postsynaptic density. In particular, we observe changes in numerous cytoskeletal proteins, including the Arp2/3 complex. Ongoing work is investigating the functional consequence of these changes to Arp2/3 accumulation in dendritic spines and neurotransmitter receptors on the neuronal surface.

SG292

**CDKL5 signalling in neuronal development.**

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CDKL5 is a serine/threonine kinase with enriched in the brain. Loss of function mutations in the X-linked CDKL5 gene cause a severe developmental encephalopathy with early-onset seizures and general neurodevelopment delays, named CDKL5 Deficiency Disorder (CDD). Roles of CDKL5 in brain development is not well-understood. We have discovered several physiological substrates of CDKL5 in the brain using phosphoproteomics. CDKL5 phosphorylates microtubule binding proteins affecting microtubule dynamics and dynein-based transport. CDKL5 also phosphorylates the voltage-gated calcium channel Cav2.3 and modulates the function. Despite significant improvements in the molecular understanding of CDKL5 function, how CDKL5 orchestrates these events and their role in neuronal development remains unclear. Our mechanistic insights on CDKL5's role in microtubule-based cargo transport and voltage-gated calcium channel regulation will be presented.

## Subgroup: Beyond Pretty Pictures: Quantitative Methods for Learning Cell Biology from Microscopy Datasets

SG293

**Integrating Deep Learning Morphology Profiling and Single Cell RNA-Seq Reveals Tumor Heterogeneity and Enriches Sub-populations.**

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Technological advances have ushered in a new multi-omics era that has elucidated more granular information on cell types and functions at single cell resolution. In particular, methods to capture tumor heterogeneity are crucial for better understanding and treatment of cancer. Current isolation of tumor cells from tissue typically relies on targeting biomarkers, such as EpCAM, resulting in potentially biased isolation of tumor population subsets used for further molecular and functional analysis. Here, we integrate deep learning and high-dimensional morphology as a readout of cell identity, state, and function to isolate tumor cells without biomarker labels. We developed a self-supervised deep learning foundation model, 'Human Foundation Model' (HFM), that combines deep learning with morphometrics for high-dimensional morphology analysis to provide biologically interpretable features on a diverse range of human cells and sample types.

We applied the HFM as part of the REM-I platform to identify and enrich malignant cells from non-small cell lung cancer (NSCLC) dissociated tumor cell (DTC) samples. Results demonstrate the ability to identify different cell types (epithelial, stromal, immune, and endothelial cells) and distinguish malignant cells from normal healthy cells based on unannotated brightfield images. Enriched NSCLC cells are label-free, unperturbed, and viable, making them amenable to downstream molecular and functional analyses. We verified enrichment of malignant cells by performing scRNA-Seq analysis on sorted samples. Sorted malignant cells showed high levels of tumor cell gene expression signature relative to initial patient specimens. Multiple clusters of morphologically unique tumor cell populations were detected by UMAP analysis of deep learning embeddings and computer vision morphometric features. Retrieval and subsequent scRNA-Seq analysis of these morphologically distinct populations revealed molecular differences between these subgroups, suggesting morphology insights may reflect molecular profiles.

Further work is planned to evaluate the link between the morphological, molecular, and functional characteristics of each subpopulation.

SG294

**Illuminating organelle remodeling during neuronal differentiation.**

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Organelles undergo dramatic changes in shape, position, dynamics, and interactions with other organelles (together termed morphodynamics) in response to changing environmental or developmental conditions. We developed a method for multispectral imaging of up to eight organelles simultaneously in live cells and used this method to visualize organelle morphodynamics along neuronal differentiation. We transfected induced pluripotent stem cells (iPSCs) and iPSC-derived cortical neurons (iNeurons) with genetically encoded organelle markers and collected multispectral z-stack and timelapse images at five timepoints throughout neuronal differentiation and maturation: iPSCs, and iNeurons at day 7, day 14, day 21, and day 30. Images were subjected to linear unmixing and run through a pipeline for segmentation and analysis of approximately 400 metrics including organelle number, size, distribution, shape, and inter-organelle contacts. To analyse organelle shape, we used O2-VAE, a custom unsupervised learning method for orientation-invariant morphologic profiling of cells and organelles. We observed dramatic remodeling of organelles during differentiation of iPSCs into iNeurons. For example, mitochondrial tubulation increased as iPSCs differentiated into cortical neurons, consistent with increased oxidative phosphorylation. At early stages of differentiation, we observed rearrangement of mitochondria-organelle contacts, followed by changes in peroxisomes-organelle contacts during neuronal maturation. We also observed an increase in higher order contacts (4- and 5-way contacts) as iNeurons matured. Contacts between organelles can be modulated by tether proteins. We found that expression of VAP-B, which is implicated in endoplasmic reticulum (ER)-peroxisome, ER-mitochondria and ER-Golgi contacts, increases as iPSCs differentiate into iNeurons. VAP-B knockdown caused a reduction in neurites and synapses during iNeuron differentiation. Our results suggest that extensive rewiring of organelle contacts is necessary to support metabolic changes throughout differentiation, and that the resulting organelle signature is required to sustain neuronal functions.

SG295

**O2-VAE: Robust unsupervised cell and organelle profiling using orientation-invariant autoencoders.**

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Cell and organelle shape are driven by diverse genetic and environmental factors and thus accurate quantification of cellular morphology is essential to experimental cell biology. A popular method for unsupervised biological image analysis is autoencoders (AE's). Autoencoders learn a low-dimensional representation that maps images to feature vectors to generate a semantically meaningful embedding space of morphological variation. The learned feature vectors can also be used for clustering, dimensionality reduction, outlier detection, and supervised learning problems. Morphology properties do not change with orientation, and thus we argue that representation learning methods should encode

this orientation invariance. We show that conventional autoencoders are sensitive to orientation, which can lead to suboptimal performance on downstream tasks. To address this issue, we develop O2-variational autoencoder (O2-VAE), an unsupervised learning method that learns robust, orientation-invariant representations.
We demonstrate how to integrate O2-VAE into workflows for microscopy analysis. The object of interest - cells or organelles - are segmented and added to a training set. The O2-VAE model can be trained from scratch to learn profiles of shape; alternatively profiles can be extracted from a model that is pretrained on a large dataset. The pretraining approach removes the need for practitioners to do time-consuming training, and it enables profiles to be extracted for datasets that are usually too small for learning. The object profiles (vectors) can be analyzed using classical statistical methods like clustering, outlier detection, dimensionality reduction, and supervised learning. We use O2-VAE to discover novel morphology subgroups in segmfented cells and mitochondria, detect outlier cells, and rapidly characterize cellular shape and texture in large datasets.

SG296

**Uncovering the regulation and role of tissue wide $Ca^{2+}$ signaling in the regenerating epidermis.**

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Skin homeostasis is maintained via constant regeneration by stem cells, which must communicate to balance self-renewal and differentiation. Yet how stem cells carry out this signaling across regenerative tissues remains unknown due to challenges in studying signaling dynamics in live mice. We combined live imaging in the mouse basal stem cell layer with machine learning tools to analyze patterns of $Ca^{2+}$ signaling. Specifically, we developed Geometric Scattering Trajectory Homology (GSTH) to analyze and model high-dimensional signaling dynamics to understand $Ca^{2+}$ signaling in this context. Importantly, this computational pipeline is broadly applicable. We applied GSTH to analyze and model complex dynamics across other signaling pathways (MAPK/ERK), in non-homeostatic contexts (aging, oncogenes, injury), and across systems (neurons, in vitro cell culture vs. mouse vs. fly, etc.). This allowed us not only to discover the emergent property of long-range coordinated $Ca^{2+}$ signaling across the epidermal stem cell layer, but to also open up avenues of exploration in understanding spatiotemporal signaling dynamics in diverse areas of cell biology. In the skin stem cell layer, we find that local $Ca^{2+}$ signals are coordinated across thousands of cells. We demonstrate that G2 cells are required to initiate normal levels of coordinated $Ca^{2+}$ signaling, while connexin43 connects basal cells to orchestrate tissue-wide coordination of $Ca^{2+}$ signaling. Finally, we find that $Ca^{2+}$ signaling promotes cell cycle progression, revealing a communication feedback loop. This work provides insight into how stem cells at different cell cycle stages coordinate tissue-wide signaling during epidermal regeneration.

SG297

**Capturing spatiotemporal signaling patterns in cellular data with geometric scattering trajectory homology.**

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Cell signaling plays a critical role in orchestrating the complex interactions and processes necessary for the proper functioning and survival of organisms. This intricate communication system allows cells to

perceive and respond to their ever-changing environment by transmitting and processing information through a series of biochemical reactions. Although many signaling molecules and pathways have been identified, the dynamics of signaling processes, including their initiation, propagation, termination, and adaptation, are not yet fully understood. To facilitate quantitative understanding of complex spatiotemporal signaling activity, we developed Geometric Scattering Trajectory Homology (GSTH), a general framework that integrates geometric scattering and topological data analysis (TDA) to provide a comprehensive understanding of complex cellular interactions. This combination allows for the effective capture of both local and global patterns, as well as a robust analysis of the underlying topology. By accounting for the multi-scale nature of cellular interactions and the temporal fluctuations of signaling events, GSTH uncovers the intricate mechanisms that govern cellular communication and coordination. We tested this framework using a variety of computational models and experimental data. Our findings demonstrate that the GSTH-generated trajectory is related to the degree of synchrony, speed, and quasi-periodicity of the underlying signaling pattern. We recovered model parameters and experimental conditions by training neural networks on the trajectory, showing that our approach preserves information that characterizes various cell types, tissues, and drug treatments.

SG298

**Characterizing cell states by integrating cell behavior, organization, and molecular census in migratory cells from the epithelial to mesenchymal transition (EMT) of human induced pluripotent stem cells (hiPSCs).**

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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells establish and maintain robust dynamic localization of cellular structures, and how cells transition between states during differentiation and disease. The epithelial-to-mesenchymal transition is a state change that occurs in both normal contexts such as development and pathological contexts such as cancer metastasis. The EMT has been described as a change in cell behavior from largely non-motile to migratory cells, a change in cell shape characterized by a decrease in cell height, a change in cell organization from apical-basal to front-rear polarity, and a change in the profile of protein expression. However, the dependencies and relationships between these aspects of EMT are not well understood and require a large-scale, multi-modal data integration approach. We have standardized an imaging pipeline to generate 90-minutes, 3D live-cell time-lapse images of hiPSCs from the Allen Cell Collection (allencell.org) undergoing EMT prior to 4i (Iterative Indirect Immunofluorescence Imaging) 14-plex immunostaining. We are developing an image and data analysis framework to investigate the temporal dynamics of migratory cell behavior during EMT. We have found that migratory cells can be grouped into distinct categories based on cell displacement metrics (such as cell speed), cell location with respect to the colony, and cell-cell interactions. We have also incorporated an elastic metric of deformation into measurements of cell displacement to account for changes in cell shape. with this framework, we can explore the relationship between quantified migratory cell behaviors and the observed heterogeneous protein expression profiles found within a population of these migratory cells (including e.g., Sox2, LEF1, BiP, WNT5a, Eomes, DKK1). For example, Sox2 expression was detected in slow-moving cells, but not in fast-moving cells. We aim to use this integrated approach to determine whether migratory cell behavior is a predictor of protein expression or vice versa. Overall, we believe that our approach to define states of hiPSC-derived migratory cells as an integration of cell behavior, cell

organization, and molecular census of the cell, could serve as a model to define cell states more generally.

SG299

**Physics-informed computation of label-free and fluorescence microscopy data improves contrast, information content, and biophysical interpretation.**

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Label-free and fluorescence microscopes are invaluable tools for biologists, but physics limits the quality of the images these tools can collect. In addition to the well-known diffraction limit on the resolution of features, microscopes are subject to other less-appreciated limitations: varying contrast among features of different sizes, inverted contrast for features above and below focus, and raw measurements in the form of "counts" instead of direct biophysically interpretable values.

We address these limitations by applying physics-informed computations to our data. We develop physical models of widely available instruments, express these physical models mathematically, and create computational tools to transform raw label-free and fluorescence data into reconstructions with improved physical interpretability and uniform contrast. Here, we demonstrate that these transformed images facilitate easier interpretability for both human and machine observers by providing direct biological insight. Notably, these `q1wq computation-enabled measurements allow the quantification of mitotic spindle helicity, improved virtual staining of membranes and nuclei from label-free data, and enable high-throughput phenotyping of viral infection.

To facilitate broader adoption, we make all of our computational tools available via open-source repositories (Github: mehta-lab/recOrder, mehta-lab/waveorder). We target a diverse audience, from biologists without coding expertise to expert machine-learning pipeline builders, as we seek to reduce the effort required to learn the physical limits that constrain microscopy data.

SG300

**Integrating Motility and Morphology for Unraveling Cellular Phenotypic Heterogeneity in Breast Cancer Stem Cells.**

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Cellular motility and morphology play crucial roles in providing valuable insights into the physiological and signaling states of cells. Understanding these dynamic cellular behaviors offers significant implications for diverse cellular states, including tissue regeneration and cancer invasiveness. However, the integration of motility and morphology to generate cellular phenotypes remains challenging due to inherent cellular heterogeneity. In this study, we address this challenge by applying our novel feature selection strategy, PHet (Preserving Heterogeneity), designed to preserve subtype heterogeneity while discriminating known cellular states. PHet utilizes iterative subsampling analysis of interquartile range (IQR) and mean differences between known classes, resulting in a minimal set of heterogeneous-preserving discriminative features. This approach maximizes the quality of subtype clustering, allowing us to extract meaningful cellular phenotypes integrating motility and morphology. To demonstrate the effectiveness of our method, we applied PHet to live cell movies of breast cancer stem cells (CSCs), a rare and crucial subpopulation within cancer cells. CSCs possess unique characteristics, driving tumorigenicity through self-renewal and differentiation, and exhibit distinct behaviors such increased

chemoresistance and proliferation. Through time-lapse microscopy data analysis of breast CSCs and non-CSCs, we extracted motility and morphological features. The subsequent PHet-based feature selection revealed a rare subpopulation (~10%) that exhibited a predominantly higher proportion of CSCs compared to non-CSCs. Remarkably, this specific phenotype remained undetected when analyzing motility or morphology features in isolation. However, our integrative approach successfully revealed its distinct characteristics by combining both aspects. The unique profile of this CSC subpopulation suggested these cells possessed protrusive dynamic morphology and exhibited exploratory motility behaviors, potentially playing a crucial role in the formation of chemo-resistant cell clusters. Our study highlights the efficacy of our feature selection, enabling the identification of cell phenotypes that integrate motility and morphology by preserving the essential amount of heterogeneity in discriminative features. The insights gained from this research contribute to a deeper understanding of the complex dynamics of CSCs and hold promise for developing targeted cancer therapies. Furthermore, this integrative approach of analyzing motility and morphology can be broadly applicable to various fields where cellular heterogeneity plays a pivotal role.

SG301

**A machine learning-driven approach for systematic quantification of*in vivo*cell geometries during embryo development.**

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Embryo development involves coordination of multiple processes including cell proliferation, cell migration, patterned gene expression and cell shape changes. To characterize the complex trajectories of development, and to extract mechanisms, these processes need to be quantitatively tracked. Recent advances in spatially resolved single-cell transcriptomics have elucidated embryo gene expression patterns, and a recent analysis classified cell shapes in cultured cell systems (Viana et al., 2023). However analysis of cell shape patterning in developing organisms remains primarily qualitative.
Here we present a quantitative, machine learning-driven approach for systematic analysis and classification of *in vivo* cell shapes in early embryos. The early *Drosophila* (fruit fly) embryo contains 5000-6000 epithelial cells, many having having similar columnar shapes prior to gastrulation onset. In a recent study, the gastrulation episode was cataloged at single-cell resolution using light sheet microscopy and 3D cell segmentation (Stern et al., 2022). Here we analyzed this data to systematically classify cell shapes across the embryo during gastrulation.
After aligning cells according to their apical-basal orientation, 3D shape features for each cell were extracted, from which cell shape can be accurately reconstructed. An unsupervised clustering algorithm was used to identify multiple shape classes during gastrulation, including long columnar, short columnar, tilted columnar and cuboidal. Thus we could define a time-dependent morphological cell patterning of the embryo, defined purely by cell shape. Combined with established gene expression patterning, this provides a far more comprehensive description of embryo development. For example, we identified two emerging patches of tilted columnar cells at the edge of the invaginating ventral furrow. Previous experiments show this morphological pattern is causally downstream of both the known dorsal-ventral gene expression patterning, and the ventral mechanical stress patterning determined by activated myosin. Another finding is clustering of short columnar cells at the embryo head and tail, presumably accommodating high local intrinsic curvatures. Overall, systematic measurement of cell morphology patterning will complement genome, transcriptome and proteome data to furnish a fuller description of embryo development.

SG302
**Systems for scaling quantitative bioimage analysis.**
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While bioimage analysis is increasingly recognized as required for quantitative microscopy experiments, best practices are still being shaped. Analyses are most (and some might say only) helpful when they are reproducible, but the ability to create reproducible analysis workflows in most tools requires significant computational expertise that individual researchers may or may not possess. As deep learning and artificial intelligence approaches proliferate in bioimage analysis, this problem is likely to worsen, since such networks are often difficult to install or transfer across researchers.
A major strategy for reproducible computing are software containers such as Docker and Singularity, which package tools, tool dependencies, and optionally data together so that a workflow can be run on any computer of any operating system as long as it can run Docker or Singularity containers. While popular in disciplines such as genomics, containers are still rarely adopted in bioimage analysis. Here we will discuss the advantages of software containerization for both developers and users, ways to integrate software containers into existing tools, plugins that make containers easy to run in the browser, and new tools that make it easy to scale and parallelize containers in the cloud. Adoption of such approaches will bring quantitative bioimage analysis to the community far more easily than is often currently possible.

## Subgroup: Biophysical Modeling of the Cell

SG303
**Collective stability of epigenetic marks: insights from statistical inference and biophysical modeling.**
**E. Read**, T. Downing; University of California, Irvine, Irvine, CA.

DNA methylation is a ubiquitous epigenetic modification. In the mammalian genome, DNA methylation occurs predominantly on cytosine-phosphate-guanine (CpG) dinucleotides, and methylation patterns are dynamically maintained over mitotic cell division by methyltransferases in concert with other enzymes. It has been posited that coupling of enzymatic processes among neighboring CpGs is critical to epigenome stability, however the mechanisms underlying this coupling are not well understood. In this study, we combine biophysical modeling with data-driven statistical approaches to shed light on correlations observed in methylome measurements. We develop a computationally efficient spatial stochastic modeling framework for simulating methyltransferase activity. By playing out alternative mechanistic hypotheses, the simulations provide insight to both short-range (<1 kb) and long-range methylation correlation observed in cell-based experiments. Broadly, our study provides an example of how biophysical modeling can aid interpretation of high-throughput sequencing data.

SG304
**The actomyosin cortex requires friction to avoid constrictional shape instability.**
H. Zhu[1], R. Alonso-Matilla[2], **B. O'Shaughnessy**[1]; [1]Chemical Engineering, Columbia University, New York, NY, [2]Biomedical Engineering, University of Minnesota, Minneapolis, MN.

The actomyosin cortex is essential to the life of a cell. Contractile myosin forces in the thin (~ 0.2 microns) cortical layer generate cortical tension for cytokinesis, shape regulation, cell polarization,

exocytosis and for collective cell behaviors such as tissue reshaping. A fundamental threat is actomyosin contractile instability (ACI), when contractile forces trigger myosin inhomogeneities that amplify contractility in a runaway process that destroys actomyosin architecture (Thiyagarajan et al., 2022). Here we use quantitative modeling to show that a stable actomyosin cortex surface requires frictional interactions with the plasma membrane or extracellular matrix (ECM). Lacking frictional stabilization, the cortex layer suffers catastrophic constrictional instabilities due to ACI. Our findings explain experiments on *Drosophila* tracheal tubules that suffer constrictional tubule pearling instabilities in mutants lacking ECM frictional stabilization (Hannezo et al., 2015). We also explain the furrowed actoymosin cortical architecture with 5-fold symmetry that encloses and squeezes giant drosophila salivary gland vesicles during exocytosis (Rousso et al., 2016). We used an active gel framework with differential geometry (Mietke et al., 2019). with physiological values for the amount of cortical myosin and other parameters, we find a cortical surface lacking frictional resistance to actomyosin motions suffers constrictional shape instability. However, with physiologically realistic friction due to transmembrane proteins and other entities interacting with the cortex, constriction is rescued and instead the cortex develops multiple stable furrows because friction severely suppresses long wavelength variations, stabilizing shorter-wavelength variations. These results explain multiple shallow furrows seen in wild-type *Drosophila* tracheal tubules, whereas the functional tubule shape is destroyed by constrictional pearling instability in *kkv* mutants with lowered cortex-ECM friction (Hannezo et al., 2015). We also explain multiple furrows in actomyosin coats that grow on giant exocytic vesicles in *Drosophila* salivary glands (Rousso et al., 2016). High myosin is needed to squeeze huge vesicles, so friction must be present, explaining multiple furrowing. Some vesicles suffer constriction instability and failed exocytosis, suggesting accidentally lowered friction.

SG305

**Nascent adhesions shorten the period of lamellipodium protrusion through the Brownian ratchet mechanism.**

**M. Mendoza**; Oncological Sciences, University of Utah, Salt Lake City, UT.

Directional cell migration is driven by the conversion of oscillating edge motion into lasting periods of leading edge protrusion. Actin polymerization against the membrane and adhesions that link the actin to the cell substrate drive edge velocity. However, the pro-migration signals extracellular-regulated kinase (ERK) promotes actin assembly and adhesion disassembly. We interrogated how these two outputs together promote migration. Adhesion tracking in migrating cells showed that ERK reduces the number of nascent adhesions and shortens the adhesion lifetime. Nascent adhesions are small, transient adhesions that form at the very edge of protruding cells. We asked how ERK's shortening of adhesion lifetime would integrate with its increase of actin assembly to promote edge protrusion through computational modelling. We built a model in which polymerization of actin filaments against a deformable membrane and variable adhesion dynamics support edge motion. The model incorporated, for the first time, both the Brownian ratchet and the molecular clutch mechanisms (BR-MC) using discrete elements for actin, membrane and adhesions. Consistent with previous reports, the model showed that actin polymerization and adhesion lifetime power protrusion velocity. It also revealed that increasing adhesion lifetime decreased the protrusion period. Measurements of adhesion lifetime and edge motion in migrating cells confirmed that adhesion lifetime is associated with and promotes protrusion velocity, but decreased duration. Adhesions' control of protrusion persistence originated from the Brownian ratchet mechanism for actin filament polymerization, as removing the BR from the model removed the control of protrusion duration. We found that longer adhesion lifetime and

increased adhesion density resulted in an increased proportion of actin filaments tethered to the substrate. This reduced filament-membrane distance, which increased pushing force for high edge velocity, but limited further polymerization needed for protrusion duration. Thus, adhesion strength regulates actin filament polymerization to control the period of leading edge protrusions. This suggests that ERK's joint action inducing actin polymerization and adhesion disassembly balances protrusion velocity and duration to convert edge protrusions into productive migration.

SG306

**Advancing cell biology by harnessing open-source modeling and facilitating broad community collaboration.**

**B. Lyons*, G. T. Johnson*, J. S. Yu*,.** Allen Institute for Cell Science, N. Bagheri; Allen Institute for Cell Science, Seattle, WA.

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 (allencell.org). We also strive to democratize tools that expedite research and foster community-driven biological discovery. Recognizing that the blue-sky vision of modeling dynamic living cells will demand a concerted effort, we are committed to catalyzing this with reusable models, extensible frameworks, usable tools, and broad community collaboration and training.

To understand mechanisms underlying cell shape dynamics, we integrate imaging data with iterative model development in a framework that combines an agent-based model (ABM) of agents (cells) following rules (theoretical biological mechanisms) with a 3D Cellular Potts Model (CPM) of cell shape (github.com/bagherilab/ARCADE). Simulating the ABM with varying rule sets, we compare size and shape distributions with distributions calculated directly from our dataset of over 200,000 3D live hiPSC images. Preliminary simulations reveal that basic CPM rules—cell-cell adhesion, volume, and surface area—don't reproduce observed distributions, while additional rules, like substrate adhesion, impact emergent distributions differently. Comparing simulated and observed distributions across rule sets, we gain insights into mechanisms driving cell shapes to inform model development and hypothesis generation.

The Simularium project (simularium.allencell.org) collaborates with model and framework developers and users, to engage the broader biology community in building and analyzing spatial mechanistic simulations. Our user-friendly, open-source viewer allows easy sharing and exploration of 3D biological simulations in a web browser. We have begun prototyping integrations with Vivarium (vivarium-collective.github.io) to create multiscale and multi-method models, with interfaces enabling dynamic transitioning between various open-source simulation packages. Our goal is to develop multiscale models with adaptive resolution and calculation methods, facilitating comparisons between models, enhancing reproducibility, and ultimately fostering collaborations between wet lab biologists and computational modelers.

We will outline plans to couple these efforts and resources with training and education, collaborating with the community to shape the next generation of virtual cells.

* 3 authors will present as a team

SG307

**Modelling Form and Function at the Synapse.**

**M. Stefan**; Medical School Berlin, Berlin, GERMANY.

Learning happens through long-term changes in neuronal connectivity. These changes involve both changes in the activation and biochemical interaction patters of synaptic proteins, as well as changes in their localisation and the structure of the synapse itself.
It is increasingly clear that a protein's spatial microenvironment and its biochemical function interact in complex ways that we are only just beginning to understand. For instance, the probability of a protein binding to a ligand will not only depend on their dissociation constant and concentrations, but also on factors such as the presence of other proteins competing for the same ligand, the spatial distributions of both binding partners, and the extent to which both can diffuse freely. Thus, the function of a protein depends on its precise location in the synapse. The reverse is also true: The functional state of a protein (in terms of ligand binding, conformation, or post-translational modifications) will determine what other molecules it can bind to, and thereby help localise it to specific domains.
Over the last decade or so, there have been exciting developments in computational tools for spatial stochastic modelling of biomolecules, as well as tools that allow modelling of dynamically changing cellular shapes. Efforts in my group have been geared towards combining the two, in order to build an "in silico synapse" that accounts for both biochemical interactions and spatial changes, and how the two affect each other. In this talk, I will present some of our results so far, focusing specifically on the role of actin filaments in the postsynaptic dendrite: I will first compare various platforms and methods we have used to model actin filament dynamics in dendritic spine in MCell, Morpheus, and BioDynaMo. I further show that one of the roles of CaMKII binding to actin filaments in the unpotentiated synapse may be to allow precise control of the relative concentrations of calmodulin and CaMKII, which need to change over time in order to allow for CaMKII activation. I hope to convince you that effects of a protein's microenvironment on its funtion are often counterintuitive and therefore merit careful consideration in modelling.

SG308

**Scale-independent topological interactions drive the first fate decision in the Drosophila embryo.**

**A. Mukherjee**[1], W. Hur[2,3], M. Vergassola[1], S. Di Talia[3]; [1]Physics and QBio, ÉCOLE NORMALE SUPÉRIEURE, Paris, Paris, FRANCE, [2]Rockefeller University, New York, NY, [3]Duke University, Durham, NC.

During embryogenesis of many species, the earliest cell fate decision is tightly linked to nuclear positioning. Control of nuclear positioning arises from the integration of the cell cycle oscillator and associated cytoskeletal dynamics. Yet, the mechanisms that ensure that the correct number of nuclei move to the appropriate place remain poorly understood. Here, using light sheet microscopy, we show that in *Drosophila* embryos spindle orientation controls which nuclei migrate towards the cortex and which ones remain inside the embryo, determining the fate of the nuclei and the number of cells undergoing development. Combining computational methods inspired by integral geometry and genetic manipulation of cellcycle genes, we show that spindle orientation is controlled by topological spindle-spindle interactions and not by internuclear distance. Using arguments describing the behaviour of space-filling systems, we develop a general theory for topological dependency in microtubule structures. Our work reveals a unique topological interplay of microtubule mechanicsin density homeostasis and cell fate determination.

SG309

**Vacuole-nucleus packing interactions in Saccharomyces cerevisiae revealed by soft X-ray tomography.**

**M. Mirvis**, W. F. Marshall; Biochemistry & Biophysics, University of California, San Francisco, San Francisco, CA.

Cell anatomy, the cumulative morphology and relative arrangement of subcellular structures, forms distinct spatio-geometric patterns. On the mesoscale, these patterns arise from complex organelle interactions, the biochemical basis of which has been well-studied. However, the spatial and morphological interdependencies between whole organelles, and their roles in establishing global cell anatomy, are poorly understood. To what extent is cell anatomy a reflection of organelle confinement within the limited space of the crowded cytoplasm? We collected whole-cell tomograms of *S. cerevisiae* cells imaged with isotropic nanometer resolution by soft X-ray tomography in collaboration with the National Center for X-ray Tomography, with organelles (nucleus, vacuole, lipid droplet) segmented by a custom machine learning algorithm. 3D analysis of this high quality morphological data reveals that subcellular space constraint induced by vacuole enlargement is associated with disrupted size scaling trends of the nucleus and lipid droplets. We observe a tendency for nuclear flattening in large-vacuole cells, and joint 3D shape analysis of apposing vacuole-nucleus pairs also shows correlated curvature at the interface in some of these cells. These results suggest that asymmetric deformations may arise due to vacuole-nucleus packing. Finally, we adapted the Allen Institute's integrated modeling pipeline to yeast cells in order to quantify the key constrained and variable features in yeast and to determine the effects of vacuole enlargement on whole-cell anatomy. Dimensionality reduction of joint spherical harmonics-based shape representations of cell-nucleus-vacuole complexes point to relative organelle size, positioning, shape, and orientation as principal components of cell anatomical variation in wild-type and large-vacuole mutant cells. Further analyses of these co-varying geometric features, and biophysical deformation simulations based on observed shapes in interacting organelle pairs analysis between WT and large-vacuole mutants, are currently being developed. This work will ultimately aim to quantify the magnitude of disorganization in a highly packed cytoplasm and characterize the impacts of shifting physical interdependencies between organelles on global cell anatomy.

SG310

**Integration and decoupling of adhesion, signaling, and actin dynamics in mesenchymal cell migration.**

**J. Haugh**[1], R. Appalabhotla[1], M. Butler[2], A. Chandra[1], J. Bear[2]; [1]North Carolina State University, Raleigh, NC, [2]University of North Carolina, Chapel Hill, NC.

Orchestration of cell migration is essential for proper wound healing, embryonic development, and immune responses and involves the integration of adhesion, signaling, and cytoskeletal subprocesses across spatial and temporal scales. In mesenchymal cells such as fibroblasts, which are chiefly responsible for wound repair, integrins engage extracellular matrix (ECM) proteins and cluster to form nascent adhesion complexes, which mediate signal transduction as well as physical interaction with the F-actin cytoskeleton required for protrusion of the cell’s leading edge. Mathematical modeling has proven valuable in understanding both isolated and integrative aspects of leading-edge motility. In this talk, two related efforts combining partial differential equation models and live-cell microscopy experiments will be presented. The first is an attempt to integrate, at a coarse level of molecular granularity, the dynamics of nascent adhesions, signal transduction pathways, and actin dynamics and mechanics. The model explicitly connects adhesion-based signaling to F-actin branch nucleation and generation of barbed ends that share mechanical load, promoting polymerization of F-actin that stabilizes nascent adhesions. Accordingly, mechanical failure of this feedback loop can occur via

destabilization of adhesion/F-actin linkages or/and stalling of actin polymerization under load. Stress on the F-actin network applied by Non-muscle Myosin II can contribute to these failure modes. Another major insight concerns the role of F-actin turnover, which on the one hand reduces total F-actin available for stabilizing adhesions while also resupplying actin monomers. Analysis of the model suggests that monomer recycling is the dominant effect; the loss of distal adhesions is less important than the flux of G-actin back to the leading edge. The second part of the talk will present an experimental strategy to decouple these dynamics and to examine G-actin transport in particular. Fibroblasts allowed to adhere to ECM-coated islands of a particular size, surrounded by a non-adhesive coating, are compelled to adopt a thin 'spillover' extension, throughout which actin treadmilling is rapid and steady. The steady-state nature of the system dramatically simplifies modeling of the actin dynamics in the non-adhesive region. In conjunction with fluorescence recovery after photobleaching (FRAP), we quantified the opposing fluxes of F- and G-actin, revealing that diffusion alone cannot account for the transport of G-actin to the leading edge. The framework we have established can be used to explore other biological questions concerning actin network dynamics.

## Subgroup: Chromatin Dynamics in DNA Damage, Repair, and Chromosome Segregation

SG311

**Impact of replication stress on human centromere inheritance.**

**D. Foltz**, A. S. Lee, J. Bodner, P. Vadlamani, N. Kelleher; Biochemistry and Molecular Genetics, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Human centromeres control the proper segregation of chromosomes during mitosis by specifying single region of the chromosome to act as a linkage to the microtubule spindle during mitosis. Centromeric chromatin is defined by the assembly of centromere-specific nucleosomes containing the histone H3 variant CENP-A. The centromeric nucleosome contributes to the recruitment of the constitutive centromere associated network of proteins present throughout the cell cycle. Replication of DNA necessitates the disassembly of nucleosomes, both canonical and centromeric. Replication stress is associated with the uncoupling of replicative machinery and the formation single stranded DNA, and occurs in response to chemical inhibition of DNA replication and through activations of oncogenes such as mutated RAS and overexpression of Cyclin E or Myc. Our data show that replication stress leads to loss of CENP-A during DNA replication. Likewise, the centromere and CENP-A nucleosomes exhibit changes in their interaction partners in response to replication stress. The effects of these altered interactions and persistence of the effects on centromere identity will be discussed. In addition, the overexpression of CENP-A occurs in many cancers and is correlated with the upregulation of the FoxM1 and MybL2 transcription factors. In cell culture, this leads to the incorporation of CENP-A into the arms of chromosomes, and the chaperones responsible have been descried previously. The effects of CENP-A nucleosomes occupying general chromatin is unclear. Our current data show that CENP-A interferes with the DNA damage response, leading to reduced H2A.X phosphorylation. The mechanisms by which CENP-A is disrupted in cancer, the effects of ectopic CENP-A will be discussed.

SG312

**The histone co-chaperone DNAJC9 prevents CENP-A mislocalization and chromosomal instability.**

V. Balachandra[1], **M. Basrai**[1], R. Shrestha[1], C. Hammond[2], S. Lin[1], I. Hendriks[2], L. Chen[1], N. Caplen[1], R. Chari[1], T. Karpova[1], K. Cheng[1], M. Nielsen[2], A. Groth[2]; [1]National Cancer Institute, NIH, Bethesda, MD, [2]University of Copenhagen, Copenhagen, DENMARK.

The histone H3 variant CENP-A mediates faithful chromosome segregation by defining the centromeric (CEN) chromatin which is required for the assembly of kinetochore. The CEN localization of CENP-A is essential for chromosomal stability. Overexpression of CENP-A is observed in many cancers, and we and others have shown that overexpressed CENP-A mislocalizes to non-CEN regions and promotes chromosomal instability (CIN). Despite the clinical significance, pathways that prevent mislocalization of CENP-A have not been fully explored. Here, we performed a genome-wide RNAi screen to identify gene depletions that exhibit increased nuclear CENP-A levels which correlates with CENP-A mislocalization. Among the top hits of the screen, we identified CHAF1B/p60, a component of the CAF-1 complex that facilitates the delivery of newly synthesized H3/H4 dimers to the replication fork during DNA synthesis and DNAJC9, a molecular co-chaperone. We recently reported that CHAF1B prevents mislocalization of CENP-A and CIN. Here we focus on DNAJC9, a molecular co-chaperone with newly identified roles in supplying properly folded histone H3.1/2/3-H4 dimers during replication and transcription. We hypothesized that defects in the availability of properly folded H3 upon knockdown of DNAJC9 favors a chromatin landscape conducive for CENP-A mislocalization. Our results showed that cells with DNAJC9 KD showed an enrichment of CENP-A in the chromatin fraction and, mislocalization to non-CEN regions. DNAJC9 KD did not affect CENP-A transcript levels rather increased the stability of CENP-A. Consistent with a correlation between CENP-A mislocalization and CIN, we observed a higher incidence of micronuclei and defective chromosome segregation in DNAJC9 KD cells. One of the lead candidates from interactome studies that showed increased interaction with CENP-A upon DNAJC9 KD was MCM2, which along with DNAJC9 forms a H3-H4 chaperone complex. Depletion of MCM2 in DNAJC9 KD cells reduced the mislocalization of CENP-A suggesting that MCM2 contributes to the mislocalization of CENP-A in DNAJC9 KD cells. Furthermore, cells overexpressing catalytically inactive DNAJC9 which traps H3-H4 in a presumably misfolded state, showed increased CENP-A mislocalization. Taken together, we provide evidence to support our hypothesis that defects in the availability of properly folded H3-H4 favors CENP-A mislocalization upon DNAJC9 KD. In summary, we have used a comprehensive approach to identify factors that prevent mislocalization of CENP-A and defined a novel role for DNAJC9 in preventing mislocalization of CENP-A and, CIN. Our studies provide insights into pathways that prevent mislocalization of overexpressed CENP-A and how defects in DNAJC9 function may promote CIN in human cancers.

SG313

**Liquid-liquid phase separation of DNA damage proteins impacts DSBs mobility and repair efficiency.**

**M. Locatelli**[1], J. Lawrimore[1], H. Lin[2], S. Sanaullah[3], G. Holzwarth[3], K. Bonin[3], J. Liu[2], P. Vidi[4], K. Bloom[1]; [1]University of North Carolina At Chapel Hill, Chapel Hill, NC, [2]Department of Physics, Indiana university-Purdue University, Indianapolis, IN, [3]Wake Forest University, Winston Salem, NC, [4]Laboratoire InGenO, Institut de Cancérologie de l'Ouest, Angers, FRANCE.

Chromatin motions change in response to DNA damage and may be an important factor for genome maintenance. The molecular determinants of chromatin mobility, and the relation between mobility and DNA repair outcomes are however still poorly understood. We previously measured the motions of

chromatin microdomains in mammalian cells expressing histones with photoactivatable probes and found that chromatin motions are correlated within a 1.5 - 2 μm range. After DNA damage, chromatin motions become less correlated. This loss of correlation requires liquid-liquid phase separation (LLPS) of the 53BP1 repair factor. To better understand the role of LLPS in the DNA damage response and the subsequent DNA repair efficiency, we are using a yeast model system featuring LacO and TetO DNA arrays surrounding an inducible DNA break site and expressing LacI-GFP and TetR-CFP. This system enables us to measure the distance separating broken DNA ends and to track chromatin motions in the presence or absence of a DNA break. Perturbing LLPS with 1.6 hexanediol increases the spatial separation between the break ends. Those spatially separated extremities show increased mobility compared to those that stay in close proximity when LLPS is not perturbed. Strikingly, phase separation disruption decreases the efficiency of break resolution, demonstrating its crucial role in DNA repair processes. To decipher more specifically the role of LLPS in chromatin mobility and repair, we are repeating our measurements in yeast mutants with impaired molecular condensation for specific repair factors. Altogether, we demonstrate a fine-tuned spatiotemporal control of chromatin motions and an important role of molecular condensation of DNA repair proteins. Those events may facilitate repair at the break sites and prevent deleterious contacts of DSBs, thereby reducing the risk of genomic rearrangements.

SG314

**Identification of Regulators of 3D Centromere Organization.**

**K. Guin**, A. Keikhosravi, L. Ozbun, G. Pegoraro, T. Misteli; National Cancer Institute, NIH - Bethesda, MD, Bethesda, MD.

Centromeres are specialized genomic loci that assemble the multi-protein kinetochore complex, which in turn interacts with the spindle apparatus to ensure faithful chromosome segregation during cell division. While the centromere function is highly conserved, centromeric DNA sequences and the structure of kinetochores can be diverse across different species. Faithful centromere function enables sister kinetochores to correctly attach to the spindle fibers from opposite poles and to congregate into the metaphase plate in a timely manner for an error-free completion of cell division. The physical location of centromeres in the 3D space of the interphase cell nucleus is thus a crucial determinant of genomic stability. Intriguingly, the 3D organization of centromeres is also highly variable across species. While in many yeast species all centromeres form a single cluster at the nuclear periphery, multiple heterochromatin embedded clusters of centromeres are observed in *Drosophila* and mice. In human cells, however, clustering is less pronounced, with some centromeres clustering near the nucleolus in multiple cell types. The molecular mechanisms that determine the 3D location and extent of clustering of centromeres in the human cell nucleus are largely unknown. By quantitatively measuring the degree of centromere clustering in several cell lines, we find cell-type specific differences in the 3D organization of centromeres. For example, in colon cancer epithelial cells have a central arrangement of centromeres, while centromeres are well distributed in retinal epithelial cells. To identify the molecular regulators of centromere clustering, we performed an arrayed, high-throughput imaging based CRISPR knockout screen for 1068 chromatin-associated proteins in these two cell types. The results identified several gene groups encoding components of the nucleolus, kinetochore, the cohesin-condensin complex, nuclear pores, and the replication machinery as factors that either increase or decrease clustering of centromeres. Changes in the centromere clustering phenotype for most of these centromere clustering factors is cell-cycle dependent. These observations provide novel insights into mechanisms of centromere clustering and of higher order genome organization in general.

SG315

**Nuclear Envelope Reformation is linked to Centromere Assembly.**

**A. Landeros**[1], D. Wallace[1], C. Magdongon[1], A. Rahi[1], P. Suraneni[1], M. Amin[1], M. Chakraborty[1], S. Adam[1], D. Foltz[2], D. Varma[1]; [1]Cell and Developmental Biology, Northwestern University Feinberg School of Medicine, Chicago, IL, [2]Biochemistry and Molecular Genetics, Northwestern University Feinberg School of Medicine, Chicago, IL.

It is known that many Lamin A-associated proteins (LAAPs) that are key constituents of the nuclear envelope, assemble at the "core" domain of the chromosomes during nuclear envelope reformation and mitotic exit. However, the identity and function of the chromosomal core domains remained ill-defined. It has been shown that the expression of a mutant Lamin A/C in cells causes defective distribution of the centromeres within the interphase nuclei. Here, we show that a distinct section of the core domain overlaps with the centromeres/kinetochores of the chromosomes during mitotic telophase. The core domain can thus be demarcated into a kinetochore proximal core (KPC) on one side of the segregated chromosomes and the kinetochore distal core (KDC) on the opposite side, close to the central spindle. We thus tested of if centromere assembly is connected to nuclear envelope re-formation. We find that centromere assembly is markedly perturbed after the inhibition of function of Lamin A/C and the core-localized LAAPs, BANF1 and Emerin. We also find that the LAAPs exhibit a biochemical interaction with the centromere and inner kinetochore proteins. Consistent with these observations, normal mitotic progression and chromosome segregation was severely impeded after this inhibition of LAAP function. Intriguingly, the inhibition of centromere function also interferes with the assembly of the LAAP components at the core domain, suggesting a mutual dependence of LAAP and centromeres for their assembly at the core domain. Finally, we find that the localization of CENP-A chaperones and accessory proteins, including the Mis18 complex and HJURP to the centromeres in both telophase and interphase was markedly affected in LAAP-inhibited cells. Our evidence points to a model where LAAP assembly at the core domain might serve a key function in the loading of new copies of centromeric proteins to this site during or immediately after mitotic exit.

SG316

**Misregulation of cell cycle dependent methylation of budding yeast CENP-A causes kinetochore dysfunction and chromosome instability.**

**P. Mishra**[1], W. Au[1], P. Castineira[1], N. Ali[2], J. Stanton[3], L. Boeckmann[1], Y. Takahashi[1], M. Costanzo[4], C. Boone[4], K. Bloom[3], P. Thorpe[2], M. Basrai[1]; [1]NCI, NIH, Bethesda, MD, [2]Queen Mary University, London, UNITED KINGDOM, [3]UNC, Chapel Hill, NC, [4]University of Toronto, Toronto, ON, CANADA.

Centromere (*CEN*) identity is marked epigenetically by specialized nucleosomes containing evolutionarily conserved *CEN*-specific histone H3 variant Cse4 (CENP-A in humans), an essential component for high-fidelity chromosome transmission. However, the epigenetic mechanisms modulating Cse4 function have not been fully characterized. Here, we show that cell cycle mediated methylation of Cse4-R37 regulates kinetochore integrity and faithful chromosome segregation. We determined that methylation of Cse4 is cell cycle regulated with maximum enrichment of methylated Cse4-R37 and its accumulation at *CEN* chromatin occur in the mitotic cells. Methyl-mimic *cse4-R37F* mutant exhibits reduction in the levels of *CEN*-associated kinetochore proteins, synthetic lethality with kinetochore mutants and chromosome instability (CIN) phenotype, suggesting that constitutive methylation of Cse4-R37 throughout the cell cycle is detrimental to kinetochore function and chromosome segregation. We further show that SPOUT

methyltransferase Upa1 contributes to methylation of Cse4-R37 and its overexpression results in increased levels of Cse4-R37 methylation in mitotic cells, and CIN phenotype. Overall, our results provide new insights into molecular mechanisms by which epigenetic modifications such as methylation of kinetochore proteins play an important role in preventing CIN.

SG317

**Endogenous selfish yeast plasmid utilizes condensed chromosomal sites to ensure its stable propagation.**

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The 2-micron plasmid, a multicopy selfish genetic element (~40-100 copies), is a ubiquitous resident of the budding yeast nuclei with high mitotic stability. Interestingly, the plasmid appears to challenge the evolutionary logic by being present in a high copy number irrespective of any fitness advantage conferred to the host. It imposes a selective growth disadvantage of 1-3 % on yeast cells over plasmid-free cells. More mechanistic insight has revealed that the plasmids couple their segregation with the host chromosome via host-coded proteins involved in chromosome segregation by hitchhiking themselves at the chromosomal sites. We have demonstrated that these sites are not random. Instead, they are the condensed and repetitive regions of chromosomes: Telomeres (*TELs)*, rDNA, and tRNA gene clusters. To pinpoint the host proteins involved in the hitchhiking process, we have shown that condensin and *RSC2* chromatin remodeling complexes contribute to this 3D organization of the 2-micron plasmid in the yeast genome. Our findings provide evidence that these proteins, along with the plasmid-encoded Rep proteins, are required for the plasmid-chromosome association and for successful segregation. Thus, our work unravels the mechanistic insight of the remarkable stability of the 2-micron plasmids in budding yeast. This knowledge will turn out to be crucial in understanding the analogous strategies utilized by other episomal elements, including episomes of certain mammalian viruses, and how hitchhiking of selfish genetic elements plays a role in the course of evolution.

SG318

**FANCD2 binds to multiple DNA substrates during double-strand break repair.**

**C. Joyce**, A. Wang, J. Bacal, C. Cruz, K. Bains, Z. Rodriguez, C. D. Richardson; Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA.

Eukaryotic cells repair double strand breaks (DSBs) according to two main mechanisms: (1) non-homologous end-joining (NHEJ) and (2) homology-directed repair (HDR). NHEJ, the default pathway throughout the cell cycle, is contingent on ligation of the two strands of the DNA double helix. In contrast, HDR is active during S and G2 phases and requires a DNA template molecule from which a polymerase can synthesize the damaged DNA. Previous work by our lab identified that the Fanconi Anemia (FA) pathway, and especially FANCD2, plays a key role in DNA repair, influencing DSB repair outcomes. As such, the objective of this study was to identify the DNA substrates of FANCD2 during DSB repair in order to identify the mechanism by which the FA pathway promotes HDR. We found that FANCD2 localizes to both the DSB molecule and the template molecule during Cas9-mediated DSB formation and that this localization depends on FANCD2 ubiquitination by the FA core complex. We report that FANCD2 performs distinct activities on each substrate. The binding of FANCD2 to extrachromosomal template molecules improves template stability and nuclear abundance. These

results support a model in which FANCD2 is loaded onto template molecules independently from DSB repair processes. At the DSB, FANCD2 localization does not contribute to resection activity, nor promote the formation of recombination intermediates, nor influence chromatin openness. Instead, our results are consistent with a model in which FANCD2 associates with the chromatin mark H4K20me2 and promotes acetylation of H4K16. This acetylation restricts the accumulation of the NHEJ factor 53BP1 at the site of the DSB. We show that reduced 53BP1 at the site of the DSB correlates with increased FANCD2 abundance and HDR outcomes. Altogether, our findings provide mechanistic explanations for how the FA pathway promotes HDR.

SG319

**DNA damage drugs cause nuclear softening, blebbing, and rupture.**

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The nucleus must maintain stiffness to protect shape and compartmentalization of the nucleus all in order to ensure proper function. Defects in nuclear stiffness caused from chromatin and lamin perturbations produce abnormal nuclear shapes common in diseases such as Hutchinson-Gilford progeria, prostate cancer, and Breast cancer. Loss of nuclear shape via protrusions called blebs result in nuclear rupture well-reported to lead to nuclear dysfunction. DNA damage is the most well-reported dysfunction that arises from nuclear rupture that results in the diffusion of nuclear contents into the cytoplasm and vice versa. However, it remains unknown how increased DNA damage effects nuclear stiffness, shape, and ruptures, which could create a negative feedback loop. To determine if increased DNA damage alters nuclear physical properties, we treated MEF cells with DNA damage drugs Bleomycin and Cisplatin at low and high concentrations. Micromanipulation force measurements of high concentrations of both bleomycin and cisplatin resulted in decreased chromatin-based nuclear mechanics but no change in lamin A strain stiffening at long extensions relative to wild type. Interestingly, low levels of cisplatin did not alter nuclear stiffness. Time lapse imaging of nuclei treated with high levels of Bleomycin and Cisplatin results in increased nuclear blebbing and ruptures, while low level maintain shape and compartmentalization. Time lapse imaging also reveals that the loss of nuclear shape and rupture occurs during interphase and is independent of mitotic failure well-reported in these DNA damage drugs. To determine the mechanism of the effect of DNA damage on nuclear mechanics, we examined the level of euchromatin, heterochromatin, and lamin A was measured via immunofluorescence in cells treated with Bleomycin and Cisplatin. Our data shows a decrease in both euchromatin and heterochromatin levels which puzzled us at first. We then identified that bulk levels of histones were decreased in DNA damage drug treatments. Thus, DNA damage drugs cause nuclear softening, blebbing, and rupture through a chromatin decompaction mechanism through loss of histones.

SG320

**RHINO directs MMEJ to repair DNA breaks in mitosis.**

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DNA double-strand breaks (DSBs) are toxic lesions that can lead to genome instability if not properly repaired. Breaks incurred in G1 phase of the cell cycle are predominantly fixed by non-homologous end-joining (NHEJ), while homologous recombination (HR) is the primary repair pathway in S and G2.

Microhomology-mediated end-joining (MMEJ) is intrinsically error-prone and considered a backup DSB repair pathway for HR and NHEJ. In this study, we uncover MMEJ as the major DSB repair pathway in M phase. Using CRISPR/Cas9-based synthetic lethal screens, we identify subunits of the 9-1-1 complex (RAD9A-HUS1-RAD1) and its interacting partner, RHINO, as critical MMEJ factors. Mechanistically, we show that the well-established role of 9-1-1 and RHINO in ATR signaling does not fully explain its function in MMEJ. Our findings reveal an unexpected and indispensable role of RHINO in guiding mutagenic repair to M phase. We demonstrate that phosphorylation of RHINO by PLK1 stimulates its interaction with Polymerase theta (Polθ) and facilitates the recruitment of the polymerase for repairing DSBs in mitosis. Additionally, we provide evidence that mitotic MMEJ repairs persistent DNA damage that originates in S phase but is not repaired by HR. The latter findings could explain the synthetic lethal relationship between *POLQ* and *BRCA1/2* and the synergistic effect of Polθ and PARP inhibitors. In summary, our study identifies MMEJ as the primary pathway for repairing DSBs during mitosis and highlights an unanticipated role for RHINO in directing mutagenic repair specifically to M phase.

SG321

**3D genome dynamics during double-strand break repair.**

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The mammalian genome is folded into a complex three dimensional structure that modulates DNA dependent processes such as transcription, replication and DNA repair. Here, we studied the role of the 3D genome in the repair of double-strand DNA breaks (DSB), one of the most lethal forms of DNA damage. In order to do so, we developed multi-target CRISPR, a technology to efficiently induce DSBs at defined regions of the genome with high temporal resolution. We delivered multi-target CRISPR into mammalian cells and performed Hi-C to study damage-induced changes in 3D genome organization. Interestingly, we found that Cas9 breaks induce an increase in chromatin contacts between the cut site and its neighboring chromatin, resembling a loop extrusion event with the cut site acting as a loop anchor. Knock-down of the Rad21 cohesin subunit abolished the formation of these cut-centered contacts, pointing to cohesin as the loop extruding factor. Treatment with a DNA-Pkcs inhibitor promoted the homologous recombination (HR) pathway and enhanced loop formation at DSBs; whereas pharmaceutical inhibition of MRE11 - a factor required for HR - abolished the loop, thus suggesting that loop extrusion is an HR event. Notably, auxin-induced CTCF depletion showed no effect on DSB-induced loop formation, indicating that DSB act as CTCF-independent loop anchors. Finally, using time-course Hi-C measurements, we found that loop extrusion occurs at late stages (>1h) of the repair process, shortly after formation of the recombination filament. Based on this data, we propose a model where cohesin loop extrusion mediates the scanning of resected DNA ends for their neighboring chromatin, in order to facilitate a synapsis between the DSB and its homologous template.

SG322

**Mapping DNA damage propensity by low and high LET ionizing radiation with respect to epigenetic states.**

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Space radiation, which includes gamma rays and galactic cosmic rays, presents one of the most difficult challenges to the health of astronauts engaged in long-term space travel. Chromatin, the protein-bound form of genomic DNA, protects DNA from ionizing radiation induced DNA damage. Simulations made using the code RITRACKS (Relativistic Ion Tracks)[1] show histone protection as evidenced by the overall lower number of damage events, and a higher number of damage sites in the solvent accessible surface area (SASA)[2] of DNA packaged into a nucleosome. However, the relationship between the local organization of chromatin and the sensitivity of the underlying genomic locus to radiation damage is not yet well understood. To investigate how chromatin structure shapes patterns of DNA damage caused by ionizing radiation, we measured the density of breaks in DNA across different functional genomic compartments defined by histone marks, nuclear localization, or accessibility to enzymatic cleavage in irradiated human fibroblasts (BJ cell line) and pooled-donor human umbilical vein endothelial cells (HUVECs). We irradiated cells with $^{16}O$ and $^{56}Fe$ at the NASA Space Radiation Laboratory at Brookhaven National Lab, and measured cell proliferation and DNA repair dynamics at various timepoints after irradiation. We also used RICC-seq and End-seq, two DNA sequencing-based methods, to measure genome-wide single strand DNA breaks (SSBs) and double strand DNA breaks (DSBs), respectively. Under X-ray and gamma ray irradiation, we see that heterochromatic regions show a lower break density compared to controls, whereas regions associated with euchromatic, ATAC-seq accessible regions have higher break density for both ssDNA and dsDNA breaks. These data will provide the basis for future chromatin state-specific nanodosimetry and evaluation of explicit simulations of DNA damage events by simulation codes like RITRACKS.

SG323

**Cohesin-dependent control of genomic stability during β-cell growth and maturation.**

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Accumulation of DNA damage has been implicated as a key early event in dysfunction of the insulin producing pancreatic β-cell in diabetes, but the underlying molecular mechanisms remain unclear. β-cells rely on replication for their growth during neonatal life, followed by cell-cycle exit and functional maturation. As replication is extremely prone to DNA damage, we hypothesize that β-cells may be highly vulnerable to DNA damage in neonatal life, which if unresolved could impact their growth and long-term health. Using morphometric quantification of β-cell replication and DNA damage in early (p7) and late (p21) neonatal mice, we established that the replicating neonatal β-cells are highly prone to DNA damage. Single-cell RNA seq in neonatal islets further confirmed the high DNA damage vulnerability of replicating β-cells. High-resolution imaging of replicating β-cell nuclei showed that DNA damage foci were most abundant in early mitosis, suggesting its resolution with cell-cycle progression. Replicating β-cell nuclei also displayed phase-specific loss and re-emergence of Cohesins (regulators of chromatin

architecture) with mitotic progression. The cohesin complex comprising Smc3, Smc1a/b, and Rad21 is crucial for DNA repair, replication fidelity, and transcription, yet its role in β-cells has not been investigated. Genome-wide profiling of Cohesin recruitment using ChIP-seq with Smc1a revealed that Cohesin programs the transcriptional control of β-cell genomic stability, growth, and functional maturity during the transition from neonatal life to adulthood. β-cell specific knockout of the Smc3 (Smc3BKO) in mice led to high DNA damage and altered nuclear morphology in replicating β-cells, resulting in β-cell deficit through reduced proliferation and increased apoptosis. Smc3BKO mice also exhibited compromised β-cell identity and progressive glucose intolerance. Notably, loss of Smc3 led to the depletion of the entire Cohesin complex (absence of Smc1a and Rad21) in β-cell. Finally, ChIP analysis of human T2D islets showed dysregulation of Cohesin binding at regions associated with β-cell identity, DNA damage, and senescence, highlighting the importance of Cohesins in maintaining β-cell genomic stability and health. Our study establishes that Cohesins protect the β-cell genome during the highly vulnerable neonatal growth phase and Cohesin defects in neonatal life not only impair β-cell growth but may also predispose to future functional failure and diabetes.

SG324

**DNA damage response to double-strand breaks in postmitotic neurons.**

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Every day, tens of thousands of DNA lesions occur in the genome of every mammalian cell. Among these, DNA double-strand breaks (DSBs) are a particular threat to genome stability. Neurons are especially vulnerable due to their long lifespan and limited regenerative capacity. Persistent or inaccurately repaired damage could irreversibly disrupt brain function and homeostasis. Understanding the fundamental processes that help safeguard neuronal genomic information could help identify potential targets for the prevention and treatment of age-related brain disorders. Yet, much of what we know about DNA repair stems from studies of dividing cells, and how postmitotic neurons maintain their genome is still poorly understood. To address this critical gap in knowledge, we have developed new experimental approaches, both *in vitro* and *in vivo* in mice, to selectively inactivate Non-Homologous End Joining (NHEJ)-mediated DSB repair in postmitotic neurons. Specifically, the core NHEJ factor Xrrc4 is ablated which completely inactivates the final DSB ligation step. We use multidisciplinary approaches, including super-resolution light microscopy, cryo-electron tomography, and proteomics to characterize the molecular composition and architecture of these DSB repair foci and to elucidate how neurons respond to DSBs. We find that NHEJ inactivation in postmitotic neurons results in the spontaneous accumulation of large DSB repair foci. A subset of these DSB repair foci can be resolved upon Xrcc4 re-expression. Strikingly, our preliminary analysis has revealed no signs of premature aging, such as senescence, inflammation, or cell death in mice with genetic inactivation of *Xrcc4.* These latter findings suggest that neurons show a fundamentally different response to DNA damage than diving cells.

SG325

**A mortality timer based on nucleolar size triggers loss of nucleolar integrity and catastrophic genomic instability.**

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Increasing age is the highest risk factor for cancer, metabolic disorders, cardiovascular disease, and neurodegenerative disorders, yet the molecular alterations that drive aging are unclear. Genome

instability is a hallmark of aging, with the highly repetitive ribosomal DNA (rDNA) within the nucleolus being particularly prone to genome instability. Nucleolar enlargement accompanies aging in organisms ranging from yeast to mammals while treatment with many antiaging interventions results in small nucleoli. Here we report that an engineered system to reduce nucleolar size robustly extends yeast replicative lifespan i.e., the number of times a cell divides, in a manner independent of protein synthesis or rDNA silencing. Instead, we find that when nucleoli expand beyond a size threshold, its biophysical properties are altered allowing entry of proteins that are normally excluded from the nucleolus, including the homologous recombinational repair protein Rad52. This triggers rDNA instability due to aberrant recombination, catastrophic genome instability and imminent death. These results establish that nucleolar expansion is sufficient to drive aging. Moreover, nucleolar expansion beyond a specific size threshold is a mortality timer, as the accompanying disruption of the nucleolar condensate boundary results in catastrophic genome instability that ends replicative lifespan.

SG326

**A post translational switch for linker histone H1 coacervation at stalled replication forks.**

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The H1 linker histone family is the most abundant group of eukaryotic chromatin-binding proteins, maintaining the epigenetically silent heterochromatin regions of the nucleus. Canonically, it is thought to function by binding at the nucleosome dyad axis and inducing local chromatin compaction. However, our understanding of the nature of the H1:DNA interaction remains incomplete. We previously described an additional, unexpected coacervation of H1 with single-stranded nucleic acids observed through *in vitro* biophysical methods and *in vivo* live-cell imaging experiments. Through single-molecule fluorescence and force microscopy, we directly visualized H1 coalescing around and stabilizing single-stranded DNA in a model replication fork. We further demonstrated in live-cell imaging experiments that inducing replication fork stalling in cells causes an increase in H1 puncta that can fuse in a manner characteristic of liquid-liquid phase separation (LLPS) and colocalize with PCNA present at replication forks. Notably, this coalescence occurred when ATR kinase was inhibited, suggesting a function for H1 independent of the previously described ATR-mediated heterochromatinization.In our following mechanistic investigations, we have identified post-translational modifications of the disordered H1 C-terminal domain which tune this LLPS interaction. Moreover, this tuning is abrogated in carcinoma-associated mutations of H1, implicating these PTMs in maintaining genome stability. To further characterize the interactions of modified vs unmodified H1s, we have developed an intein splicing based methodology to precisely and specifically label our H1 variants of interest with a photocrosslinkable moiety. Using this, we have been able to trap and pull down H1 interactors for mass spectrometry identification. We are now working to complete our understanding of this phenomenon to understand H1's underlying role in safeguarding DNA replication.

SG327

**Protection of DNA from mechanical stresses at the nuclear envelope.**

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**Protection of DNA from mechanical stresses at the nuclear envelope.** Eukaryotic cells encapsulate DNA molecules within a protein-membrane scaffold called the nuclear envelope, in part to protect the genome from mechanical stresses caused by internal and external forces as a consequence of cell migration, cell division or tissue compression. While it is known that an improperly assembled nuclear envelope is associated with DNA damage, cancer and laminopathies it remains unclear how the nuclear envelope organises DNA to provide mechanical resistance in the first place. Here, we show how protein components of the nuclear envelope organise DNA to increase the mechanical stability of DNA molecules to withstand forces that would otherwise damage double-stranded DNA. We use a bottom-up reconstitution approach coupled with optical tweezer experiments, theoretical physics and cell biology to investigate how the chromatin cross-bridging factor BAF the inner nuclear membrane protein LEM2 and DNA self-organize. Our data point to a mechanism where BAF-LEM2 organize DNA into regular loops and further jointly compact into large fluid-like dna-protein co-condesates. As a result, DNA is mechanically protected in several ways: Firstly, DNA respond to increasing external forces with increased compaction forces, suggesting that the protein-bound DNA molecule is mechanically stabilized with an emerging increase in DNA-protein stiffness. Secondly, forces with the potential to damage DNA percolate through the co-condensate, reducing the mechanical stress on individual DNA strands. Finally, the formation of protein-DNA co-condensates can actively bridge multiple DNA strands in *cis*-configuration, indicating further modes of protective mechanism by BAF-LEM2 complex. Our study quantitatively describes a conserved and previously unknown molecular mechanism by which cells use the nuclear envelope as a self-assembling buffer against spontaneously occurring mechanical stress to protect DNA from damage, suggesting a first preventive mechanism that adds to the cell's ability to repair DNA damage or responsively change chromatin properties.

SG328

**Replication-dependent histone (Repli-Histo) labeling specifically visualizes physical properties of euchromatin/heterochromatin in living human cells.**

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Recent advanced imaging studies have revealed that euchromatin in higher eukaryotic cells mainly forms condensed liquid-like domains, rather than "open" ones [1-3]. While such chromatin organization provides a higher-order regulation of genome functions, it raises intriguing questions: How are euchromatin and heterochromatin physically different in living cells? How relevant is the difference to the regulation of genome functions?

To approach these questions, we have developed two imaging techniques. The first is single-nucleosome imaging and tracking [2, 4], which present the physical nature of chromatin in living cells. Using this, we have demonstrated that nucleosomes in living human cells fluctuate dynamically, mainly driven by thermal fluctuation [5]. The second technique is a novel chromatin labeling method, replication-dependent histone labeling (Repli-Histo labeling), which can specifically label four groups of genome

regions from euchromatin (early replicated regions) to heterochromatin (late replicated regions) based on DNA replication timing. Combining our Repli-Histo labeling with the single-nucleosome imaging, we have found that more euchromatic regions show larger local chromatin motion, whereas more heterochromatic regions have smaller motion. This observation reveals a nearly one-to-one correlation between replication timing and local chromatin motion. Since local chromatin motion can regulate the chromatin accessibility [6, 7], our findings suggest that the genome-wide landscape of local chromatin motion in euchromatin and heterochromatin can regulate DNA replication timing.

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SG329

**Chromatin mobility is altered by DNA damage and is determinant for DNA repair.**

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Chromatin mobility is influenced by and may regulate genome functions, including the DNA damage response. We mapped the motions of chromatin microdomains in mammalian cells using structured illumination of photoactivatable histone probes. We found that these motions are highly heterogenous between cells and within individual cell nuclei. DNA damage reduces heterogeneity and alters chromatin motions: motions are globally reduced but higher mobility is retained at break sites. These effects are driven by context-dependent changes in chromatin compaction. Using an X-ray system integrated to the microscope, we detected a rapid decrease in global chromatin motions, occurring within minutes following break induction. This miniature irradiation system enables us to associate single-cell analyses of chromatin motions and DNA damage sensing and resolution, visualized with a fluorescent DNA double-strand break sensor. We also identified a small molecule altering chromatin dynamics in mammalian and yeast cells. Results from these combined approaches indicate that the capacity of cells to process radiation-induced DNA damage as well as DNA repair outcomes depend on chromatin mobility. Overall, our data show that chromatin motions are finely tuned after genomic insults and that chromatin motions influence DNA repair.

SG330

**Labeling of blocked replication fork proteome by BLOCK-ID identifies the chromatin reader protein TRIM24 in promoting Alternative Lengthening of Telomeres.**

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Chromatin-based DNA damage response (DDR) pathways are vital for the maintenance of genome integrity, including during DNA replication. Obstacles within the genome can obstruct DNA replication,

contributing to genome instability, cell fitness and human diseases. Emerging evidence has highlighted the vital functions for chromatin reader proteins in these pathways that act by recognizing modified histone and non-histone proteins in the vicinity of DNA damage to promote recognition and resolution of the lesion. As an example, acetylated lysines are recognized by bromodomain-containing "reader" proteins and our previous work has demonstrated that over 1/3rd of the 42 human BRD proteins are recruited to sites of DNA damage and are required for efficient DNA double-strand break (DSB) repair. To extend these findings beyond DSB repair, we have developed a new technique called BLOCK-ID, which allows for label free proteomic profiling using proximity ligation and visualization of proteins at blocked replication forks generated at LacO arrays bound to Lac repressor (LacI) fused to a tagged Bio-ID enzyme. Through BLOCK-ID, we identified and validated nearly a third of human BRD proteins associated with blocked replication forks in human cancer cells. These include the BRD protein TRIM24, which we demonstrate to be involved in replication stress response pathways. Further proteomic analyses identified TRIM24 involvement in Alternative Lengthening of Telomeres (ALT). TRIM24 deficiency resulted in telomere loss, reduced telomere sister chromatid exchanges and reduced hallmarks of ALT including c-circles and ALT-associated PML bodies (APB). Mechanistically, TRIM24 ALT activity is governed by post-translational modifications (PTMs), including histone acetylation by the histone acetyltransferases p300/CBP, which were found to be required for TRIM24 recruitment to stressed telomeres. Mechanistically, forced tethering of TRIM24 revealed PIAS1 SUMO-dependent interactions between TRIM24 and PML that function to dynamically associate telomeres with APB bodies to support ALT telomere synthesis. Collectively, this study delivers a new methodology for surveying replication stress proteins in human cells and identifies a PTM-driven chromatin pathway reliant on the acetylation effector TRIM24 that promotes ALT. This study provides a rationale for therapeutically targeting this PTM-mediated TRIM24 pathway in ALT cancers.

## Subgroup: Cytoskeleton Dynamics in the Nervous System: From Molecular Mechanisms to Therapeutic Interventions

SG331

**Regulation of dendritic microtubules by novel crosslinker proteins.**

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Dendrites of vertebrate neurons contain microtubules of both orientations. In some neurons, dendritic microtubules of each orientation are interspersed with one another, but in hippocampal neurons, as the dendrites mature, the microtubules of differing orientations segregate. Minus-end-out microtubules form a central bundle while plus-end-out microtubules are dispersed around that bundle. This results in more efficient organelle transport and puts the plus-end-out microtubules in closer proximity to the dendritic spines, which rely on the intermittent invasion of plus-end-out microtubules. During the formation of the dendrite's microtubule polarity pattern, molecular motors slide minus-end-out microtubules into the dendrite along microtubules of the opposite orientation, but then the microtubules become less mobile due to crosslinker proteins. In the case of hippocampal neurons, among these crosslinkers are proteins that also segregate the two orientations of microtubules and regulate them differently. We are pursuing two candidate proteins never before studied in neurons, namely oMAP4 and KIFC1. oMAP4, a newly discovered splice variant of the ubiquitous microtubule-associated protein 4, has a unique projection domain that endows it with microtubule crosslinking/immobilizing properties. oMAP4 is strongly expressed in hippocampus and concentrates in

dendrites concomitantly with the segregation of microtubules of the two orientations. KIFC1, a motor protein best known for its role in mitosis, can slide microtubules, crosslink microtubules to prevent their sliding, and also interact with membrane-related proteins and microtubule end-binding proteins. We are pursuing a hypothesis whereby oMAP4 organizes the centralized bundle of minus-end-out microtubules in hippocampal dendrites, while KIFC1 organizes the dynamic plus-end-out microtubules at the periphery of that bundle. One or both of these proteins crosslink microtubules of opposite orientation at the interface of these two zones, which prevents the centralized microtubule bundle from sliding back into the cell body. In this manner, these two proteins work coordinately to generate the high efficiency microtubule array of hippocampal dendrites. Our studies have implications for how corruption of these mechanisms leads to dendritic atrophy in neurodegenerative diseases such as Alzheimer's.

SG332

**Doublecortin stabilizes and stiffens microtubules to allow growth cone advance in physiological environments.**

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Doublecortin (DCX) is a microtubule-associated protein that is critical for brain development. Although DCX is most highly expressed in the developing central nervous system, the exact molecular function of DCX in neuron morphogenesis remains unknown and controversial. We demonstrate that DCX function is intimately linked to its microtubule-binding activity. By using human induced pluripotent stem cell (hiPSC)-derived cortical i$^3$Neurons genome engineered to express EGFP-tagged DCX from the endogenous locus, we find that at physiological expression levels, DCX-MT interactions become highly polarized very early during neuron morphogenesis and that DCX is enriched only on straight microtubules in advancing growth cones. Quantitative comparison of DCX-EGFP fluorescence intensity with dodecahedron nanocages with known EGFP number further allowed us to measure how many DCX molecules are bound per growth cone microtubule unit length. We find that at this saturation microtubule-bound DCX molecules begin to impede lysosome transport to the cell periphery in RPE cells, and thus can potentially control growth cone organelle entry. In addition, by comparing control, DCX-EGFP and knockout DCX -/Y i$^3$Neurons, we find that DCX is essential for microtubules to extend into the growth cone periphery by reducing the catastrophe frequency and the depolymerization rate. Although DCX -/Y i$^3$Neuron morphogenesis was indistinguishable from control i$^3$Neurons on stiff tissue culture plastic, DCX -/Y i$^3$Neuron morphogenesis was inhibited on soft 400 Pa laminin-coated polyacrylamide gels that better mimic the viscoelastic environment of brain tissue and failed to respond to brain-derived neurotrophic factor (BDNF) gradients in passive microfluidic devices. Together with traction force microscopy data, we propose a model in which DCX-decorated, mechanically stiff growth cone microtubules provide an essential counterforce to actomyosin generated contractile forces to allow growth cone advance and neurite elongation that otherwise cannot be explained by very weak and transient adhesion-mediated forces in the periphery of human i$^3$Neuron growth cones. These data provide a new mechanistic understanding of how DCX mutations cause lissencephaly-spectrum neurodevelopmental disorders by impacting growth cone dynamics during neuron morphogenesis in physiological environments.

SG333

**Spastin locally amplifies microtubule polymerization to pattern the axon for presynaptic cargo accumulation.**

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Neurons rely on long-range trafficking of synaptic components to form and maintain the complex neural networks that encode the human experience. with a single neuron capable of forming thousands of distinct en passant synapses along its axon, spatially precise delivery of the necessary synaptic components is paramount. How these synapses are patterned, and how efficient delivery of synaptic components is regulated, remains largely unknown. Here, we reveal a novel role for the microtubule severing enzyme spastin in locally enhancing microtubule polymerization to influence presynaptic cargo pausing and retention along the axon. In human neurons derived from induced pluripotent stem cells (iPSCs), we identify sites stably enriched for presynaptic components, termed “protosynapses”, which are distributed along the axon prior to the robust assembly of mature presynapses apposed by postsynaptic contacts. These protosynapses are capable of cycling synaptic vesicles, are enriched with spastin, and are hotspots for new microtubule growth and synaptic vesicle precursor (SVP) pausing/retention. Disruption of neuronal spastin, either by CRISPRi-mediated depletion or transient overexpression, interrupts the localized enrichment of dynamic microtubule plus ends and diminishes SVP accumulation at protosynapses. Using an innovative human heterologous synapse model, where microfluidically isolated human axons recognize and form presynaptic connections with neuroligin-expressing non-neuronal cells, we reveal that neurons deficient for spastin do not achieve the same level of presynaptic component accumulation as control neurons. We propose a model where spastin acts locally as an amplifier of microtubule polymerization to pattern specific regions of the axon for synaptogenesis and guide synaptic cargo delivery.

SG334

**Polarized microtubule remodeling transforms the morphology of reactive microglia and drives cytokine release.**

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Microglial reactivity is a pathological hallmark in many neurodegenerative diseases. During stimulation, microglia undergo complex morphological changes, including loss of their characteristic ramified morphology, which is routinely used to detect and quantify inflammation in the brain. However, the underlying molecular mechanisms and the relation between microglial morphology and their pathophysiological function are unknown. Here, proteomic profiling of lipopolysaccharide (LPS)-reactive microglia identified microtubule remodeling pathways as an early factor that drives the morphological change and subsequently controls cytokine responses. We found that LPS-reactive microglia reorganize their microtubules to form a stable and centrosomally-anchored array to facilitate efficient cytokine trafficking and release. We identified cyclin-dependent kinase 1 (Cdk-1) as a critical upstream regulator of microtubule remodeling and morphological change *in-vitro* and *in-situ*. Cdk-1 inhibition also rescued tau and amyloid fibril-induced morphology changes. These results demonstrate a critical role for microtubule dynamics and reorganization in microglial reactivity and modulating cytokine-mediated inflammatory responses.

SG335

**Molecular mechanism of Epothilone B-induced CNS axon repair by cryo-ET.**

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Neurons of the central nervous system (CNS) often fail toregenerate after traumatic brain injury and result in long-term complicationsor even death of the individual. We know that this failure to regenerate is dueto external factors like lesion scar or lack of an intrinsic growth potentialof the neuron. Understanding molecular basis of axonal regeneration will aid inbetter designing the therapeutics to alleviate injury associated complicationsin patients. Many studies have attempted to understand the clues of inducingaxon repair in central nervous system; however, the molecular basis of axonregeneration is sparsely known. Here, we present the molecular mechanismunderlying thalamic neuron regeneration induced by an FDA-approved drug,Epothilone B (EpoB). We set up a cryo-electron tomography-based experimentalpipeline to examine thalamic neuronal regeneration after axotomy. Strikingly,our cryo-ET revealed that the regeneration was facilitated by the shooting-outof the microtubule bundles beyond the injury site. Our cryo-EM analysis showedthat the lattice of the microtubules-shoots were stabilized by EpoB until a newgrowth cone was established. During the repair, various types of vesicles andER, also tubulin clusters were shuttled using the stabilized microtubule-shootsas a scaffold, supplying materials necessary for axonal repair. We identified anovel axonal repair mechanism that uses the EpoB-stabilized microtubules as aninfrastructure pipeline until axon recovers from its homeostatic imbalances andwe therefore present the molecular basis for EpoB-mediated neural recovery.

SG336

**Microtubule homeostasis and response to damage at the mechanistic level and in the human neuron.**

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Microtubules (MTs) are pivotal to diverse processes ranging from cell division to ciliogenesis and underlie cellular morphology and intracellular transport. MTs are dynamic polymers that self-assemble from α and β-tubulin heterodimers. MT growth rate and abundance is therefore directly dependent on the concentration of αβ-tubulin heterodimers—also referred to as the “soluble tubulin pool”. The soluble tubulin pool, which lies at the nexus of transcription of tubulin isoforms and their translation, sequestration, turnover and incorporation into MTs, is regulated through a co-translational feedback process termed “tubulin autoregulation” whereby cells rapidly degrade tubulin mRNAs upon an influx of soluble tubulin. The response is initiated by the protein TTC5 which binds to the translating ribosome and the first conserved amino acids of β-tubulins as they emerge from the ribosome exit channel, marking the associated transcript for decay. Tubulin autoregulation is widely-conserved across organisms and mutations in TTC5 are linked to intellectual disability in humans. However, despite its importance to cellular physiology, no studies to date have examined this pathway in specific cell-types or tissues and the downstream factors mediating decay remained unknown. Here, we identify the CCR4-Not deadenylase complex as a requisite mediator of tubulin autoregulation. CCR4-Not recruitment is dependent on subunits CNOT10 and CNOT11, which form an obligate functional unit within the complex that recruits the degradation machinery to tubulin mRNAs. Using iPSC-derived cortical-like neurons, we

demonstrate that tubulin autoregulation is operative in human neurons and is TTC5- and CCR4-Not complex-dependent. By RNA-sequencing, we show that this process acts in the soma, but also in distal neurites, demonstrating that neurons locally regulate tubulin transcripts in response to excess soluble tubulin. Knock-down of TTC5 and CNOT10 or knock-in of an autoregulation-null mutant of TTC5 decreases growth cone MT dynamics and neurite outgrowth, implicating tubulin autoregulation in neuronal fitness. Understanding how tubulin abundance and MT stability are regulated has important implications for neurodevelopmental and neurodegenerative diseases characterized by cytoskeletal dysregulation as well as acute injuries that trigger rapid MT depolymerization in neuronal processes.

SG337

**Doublecortin regulates neuronal migration by editing the tubulin code.**

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Doublecortin (DCX) is a neuronal microtubule-associated protein (MAP) that binds directly to microtubules via two Doublecortin (DC) domains. The DC domains sense the nucleotide state, longitudinal curvature, and protofilament number of the microtubule lattice, indicating a role in the regulation of microtubule structure in neurons. Mutations in DCX cause lissencephaly and subcortical band heterotopia (also known as double-cortex syndrome) due to impaired neuronal migration. To better understand the role of DCX in neuronal migration, we developed a model system based on induced pluripotent stem cells (iPSCs). We used CRISPR/Cas9 to knock out the Dcx gene in iPSCs and differentiated the cells into cortical neurons. Compared to control neurons, the DCX-KO neurons showed reduced velocities of nuclear movements. The reduced velocities correlated with an increase in the number of neurites early in the neuronal development process, consistent with a neuronal migration phenotype and previous findings in a DCX-KO mouse model. Neurite branching is regulated by a host of MAPs and other factors, as well as by microtubule polymerization dynamics. However, microtubule dynamics were unchanged in DCX-KO neurons, with similar growth rates, lifetimes, and numbers. Rather, we observe a significant reduction in tubulin polyglutamylation in DCX-KO neurons. Polyglutamylation is usually abundant in neurons and regulates microtubule severing enzymes and intracellular trafficking by molecular motors. Consistently, we observe that lysosomes in DCX-KO neurons show a reduction of their processivity. We propose that the reduction of polyglutamylation leads to increased neurite branching and thus reduced neuronal migration. Our results indicate an unexpected role for DCX in the homeostasis of the tubulin code.

SG338

**Presynaptic actomyosin regulates the mechanobiology of the neuromuscular junction.**

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Neuromuscular junctions (NMJs) are evolutionary ancient, specialized contacts between neurons and muscles that facilitate the movement of animals in a variety of habitats. with an elastic modulus between two to three orders of magnitude lower than that of muscles, nervous tissue is significantly “softer”. In addition, axons and NMJs must endure the mechanical strain of a lifetime of muscle contraction. Thus, “softer” NMJs evolved to maintain shape, function, and plasticity in parallel with contact with “harder” muscles. NMJs are susceptible to aging and neurodegenerative disorders, and their loss precedes axonal degeneration and subsequent neuronal loss. However, the strategies used by neurons to manage these mechanical challenges remain unstudied. To address this question, we

examined the actin cytoskeleton and the actin-based non-muscle myosin II motor (MyoII) at *Drosophila* larval NMJs. Actin and myosin (actomyosin) mediate contractility as a highly conserved mechanism for generating and responding to cellular forces. We identified distinct actin subpopulations, including a long-lived, low-turnover presynaptic actin core traversing the NMJ, which partly co-localizes with MyoII. Neuronal RNAi of MyoII induced disorganization of the actin core, suggesting that these actomyosin structures might have contractile properties. Interestingly, neuronal RNAi of MyoII also decreased MyoII levels in the postsynaptic muscle proximal to boutons, suggesting that neuronal actomyosin rearrangements may propagate their effects trans-synaptically. Next, we looked at integrin receptors as mediators of the trans-synaptic MyoII-induced changes. We detected a decrease of integrin levels at the NMJ upon MyoII knockdown, indicating that neuronal actomyosin defects lead to rearrangement of these receptors in neurons and in surrounding muscle tissue. In sum, we demonstrate that a previously unrecognized subpopulation of presynaptic actomyosin maintains the neuronal mechanical continuum, transmits its rearrangements to the neighboring muscle tissue and thus regulate the mechanobiology of the neuromuscular junction in concert with integrin receptors.

SG339

**Coronin 1A role in TRIM67-regulated neuronal morphogenesis.**

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Neurons progress through several developmental stages during the establishment of neuronal circuitry. The dramatic neuronal shape changes during these morphological events depend on the cytoskeleton remodeling machinery. Previously, our lab discovered that the E3 ubiquitin ligase TRIM67 is highly enriched in the developing cortex and is essential for appropriate neuronal morphogenesis. We found that TRIM67 localizes to growth cone and the actin-rich filopodia structures. Interestingly, neurons lacking TRIM67 exhibit aberrant axonal growth cone morphology and have defects in netrin-dependent axon turning and branching, but the underlying molecular mechanisms by which TRIM67 regulates neuronal morphogenesis is unknown. As E3 ubiquitin ligases typically have multiple substrates, we conducted an unbiased proximity-dependent biotin identification assay to identify putative TRIM67 substrates. This identified Coronin 1A (Coro1A) as a potential TRIM67 binding partner. We validate that Coro1A and TRIM67 are interacting partners and combining biochemistry assays with computational structural/docking analysis we show that the C-terminal coiled-coil domain of Coro1A is essential for Coro1A:TRIM67 interaction. Coro1A is a conserved F-actin binding protein crucial for actin dynamics, yet its role in brain development remains elusive. We demonstrate that Coro1A protein level increases during neuronal maturation. Through TIRF microscopy and immunofluorescence staining, we find that Coro1A is enriched in growth cones of developing cortical neurons and localizes to the base of filopodia structures, particularly at the base region. Additionally, using cultured cortical neurons from *Coro1A*$^{+/+}$ and *Coro1A*$^{-/-}$ littermates, we show that Coro1A is crucial for netrin-dependent growth cone and axon branching morphology, similar to TRIM67. Our ongoing analysis is investigating the functional consequences of loss Coro1A:TRIM67 interaction in neuronal shape change. Finally, we demonstrate that Coro1A is ubiquitinated by immunoprecipitating Coro1A under denaturing conditions. *These findings suggest a novel role for Coro1A in regulating neuronal morphogenesis, possibly functioning downstream of TRIM67-mediated pathway.*

## Subgroup: Invention of New Cell Types and Multicellular Assemblies

SG340

**Coral-algal endosymbiosis characterized using RNAi and single-cell RNA-seq.**

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Corals form an endosymbiotic relationship with the dinoflagellate algae Symbiodiniaceae, but ocean warming can trigger algal loss, coral bleaching and death, and the degradation of ecosystems. Mitigation of coral death requires a mechanistic understanding of coral-algal endosymbiosis. Here we report an RNA interference (RNAi) method and its application to study genes involved in early steps of endosymbiosis in the soft coral *Xenia* sp. We show that a host endosymbiotic cell marker called LePin (lectin and kazal protease inhibitor domains) is a secreted *Xenia* lectin that binds to algae to facilitate the interaction between algae and host cells to support the formation of algae-containing *Xenia* cells. We also identified scavenger receptors and proteins involved in phagocytosis that facilitates algae uptake by *Xenia* cells and possibly immune modulation of *Xenia*. The evolutionary conservation of domains in LePin among marine anthozoans performing endosymbiosis suggests a general role in coral-algal recognition. Our work sheds light on the phagocytic machinery and posits a mechanism for symbiosome formation, helping in efforts to understand and preserve coral-algal relationships in the face of climate change.

SG341

**The rise of symbiotic cell types across coral-algal symbioses.**

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Reef-building corals rely on nutrition from dinoflagellate algae symbionts residing intracellularly in specialized gastrodermal cells. The advent of single-cell transcriptomics has allowed the identification of molecular processes at cellular resolution in many organisms, including in a few recent studies on symbiotic corals. Yet how these processes arise upon symbiosis establishment and the degree to which these are universal remain unclear. Here we leverage these technologies to shed novel insight into organismal changes during symbiosis establishment, by using single-cell transcriptomics in aposymbiotic versus symbiotic primary polyps of two species of key reef-building corals infected with the same compatible but heterologous symbiont, the Symbiodiniaceae strain *Breviolum minutum* SSB01. We find that in both *Acropora digitifera* and *A. tenuis*, gastrodermal cell types in aposymbiotic polyps appear to be converted to a completely new cell type in the symbiotic polyps once they take up algae symbionts. The algae-containing cells are highly conserved across species, and likely represent an ancient cell type with the propensity to establish photosymbiosis, contradicting current concepts about convergent evolution of symbiotic cell types in photosymbiotic cnidarians. For example, pathways for transport of sterol lipids and sugars are commonly upregulated, as are transcription factors whose function in this context remains unexplored. We also used ultra-sensitive hybridization chain reaction (HCR) RNA-FISH

to visualize expression of key marker genes in these cell types, allowing high-resolution imaging in intact coral holobionts. Taken together, such comparisons allow us to unravel deeply conserved cellular processes in these ecologically critical organisms.

SG342

**Building bilaterian brains: innovations in molecular machinery, neuron types and circuits.**

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We study the evolution of animals from a cell type perspective, with a particular focus on the origin and rise of their most fascinating trait, which is the centralized nervous system. For this, we track the evolution of neurons and other constituent cell types across animal phylogeny, focusing on slow-evolving animals. We have chosen the nereid *Platynereis dumerilii* as a powerful model for comparative studies, with morphologically similar organisms already existing as early as the Cambrian. Taking advantage of its highly stereotypic development, we have built the *PlatyBrowser,* which combines expression atlases and volume electron microscopy to establish a link between gene expression and cellular and subcellular morphologies for all cell types of the three-segmented young worm. This stage is composed of 12,000 cells only but already possesses a fully differentiated rope-ladder-like nervous system with brain and segmental ganglia, which can be regarded a blueprint of the adult nervous system.

Building on this unique resource, we have started to map single-cell objects to the *PlatyBrowser* comprising transcriptomes for more than 100k nuclei. For the nervous system, we identify separate neuron type families representing distinct neuronal circuits and brain parts, including (1) the mechanosensory girdle, (2) the motor girdle, (3) the apical nervous system (ANS), and (4) the mushroom body neurons. We find that these genetically distinct families also differ morphologically, both regarding cellular morphologies and projections. Furthermore, we are working on the alignment and/or integration of the *Platynereis* single-cell data with that of various other bilaterians, to explore the evolutionary conversation of neuron type families and circuits across larger phylogenetic distances, with particular emphasis on the evolution of sensory associative brain centers.

These efforts lead us to outline a basic set of neuron type families and circuits conserved across the bilaterian divide. We trace the assembly of the constituting neuron types into a basic set of neural circuits that make up the bilaterian brain, and postulate that modified versions of these families are found in today's brains in all major bilaterian lineages.

SG343

**Associative learning and monoamine signaling in the sea anemone *Nematostella vectensis*.**

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The nervous system is an essential feature of most animals, allowing for remarkable adaptive capacities, as well as complex behavioral and cognitive abilities. Although this complex network of specialized cells - neurons - plays a crucial role in animals, numerous intriguing questions persist regarding its origin and evolution. To address such questions, one approach relies on the comparison of features of distantly related clades in order to make assumptions on their last common ancestor. Cnidarian species, such as sea anemones and jellyfish, possess a nerve net, which lacks centralization. As the sister group to bilaterian animals, they are particularly well-suited for studying the evolution of neurons and nervous

systems. However, whilst progress has been made in describing the development of cnidarian nerve nets, the molecular mechanisms underlying behavior remain poorly understood. Here, we implemented methods to track and analyze the behavior of the sea anemone *Nematostella vectensis* to further probe its capacity to form associative memories. By showing that this brainless sea anemone can learn using an aversive conditioning approach, we not only shed light on a novel aspect of cnidarian behavior, but also root associative learning before the emergence of central nervous systems in bilaterians. Monoamines, like dopamine or serotonin, are essential neuromodulators required for cognitive functions such as learning and memory formation, as well as fundamental homeostatic processes such as sleep and feeding. Similarly, the evolutionary origin of the genes required for monoaminergic modulation is uncertain. Using a phylogenomic approach, we showed that most of the genes involved in monoamine production, modulation, and reception originated in bilaterians. Despite this assertion, we uncover the expression of a key dopamine synthesis enzyme, as well as two putative dopamine receptors, in the nervous system and pharyngeal cells of *Nematostella vectensis* polyps. Thus, our results suggest that some elements may be present and expressed in cnidarian neurons, drawing a complex picture on the evolution of the role of monoamines in animals. Altogether, this work highlights the unexpected complexity of the "simple" nerve nets of these brainless animals, and provides valuable insights on the ancestral characteristics of the first nervous systems.

## Subgroup: Physical Biology of the Cytoplasm

SG344

**Diffusive lensing as a novel means of subcellular compartmentalization.**

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The cytoplasm is a complex, heterogeneous milieu that exhibits emergent biophysical properties. These have increasingly been shown to modulate key cellular processes including cell division and stress adaptation. One such property, space-dependent diffusivity, has remained largely unexplored despite being ubiquitous in the subcellular environment. In this work, we use agent-based simulations to show that inhomogeneous diffusivity can lead to the accumulation and depletion of particle concentration in viscous and fluid zones, respectively. The resulting nonequilibrium organizing principle, which we call "diffusive lensing," requires neither membranes nor phase separation, and may have fundamental relevance to transport processes across myriad cellular environments. Our simulations also reveal cascading effects of diffusive lensing, including: i) altered clustering rates, ii) anomalous sampling of available zones for diffusion and, notably, iii) the creation of "expressways" between viscous hotspots accounting for faster dynamics of diffusing particles not seen in otherwise uniform-viscosity states. In summary, our work highlights the phenomenon of diffusive lensing as a potentially key driver of mesoscale dynamics in the cytoplasm, with possible implications for biochemical processes.

SG345

**Probing the Role of Cytoplasmic Actin in Cell Division using Cycling Xenopus Egg Extract.**

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Cell division dynamics are driven by cytoskeletal networks. In small somatic cells, cortical actin is usually thought to dominate cytomechanics, while in large embryonic cells the cortex may be less dominant. We sought to explore the role of actin networks in bulk cytoplasm during cell division in a large cell system, *Xenopus* eggs. In this system, cleavage planes are positioned by microtubule asters which grow out from spindle poles and do not reach the cortex for most of anaphase. How intracellular forces are generated to move these millimeter-scale asters without direct interactions with cell cortex has been under debate, and cytoplasmic actomyosin far from the cortex might play a role. To address these questions, we developed a *Xenopus laevis* egg extract system in which actin-intact, cycling extract was spread under oil. This system allows live imaging over multiple cell cycles and access to mechanical and biochemical perturbation. When F-actin was intact, forces exerted by a moving needle propagated for hundreds of microns and asters behaved as a deformable gel. When F-actin was depolymerized, forces did not propagate over long distances and the aster was easily fragmented by the needle. These observations suggest cytoplasmic actin is a dominant mechanical player in our system. By tracking cytoplasmic flows, we found that during anaphase with actin intact, cytoplasm and daughter centrosomes moved apart in a coherent manner. We observed inward flows in the gap between sister asters which we interpret as driven by hydrodynamic forces, suggesting that outward moving asters advect cytosol to generate hydrostatic pressure gradients. When F-actin was depolymerized, the aster cytoplasm no longer moved coherently outwards and centrosome motion was altered. We propose that microtubule asters behave as actin-dependent coherent gels capable of advecting cytosol when they move, which is a new mechanical picture. Functionally, we found that F-actin enables better segregation of chromosomes and daughter compartments; actin depolymerization between asters enables them to move apart from the future cleavage plane. We are now attempting to disentangle the mechanical contribution of myosin and intermediate filaments. By studying all three cytoskeletal networks in the cell-free system, we are generating novel insights into egg cytomechanics, in particular revealing central roles of bulk cytoplasmic F-actin.

SG346

**Robust trigger wave speed in *Xenopus* cytoplasmic extracts.**

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Positive feedback loops and spatial coupling enable complex cellular processes to rapidly amplify and propagate their activities as trigger waves through the cytoplasm. The cytoplasm contains a high concentration of macromolecules, which allows for fast reactions, but at the same time, it is crowded enough to limit diffusion. Here, we used the *Xenopus* cytoplasmic extract system, which can be systematically diluted and concentrated, to investigate the effect of cytoplasmic concentration on the spatial propagation of mitosis and apoptosis. We observed that the speeds of mitotic and apoptotic waves are robust to perturbations in cytoplasmic concentration. This robustness can be attributed to the opposing effects of active species concentration and molecular crowding. The trade-off between these two effects can be described by a theoretical curve with a flat optimum which ensures robust wave speeds across a wide range of cytoplasmic concentrations.

SG347

**Endoplasmic reticulum network connectivity directs heterogeneous protein transport and facilitates calcium signaling.**

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The endoplasmic reticulum (ER) forms an interconnected network of membrane-bound tubules that serves to process, store and distribute critical cellular components such as secreted proteins, lipids and ions. Defects in ER shaping proteins responsible for generating its archetypal reticulated structure reduce the efficiency of transport through the network and are associated with neurodegenerative diseases. Thus, understanding the connection between ER morphology and its function as a transport network is an important problem in cellular biophysics. We use numerical simulations and *in vivo* imaging to probe the impact of ER structure and network dynamics on the dispersion and delivery rates of proteins and calcium ions. Photoactivated membrane proteins in COS7 cells exhibit highly non-uniform spreading to adjacent regions, in a manner consistent with diffusion through a spatial network with variable connectivity. An interesting implication of this heterogeneous transport is the existence of 'hot spots' in the ER where diffusive reactants are more likely to find one another. In addition to its role in the early secretory pathway, the ER forms an important reservoir for calcium storage and release. We explore the effect of network structure on the transport of calcium ions and calcium-binding buffer proteins, using both simulations and genetic perturbations of ER morphogens. Our physical model demonstrates that efficient calcium release requires mobile low-affinity buffer proteins moving through a well-connected network structure. Quantification of local calcium signaling events in live cells shows that reduction in network connectivity both hinders transport and limits calcium release magnitude. Our work combines *in vivo* experiments with quantitative image analysis, numerical simulations, and mathematical modeling to reveal how the distinctive network morphology of the ER facilitates transport processes underlying a variety of cellular functions.

SG348

**Complex network morphology encourages mixing in *Physarum polycephalum*.**

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Syncytial cells have many nuclei in a shared cytosol and can be found across the tree of life from fungi to the mammalian placenta. This form of organization can generate especially large and complex cells such as seen in *Physarum polycephalum,* which in its syncytial state can grow up to centimeters in size and house thousands to hundreds of thousands of nuclei. Despite its size, *Physarum* displays highly coordinated foraging activity, allowing it to solve mazes for nutrients, and avoid environmental insults. For these large, morphologically-complex cells, diffusion is insufficient to distribute nutrients and information across a dynamic network. To circumvent this, *Physarum* rely on actin-myosin dependent cytoplasmic shuttling to integrate nutrients and information acquired at the foraging front of the cell to distal parts of the cell network. Despite these oscillatory flows, which can in some cases reach up to ~1mm/sec, it is unclear how local regions of the cytoplasm are effectively mixed with one another given the laminar flow. Conversely, it remains mysterious how these large cells balance cytoplasmic streaming

to maintain asymmetry and build a directed response to environmental stimulus. Our work suggests that populations of nuclei which exhibit lower mobility in the cytoplasmic flow may act as local regulators of cytoplasmic composition. In addition, we show that complex network topology in *Physarum,* despite incurring a significant energy cost in network flow resistance, contributes to cytoplasmic mixing. These observations lend insight into how these incredibly large cells maintain local responses, while still integrating information and nutrients at centimeter length scales.

SG349

**Single-Molecule and Super-Resolution Microscopy of Fast Intracellular Diffusion.**

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Intracellular diffusion plays a vital role in defining the kinetics and timescales of biological processes. Due to the fast motion of biomolecules and the often sub-micrometer size of intracellular structures, it remains difficult to elucidate how unbound molecules navigate through the cell. Our recently developed technique, single-molecule displacement/diffusivity mapping (SM*d*M), inherits the super-resolution capabilities of single-molecule localization microscopy (SMLM), but uniquely maps out the motion and diffusivity of freely diffusing molecules at the nanoscale. Here we present our recent SM*d*M efforts to interrogate how different physical parameters, including charge and molecular size, impact biomolecular diffusion in the living cell and *in vitro*. We thus quantify diffusion coefficients *D* up to 350 $\mu m^2/s$ for water-soluble dyes and dye-tagged nucleotides, and unveil that their intracellular diffusion is dominated by vast regions of high diffusivity ~60-70% of that *in vitro*, hence lifting a paradoxical speed limit of intracellular diffusion as suggested by previous studies. Moreover, we uncover strong net charge-driven interactions between biomolecules both in live cells and *in vitro*, showing that for both systems, the procession of positive net charges asymmetrically drives diffusion slowdown due to interaction with the predominately negatively charged environments of bio-macromolecules. In collaboration with Dr. Coral Y. Zhou and Prof. Rebecca Heald, we have further started work on *Xenopus laevis* egg and embryo extract model systems to quantify how molecular diffusion shapes fundamental biological processes in cell division, cell-size control, and intracellular organization.

SG350

**Regulation of the Biophysical Properties of the Cytoplasm in Mammalian Cells.**

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The interior of a cell is highly dynamic and crowded, yet extremely well organized. The accurate cytoplasmic organization is critical for all cellular reactions ensuring efficient molecular interaction and survival of the cell. Despite its significance, the molecular mechanisms that control the cytoplasmic organization remain poorly investigated. One limitation to investigating such mechanisms had been the lack of faithful tools and techniques to monitor changes in the cytoplasmic organization. Single particle tracking (SPT) measurements using tracer probes called passive rheology have emerged as a powerful tool to study the intracellular environment and provide a quantitative assessment of the biophysical properties of the cytosol. Here, we investigate the change in the biophysical properties of the cytoplasm of mammalian cells in different growth conditions. We probed the diffusive properties of the cytoplasm using the recently developed 40 nm genetically encoded multimeric (GEM) particles as tracer probes (Carlini et.al 2020, Delarue et.al 2018). To visualize and track the mobility of GEMs we performed lattice light sheet microscopy. We demonstrate that upon carbon source starvation and inhibition of oxidative

phosphorylation the mobility of GEMs is severely altered. Single particle tracking and mean square displacement analysis reveal that intracellular diffusion is significantly reduced in these conditions. By contrast, inhibition of translation dramatically enhances the mobility of 40 nm GEMs. In summary, our findings demonstrate that as an adaptive response to changes in their environment, cells alter the biophysical properties of their cytoplasm, and we discuss the underlying mechanisms of regulation.

SG351

**Cellular adaptations of yeast to freeze-thaw.**

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We study the adaptation of Saccharomyces cerevisiae when exposed to cycles of freeze (77K) and thaw (298 K), followed by growth. A "wild-type" population of cells has a ~2% survival rate upon exposure to a single cycle. Under experimental evolution, we found that the population survival fraction increased to ~70% within ~25 cycles. Compared to the initial wild-type cells, we found that these "evolved" cell types displayed increased mass density, lower cellular volumes and lower cytoplasmic fluidity, together with increased basal levels of a glass-forming sugar, trehalose. In addition, our measurements of the cellular growth rates and associated thermal fluxes using calorimetry indicate different nutrient utilization characteristics for the evolved cells. Combined with sequencing data, altogether, these point to an underlying mechanical adaptation of yeast to these extreme environmental perturbations.

SG352

**Mechano-dependent sorbitol accumulation supports biomolecular condensate.**

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Biomolecular condensates regulate a wide range of cellular functions from signaling to RNA metabolism, yet, the physiologic conditions regulating their formation remain largely unexplored. Biomolecular condensate assembly is tightly regulated by the intracellular environment. Changes in the chemical or physical conditions inside cells can stimulate or inhibit condensate formation. However, whether and how the external environment of cells can also regulate biomolecular condensation remain poorly understood. Increasing our understanding of these mechanisms is paramount as failure to control condensate formation and dynamics can lead to many diseases. Here, we provide evidence that matrix stiffening promotes biomolecular condensation *in vivo*. We demonstrate that the extracellular matrix links mechanical cues with the control of glucose metabolism to sorbitol. In turn, sorbitol acts as a natural crowding agent to promote biomolecular condensation. Using *in silico* simulations and *in vitro* assays, we establish that variations in the physiological range of sorbitol, but not glucose, concentrations, are sufficient to regulate biomolecular condensates. Accordingly, pharmacologic and

genetic manipulation of intracellular sorbitol concentration modulates biomolecular condensates in breast cancer - a mechano-dependent disease. We propose that sorbitol is a mechanosensitive metabolite enabling protein condensation to control mechano-regulated cellular functions. Altogether, we uncover molecular driving forces underlying protein phase transition and provide critical insights to understand the biological function and dysfunction of protein phase separation.

SG353

**Quantification and regulation of the material properties of synapsin condensates in vitro and in live cells.**

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The material properties of biomolecular condensates, namely surface tension and viscoelasticity have been suggested to play pivotal biological and pathological roles. Surface tension directly regulates the interaction between condensates and other cellular structures; meanwhile, viscoelasticity underlies aberrant phase transitions and controls the rates of biochemical reactions within condensates. However, the quantifications of condensate material properties, especially in living cells, remain challenging. Here, first we use micropipette aspiration (MPA) to quantify the material properties of synapsin condensates in vitro. We identified significant effects of synapsin domains, synaptic vesicles, and alpha-synuclein on the material properties of synapsin condensates. Furthermore, by integrating MPA with whole cell patch clamp, we quantified the material properties of alpha-synuclein and synapsin condensates in live cells and identified significant cooperativity between the two proteins. Our measurements shed light on the molecular mechanisms that underlie the regulation of condensate material properties in live cells.

SG354

**Macromolecular condensation buffers intracellular water potential.**

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Optimum protein function and biochemical activity critically depends on water availability inside cells. Macromolecules restrict the movement of “structured” water molecules in their hydration layers, reducing the available “free” bulk solvent and therefore the total thermodynamic potential energy of water, or water potential. Within concentrated macromolecular solutions like the cytosol, we found that modest changes in temperature greatly impact the water potential. We predicted that lower temperatures would reduce the available “free” intracellular water in a similar manner to external hyperosmotic conditions, and *vice versa* for higher temperatures and hypoosmotic conditions. We validated this duality of temperature and osmotic strength on cellular physiology: hypoosmotic

conditions mimicked high temperature in thermosensitive yeast mutants, whereas cold temperatures induced chondrocyte $Ca^{2+}$ signalling similar to hyperosmotic conditions. Most remarkably, simple manipulations of solvent thermodynamics were sufficient to prevent cell death upon extreme cold or heat shock. Physiologically, cells must sustain their activity in the face of fluctuating temperature, pressure and osmotic strength that impact water potential within seconds, but established mechanisms of water homeostasis act over much slower timescales, so we postulated the existence of a rapid compensatory response. We find this function is performed by water potential-driven changes in macromolecular assembly, particularly biomolecular condensation of intrinsically-disordered proteins, which is determined by the water potential rather than the concentration of any specific macromolecule. In cells, formation or dissolution of biomolecular condensates counteracted thermal or osmotic perturbations of water potential, which was robustly buffered in isolated cytoplasm. Our results indicate that biomolecular condensation constitutes an intrinsic biophysical feedback response that rapidly compensates for intracellular osmotic and thermal fluctuations. We suggest preserving water availability within the concentrated cytosol is an overlooked evolutionary driver of protein (dis)order and function.

## Subgroup: The Assemblies in and of Membranes: From Superresolution Imaging to Immunoengineering

SG355

**Cooperative phagocytosis of solid tumors by macrophages.**

**D. Discher**, L. Dooling; University Pennsylvania, Philadelphia, PA.

In solid tumours, an abundance of macrophages typically associates with a poor prognosis. However, clusters of macrophage in tumour-cell nests associate with survival in some tumour types. Here, by starting in vitro with cohesive 'tumouroids' of cancer cells that are opsonized via a mAb, we show that addition of macrophages leads to highly ordered clusters of macrophages which cooperatively phagocytose and eliminate the cancer cells. Efficacy requires the macrophage checkpoint CD47-SIRPα to be knocked out or deeply blocked with a second mAb, and also requires high macrophage numbers that reveal a 'Hill-like' signature of cooperativity. The approach translates to mice bearing poorly immunogenic tumours, and durable cures triggered production of tumour-opsonizing IgG that could also drive macrophage clusters in tumouroid elimination. Clustered macrophages subsequently disperse, with macrophage-macrophage interactions based on integrins activated by phagocytosis. Ongoing depletions of factors in engineered macrophages and various perturbations of cancer cells help to further clarify molecular mechanisms and also enhance potency.

SG356

**Phagocytosis via Mannose Receptors analyzed using Chemically Functionalized Lipid Particles.**

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Phagocytosis is a cellular process by which particles larger than 0.5 um are engulfed and digested after cell surface receptor triggering and actin polymerization. Phagocytic receptors that have been well studied are receptors for opsonins, like immunoglobulins (FcR) and complement (CR). By contrast, there

are some receptors whose signalling and internalization mechanisms have been overlooked, such as mannose receptor, a C-type lectin receptor that binds glycoconjugates with terminal mannose, fucose and N-Acetylglucosamine (GlcNAc), present in bacterial and yeast walls. Therefore, it is of major interest to better understand how professional phagocytic cells such as macrophages, perform phagocytosis via the mannose receptors. We have developed micrometer sized oil-in-water emulsion droplets functionalized with synthetic fluorescent amphiphilic glycolipids. Two glycolipids conjugated to a matched pair of fluorophores to perform Fluorescence Resonance Energy Transfer (FRET) were synthetised to demonstrate ligand-receptor clustering. Using this technique, coupled to Fluorescence Lifetime Microscopy (FLIM), as well as chemical inhibitors, we are investigating mannose receptor engagement in the early steps of phagocytosis, from receptor clustering to signalling and actin remodelling, in primary mouse and human macrophages. In conclusion, we have developed unique tools to shed light on the mechanisms of activation of phagocytic receptors of pathophysiological importance in relevant phagocytic cells.

SG357

**Immunoengineering can overcome the glycocalyx armor of cancer cells.**

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Cancer cells construct a glycocalyx with biochemical and physical attributes that protect against immune surveillance. Whether the structural properties of the glycocalyx also physically shield cancer cells from immune recognition has not been fully resolved. Here, we have developed an interference-based imaging tool called Scanning Angle Interference Microscopy (SAIM) to image the nanoscale physical dimensions and structural organization of the cellular glycocalyx. By combining genetic approaches and the imaging tools, we reveal how the surface density, glycosylation, and crosslinking of cancer-associated mucins contribute to the nanoscale material thickness of the glycocalyx, and further analyze the effect of the glycocalyx thickness on resistance to effector cell attack. We uncover a strong reciprocal relationship between the thickness of the glycocalyx and immune cell killing. Natural Killer (NK) cell-mediated cytotoxicity exhibits 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. Changes in glycocalyx thickness of approximately 10 nanometers can alter susceptibility to immune cell attack. Similar relationships were found for chimeric antigen receptor (CAR) NK and T cells against target cells with engineered glycocalyces. We further suggest strategies for overcoming the glycocalyx physical barrier through the cellular engineering of immune cells. Using leucine zipper-based conjugation approach, we generate engineered NK cells that tether glycocalyx-editing enzymes (GE) on the NK cell surface for improved penetration of the glycocalyx barrier and therefore induce higher NK cell-mediated killing.

SG358

**Regulation of cell surface crowding on cancer cells and immune cells.**

**D. S. W. Lee**, E. J. Y. Kim, E. A. Robey, D. A. Fletcher; UC Berkeley, Berkeley, CA.

Cells process external stimuli and interact with their environments using a wide array of macromolecules presented on their surfaces, typically at densities of ~20,000 molecules/$\mu m^2$ or more. These signals are vital for many physiological and disease-related processes, particularly in the context of the cancer, wherein potentially tumorigenic cells are identified and destroyed by the immune system

based upon their presentation of specific surface molecules. However, expression of tall, glycosylated proteins is thought to hinder recognition by immune cells in some cases. The ability of a crowded cell surface to inhibit immune recognition by preventing the engagement of ligands with receptors highlights the need to quantify not only specific interactions but also the emergent physical organization and material properties of cell surfaces.
To address this challenge, we recently developed a new molecular sensor to measure surface crowding. This probe, consisting of a DNA oligo modified with a cholesterol, a fluorophore, and a biotin moiety, can be easily integrated into live cells in suspension and used to quantify crowding by measuring what fraction of biotins are occupied by an anti-biotin antibody. First, we show that changing the composition of the surfaceome, e.g. by lentiviral overexpression of bulky surface proteins, measurably affects surface crowding. Then, we use the crowding sensor to investigate the regulation of surface crowding on cancer cells and in developing T cells. We find that tumorigenic cells tend to have crowded surfaces, mediated by glycosylated oncoproteins such as MUC-1, as demonstrated by CRISPR interference. Interestingly, as primary murine T cells mature from thymic progenitor cells into splenic CD4+ and CD8+ T cells, their surfaces become much less crowded. We propose that this may indicate large-scale surface remodeling during T-cell differentiation, with the possible purpose of maximizing ligand binding affinity for surface receptors and thus antigen recognition.

SG359

**Nanoscale organization of Eat me and Don't Eat Me signals during phagocytosis.**

**M. Morrissey**; MCDB, UCSB, Santa Barbara, CA.

Macrophages measure the amount of 'Eat Me' and 'Don't Eat Me' signals on a targets surface to determine how to respond. The mechanism for measuring, adding or subtracting these signals is not clear. Clustering of immune receptors or their ligands in the plasma membrane is a common strategy to amplify signaling, so we hypothesized that the nanoscale organization of 'Eat Me' and 'Don't Eat Me' signals may affect their potency. My lab has previously used DNA origami to control ligand pattern during phagocytosis. We determined that clustering activating Fc Receptors enhances receptor phosphorylation. Based on this knowledge we designed an optogenetic Fc Receptor, where receptor clustering is controlled by light. Using this tool, we found that clustering the Fc Receptor is sufficient to trigger phagocytosis. In separate experiments, we found that CD47, the most well-described 'Don't Eat Me' signal is also organized into nanoscale clusters in the plasma membrane of cancer cells. Activating apoptosis de-activates CD47, allowing for phagocytosis of apoptotic corpses. Apoptosis does not correlate with a decrease in CD47 surface levels, but does correlate with a change in CD47 clustering. This suggests that CD47 clustering may affect its ability to activate the 'Don't Eat Me' signaling pathway. We are currently testing the effect of this change in CD47 organization.

SG360

**Unraveling membrane invagination and CD28-mediated T cell autonomous signaling.**

**Y. Zhao**, C. Caron, Y. Chan, C. K. Lee, X. Xu, J. Zhang, T. Masubuchi, C. Wu, J. D. Bui, E. Hui; University of California, San Diego, La Jolla, CA.

**Background**: CD28 is a major costimulatory receptor that promotes T cell responses alongside T cell receptor (TCR). Aberrant CD28 signaling is a defining feature of T cell dysfunction in cancer, autoimmunity, viral infection, and aging. While CD28 is best known to be engaged by the ligands of B7-1 (CD80) and B7-2 (CD86) expressed on professional antigen presenting cells (APCs) during T cell priming

in secondary lymphoid organs, there is evidence of notable B7 expression on activated T cells, raising the possibility of T cell autonomous CD28 signaling in the absence of professional APCs. Indeed, B7 ligands can be limited in peripheral tissues, due to the general lack of B7 expression on non-immune cells, and therefore the on-board CD28 stimulation by T cell B7 could be an important mechanism to promote T cell function. However, it is still unclear whether and how B7 ligands bind CD28 on the same T cell (*cis*-interactions), as opposed to the well-established *trans*-B7:CD28 interactions between APCs and T cells. Investigating *cis*-interactions is essential to further our understanding of T cell function in professional APCs-sparse tissues, such as tumor. **Aims**: We hypothesized that CD28 can interact with B7 in *cis* on T cell membrane for costimulation. Our aim was to determine the existence, topological requirement, molecular regulation and physiological consequence of *cis*-B7:CD28 interactions on T cells. **Results:** We found B7 ligands expressed on T cells interacted with CD28 in *cis*, which is facilitated by endocytosis associated membrane invaginations. We also found *cis*-B7:CD28 interactions enriched at the immunological synapse, as a result of phosphoinositide-3-kinase (PI3K) and sorting-nexin-9 (SNX9)-mediated membrane remodeling. *Cis*-B7:CD28 interactions triggered CD28 signaling through enhancing PKCθ recruitment at immune synapse and promoted T cell survival, migration and cytokine production. In the mouse tumor model, deficiency in *cis*-B7:CD28 signaling accelerated tumor growth and decreased intratumoral T cells. **Conclusion**: in the current paradigm, CD28 is activated in *trans* by B7 ligands expressed on professional APCs in secondary lymphoid organs. Here, we showed that B7 ligands displayed by primed T cells activated CD28 in *cis* through PI3K-SNX9-driven invagination of synaptic membranes, leading to T-cell-autonomous costimulation that enhanced cytokine production, survival, migration, and anti-tumor activity of primed $CD8^+$ T cells. Our results explain how T cells sustain their functionality in APC-sparse tissues and suggest *cis*-signaling as a general mechanism for boosting T cell functionality.

SG361

**Superresolution Imaging Reveals Maturation-Dependent Membrane Fibers of Dendritic Cells.**

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Actin-associated membrane structures have emerged as crucial components in intercellular communication, holding great promise for nanomedicine. Dendritic cells (DCs) play a specialized role in initiating and regulating the adaptive immune response, which is believed to be influenced, at least in part, by actin-associated elongated membrane fiber structures. However, nanoscale evaluation of these elongated structures at the nanoscale using standard single-molecule localization microscopy (SMLM) techniques remains challenging due to the inherent fragility of these structures and the suboptimal labeling densities with conventional labeling strategies. In our study, we address these challenges by employing single-molecule labeling of F-actin with fluorophore-conjugated phalloidin. Here we investigate actin-associated membrane fiber structures in dendritic cells and their remodeling upon immune activation at the nanoscale. Single-molecule-phalloidin labeling revealed that actin-associated membrane fibers are characteristic of immature dendritic cells. Furthermore, the actin cytoskeleton undergoes remodeling upon immune activation accompanied by a recession of the membrane fiber networks. This recession may be linked to the downregulation of their antigen-uptake function. Single-molecule-phalloidin labeling offers a straightforward superresolution microscopy technique for investigating F-actin dynamics in fragile, actin-associated membrane fiber structures.

SG362

**Leukocytes use membrane tunnels to cross the vascular endothelium.**

**J. D. Van Buul**; Medical Biochemistry, University of Amsterdam, Amsterdam, NETHERLANDS.

Upon inflammation, leukocytes extravasate from the bloodstream through the endothelial cells that line the vessel wall into tissues. Leukocytes can cross the endothelium in a paracellular or transcellular manner. When using the paracellular route, it is generally accepted that junctional proteins need to move out of the way and neighboring endothelial cells need to physically disconnect to allow leukocytes to cross. When carefully examining endothelial cell junctions, endothelial cells partially overlap beyond VE-cadherin distribution. Using an endothelial-specific Confetti knock-in animal model, we show overlapping membranes in different vascular beds. Overlaps are regulated by flow as well as by actin polymerization but, although marked by, do not require PECAM-1, nor VE-cadherin. Interestingly, these endothelial membrane overlaps are preferred by leukocytes to cross the endothelium. Detailed 3D analysis of leukocyte transmigration in real-time at the highest possible resolution using lattice light-sheet microscopy revealed that the overlapping endothelial cells provide a tunnel for transmigrating leukocytes. Once penetrating the endothelium, leukocytes use the underlying endothelial overlap as a slide to migrate under the adjacent endothelial cell. Our work shows that endothelial cells do not dissociate from each other upon passage of leukocytes but rather provide endothelial-based membrane tunnels to allow leukocytes to penetrate through the endothelial monolayer. This discovery opens new insights into the multistep paradigm of leukocyte transendothelial migration.

SG363

**Emerging new roles of lysosomes in innate immune response.**

**R. Puertollano**; National Heart, Lung, and Blood Institute, NIH, Bethesda, MD.

Lysosomes have recently emerged as unique signaling hubs. Multiple types of signals are sensed and integrated at the lysosome-limiting membrane, allowing modulation of critical cellular processes, such as nutrient sensing, energy metabolism, immune response, and lysosomal damage repair. The activation of these pathways requires the regulated recruitment of signaling complexes to the lysosomal surface, as well as the subsequent activation of specific transcription factors that modulate expression of elaborate transcriptional networks to facilitate cellular adaptation and maintain cellular hemostasis. The transcription factors TFEB and TFE3 mediate lysosome to nucleus communication in response to a variety of stress conditions. Recent studies have unveiled their contribution to innate immunity and inflammation, in particular orchestrating antibacterial responses. Here we show that TFEB and TFE3 play also critical functions in response to viral infection. We found that beta-coronaviruses induce a robust and persistent activation of TFEB and TFE3. Translocation of the transcription factors to the nucleus is required for efficient expression of multiple immune regulators and contributes to viral infection-induced cell death. TFEB and TFE3 activation also increases lysosomal exocytosis, enhancing virus egress. Our results indicate that TFEB and TFE3 play a crucial role in the interface between host and beta-coronavirus.

SG364

**How force alters pathogen-host interactions.**

**Y. Yu**; Indiana University, Bloomington, IN.

Phagocytosis, a fundamental process for infection control and tissue homeostasis, involves the internalization and degradation of non-moving pathogens within phagolysosomes. Pathogens that escape immune degradation are traditionally thought to rely on virulence factors that disrupt phagoslysosomal biogenesis. However, our recent work, which I will present, demonstrates evidence that physical forces exerted by pathogens during cell entry can significantly impact their intracellular fate, diverging from the canonical phagolysosomal degradation pathway. Moreover, we show that this altered intracellular fate is determined during the formation of the phagocytic synapse. Our research highlights the importance of considering physical forces in understanding pathogen-host interactions.

SG365

**Trogocytosis in macrophages is governed by target membrane tension.**

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Macrophages are immune cells responsible for detecting and eliminating pathogenic bacteria and viruses, as well as cancerous and apoptotic host cells. While macrophages will often completely engulf their target through phagocytosis, in a growing number of examples they are observed to 'nibble' fragments from their target instead. Trogocytosis, or the selective engulfment of cell fragments, appears to occur in multiple immune cell interactions. Despite its relevance to human health and immunity, the mechanistic and functional significance of trogocytosis is poorly understood. Do the physical properties of the target membrane play a role in determining whether a target is phagocytosed or trogocytosed? to address this question, we developed a high throughput assay using flow cytometry to measure the relative amount of phagocytosis vs trogocytosis of a panel of cancer cells opsonized with IgG, which is recognized by macrophage Fcγ receptors and triggers the phagocytic machinery. For cell lines with low cortical tension, >50% of macrophage-like RAW 264.7 cells trogocytose the target cells rather than phagocytose. For cell lines with high cortical tension, full phagocytosis is on average 10x more frequent than cells with low cortical tension. We directly tested the role of membrane tension by developing a giant unilamellar vesicle (GUV) assay in which the membrane is opsonized with IgG and membrane tension can be changed osmotically or via micropipette aspiration. GUVs under high membrane tension are phagocytosed by macrophages within a matter of minutes. However, when we reduce membrane tension, we find that the highly deformable GUVs are trogocytosed. Fluorescence microscopy of the interface reveals that the broad extension of the phagocytic cup around GUVs with high membrane tension is replaced by localized deformation of the target membrane for GUVs with low membrane tension. We propose a model for trogocytosis in which mechanical feedback during extension of the phagocytic cup leads to constriction and scission of membrane fragments when membrane tension is low.

SG366

**Spatiotemporal regulation of PI(3,4,5)P3-Cdc42 signalling axis controls the podosome assembly and chemotactic migration of macrophage.**

Y. Qi, **C. Yu**; University of Hong Kong, Hong Kong, HONG KONG.

Podosomes are the key adhesion structure of macrophage and exhibit densely polymerized F-actin on the plasma membrane. In order to swiftly migrate through tissue barriers, a macrophage cell dynamically assembles numerous short-lived podosomes and degrades extracellular matrices in proximity. It is known that phosphatidylinositol and GTPase signalling are two important factors that regulate the activity of actin polymerization factor WASP at the podosome. However, their signalling crosstalk and causal molecular network remain unclearly. Here, we demonstrate that the biogenesis of PI(3,4,5)P3 lipid on the plasma membrane acts as the upstream signal to spatiotemporally promote the Cdc42-GTP level at individual podosomes of THP1 macrophage. Cdc42 activity is an effector of PI(3,4,5)P3 biogenesis, as the inhibition of Cdc42 does not alter PI3K activities. WASP-3D, the Cdc42-binding deficient mutant of WASP, is not recruited to the plasma membrane and fails to promote F-actin polymerization. Next, we combine the approaches of quantitative imaging analysis and shRNA knockdown to identify PI(3,4,5)P3-dependent Cdc42 guanine exchange factors (GEFs). Among the five major Cdc42 GEFs that exhibit higher expressions in macrophage than the non-adherent precursor monocyte, we find that Vav family are recruited to the plasma membrane in a PI(3,4,5)P3-dependent manner. Blockage of PI(3,4,5)P3-binding domain and shRNA knockdown of Vav family both result in significant reductions of podosome formation and suppress matrix degradation and chemotactic migration. Thus, the biogenesis of PI(3,4,5)P3 acts as the upstream signal to spatiotemporally recruit Vav family that activate Cdc42 on the plasma membrane. Elevated levels of Cdc42-GTP then trigger WASP-mediated F-actin polymerization, podosome assembly, and macrophage migration.

SG367

**Membrane curvature regulates integrin αVβ5-mediated cell adhesions to soft ECM fibers.**

**B. Cui**, W. Zhang, C. Lu; Stanford University, Stanford, CA.

Membrane curvature in the range of tens to hundreds of nanometers is involved in many essential cellular processes. At the cell-matrix interface, where the cells make physical contact with extracellular matrices, the membrane may be locally deformed by matrix topography or mechanical forces. We recently found that this membrane deformation promotes the formation of a new type of integrin αVβ5-mediated cell adhesions - curved adhesions (in press in Nature Cell Bio). Curved adhesions are molecularly distinct from focal adhesions and clathrin lattices. By employing nanostructures that precisely manipulate the location, degree, and sign of membrane curvatures, we identify a previously unknown interaction between integrin β5 and a curvature-sensing protein FCHo2. We find that curved adhesions are prevalent in physiological conditions and the disruption of curved adhesions abolishes the migration of cancer cells in 3D fiber matrices. These findings provide a mechanism of cell anchorage to soft protein fibers.

SG368

**Wetting of junctional condensates along the apical interface promotes tight junction belt formation.**

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Biomolecular condensates enable cell compartmentalization by acting as membrane-less organelles. How cells control the interactions of condensates with other cellular structures such as membranes to drive morphological transitions remains poorly understood. Here, we studied formation of tight junctions, which initially assemble as condensates that over time elongate around the membrane cell perimeter to form a closed junctional barrier. We discovered that the elongation of junctional condensates is driven by a physical wetting process around the apical membrane interface. Using temporal proximity proteomics in combination with live and super-resolution imaging, we found that wetting is mediated by the apical protein PATJ, which promotes adhesion of condensates to the apical membrane resulting in an interface formation and linear spreading into a closed belt. Using PATJ mutations we show that apical adhesion of junctional condensates is necessary and sufficient for stable tight junction belt formation. Our results demonstrate how cells exploit the collective biophysical properties of protein condensates and membrane interfaces to shape mesoscale structures.

## Subgroup: Design, Build, Test, Learn: Understanding the Cell Biology of Synthetic Tools and Using Those Tools to Understand Cell Biology

SG369

**A Tunable Genetically Encoded Tension Sensor Based on Engineered Proteins.**

**S. Liu**; Emory University, Atlanta, GA.

Genetically encoded tension sensors (GETSs) assist in investigating mechanical forces exerted through different proteins involved in cellular mechanotransduction. The vast majority of GETSs are analog and flank an elastic spring-like peptide with two fluorescent proteins, giving a gradual FRET response due to the extension of the elastic peptide under force. Because of ensemble averaging, the FRET signal generated by an analog sensor conceals less frequent force magnitudes outside that reported by the average. One way to address this averaging problem is to use digital force sensors that respond to a narrow range of forces (2-3 pN) and hence can identify the magnitude of mechanical events experienced by target proteins at a defined threshold of tension. However, in order for digital GETS to become useful, it is necessary to create libraries of these sensors with tunable force response thresholds. Here, we developed engineered digital tension sensors that can be fine-tuned to respond to a range of pN forces by mutagenesis. The force response threshold of these sensors is calibrated by single molecule force spectroscopy. We created a vinculin tension sensor and showed that the average vinculin tension within focal adhesions (FAs) of mouse embryonic fibroblasts is approximately 5-10 pN based on prior calibration measurements. Interestingly, a sub-population of higher force vinculin was observed that has not been reported previously. The mechanical unfolding of these engineered protein sensors was modeled using steered molecular dynamics (SMD), which showed agreement with live cell results. Together, this poster demonstrates the use of protein engineering to create tunable elements for next generation GETSs.

SG370

**Progress toward synthetic biology devices that link patterns of signaling to ultimate cell fates.**

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Techniques such as long-term fluorescence imaging can reveal how the signaling pathways active in a single cell, over time, lead to changes in that cell's fate. However, imaging can follow at most a few signaling pathways at a time. Non-optical techniques can have a higher throughput, but these all have some limitation: requiring destructive collection of cells, relying on continuous access to the cells, or being unable to tie a specific observation to a specific cell. DNA recording is a new technology that promises to overcome these limitations, enabling the simultaneous tracking of many signaling pathways in parallel in living single cells growing inside model organisms. We seek to realize this promise by engineering mammalian cells to record specific mutations in response to specific signaling events, such that the relative order and duration of these events can be interpreted merely by sequencing each cell's DNA at the end of the experiment. Our DNA recorder is not yet efficient enough to allow interpretation of order and duration from single cells. However, we have created a proof-of-principle cell line that can record the presence, absence, order, and duration of two stimuli in populations of cells. Here, we will present several large replicate experiments with this cell line, which will reveal how far the technology must be improved in order to create truly single-cell recorders.

SG371

**Computationally designed sensors for endogenous Ras activity reveal signaling effectors within oncogenic granules.**

**J. Z. Zhang**, W. H. Nguyen, N. Greenwood, S. Ong, D. J. Maly, D. Baker; University of Washington, Seattle, WA.

Genetically encoded biosensors have accelerated biological discovery, however many important targets such as active Ras (Ras-GTP) are difficult to sense as strategies to match a sensor's sensitivity to the physiological range of target are lacking. Here, we use computational protein design to generate and optimize intracellular sensors of Ras activity (LOCKR-based Sensor for Ras activity: Ras-LOCKR-S) and proximity labelers of the signaling environment of Ras (LOCKR-based, Ras activity-dependent Proximity Labeler: Ras-LOCKR-PL). We demonstrate that our tools can measure endogenous Ras activity and environment at subcellular resolution. We illustrate the application of these tools by using them to identify Ras effectors, notably Src-Associated in Mitosis 68 kDa protein (SAM68), enriched in oncogenic EML4-Alk granules. Localizing these sensors to these granules revealed that SAM68 enhances Ras activity specifically at the granules, and SAM68 inhibition sensitizes EML4-Alk-driven cancer cells to existing drug therapies, suggesting a possible therapeutic strategy.

SG372

**Controllable Actin-binding Switch Tools (CAST): Engineered Protein Switches for F-actin Binding Manipulation in Living Cells and Tissue.**

**U. Effiong**, H. Khairandish, B. Belardi; the University of Texas at Austin, Austin, TX.

The ubiquitous eukaryotic protein, actin, plays critical roles in numerous subcellular processes, including cell-cell adhesion, cell shape changes, and motility. Actin-binding proteins (ABPs) interface with the

filamentous form of actin (F-actin) to form molecular complexes that drive F-actin organization and mechanics necessary for these subcellular processes. Yet, how ABPs orchestrate their cellular activities in concert remains an outstanding question. While ABPs and their interactions with F-actin have been studied in detail, these experiments are limited as they usually involve protein depletion or the use of small-molecule F-actin disrupters. Since these methods impact F-actin or the protein-of-interest globally and are detrimental to cell and tissue integrity, a means of directly manipulating actin binding is a pressing need. To that end, we have developed three controllable actin-binding switch tools (CASTs) that can manipulate F-actin binding in a specific and spatiotemporal manner. Our CAST modules toggle between an “off” and an “on” state in response to different stimuli and achieve switching activity over a broad range of timescales. Each design relies on installing an intramolecular association to drive the “off” state of actin-binding motifs (ABM) through conformational constraint or steric occlusion of the ABM. Introduction of either a small molecule, a peptide, or incident light stimulus turns the CAST to an “on” state and leads to F-actin binding. We quantified i) the “off” state of CASTs as the fold inhibition compared to ABM alone activity and ii) the extent of activation after stimuli introduction. Together, our CASTs have achieved initial F-actin binding inhibition of 50-99% and stimuli-triggered activation up to 80%. CAST modules were engineered into an ABP to control adhesion and collective cell migration. As well, by forming an oligomerized CAST, we were able to trigger cytoskeletal re-organization. CASTs present a new approach for controlling actin binding in a direct, specific, and temporal manner, which can be beneficial for experiments seeking to probe and control the influence of actin binding on a subcellular process.

SG373

**Optogenetic control of Fc receptor signaling reveals a molecular mechanism that enhances phagocytosis.**

**A. Bond**, M. Wilson, M. Morrissey; University of California, Santa Barbara, Santa Barbara, CA.

Macrophages, an innate immune cell type, constantly surveil their environment for harmful agents such as pathogens or infected cells. Once a macrophage finds a target cell, it interprets complex signals on the cell surface to decide how to respond. One ‘eat-me’ signal, IgG, is recognized by the Fc Receptor family (FcR). If a critical threshold of Fc Receptors are activated, the macrophage will phagocytose the particle. Prior work has shown that sub-threshold Fc Receptor activation, not sufficient to activate phagocytosis, still initiates some signaling in the macrophage. What is the consequence of this low-level signaling? Can macrophages add up sequential encounters with a low level of IgG? I hypothesized that prior encounters with sub-threshold levels of IgG may generate a molecular memory that influences macrophage appetite for IgG-opsonized particles. Controlling precisely when a macrophage encounters first one target and then a second is difficult, so I designed an optogenetic Fc Receptor (optoFcR) to temporally control FcR activation. The optoFcR contains a membrane targeting sequence, the Internal Tyrosine Activating Motif (ITAM) of the FcR, and a light-sensitive homo-oligomerizing protein, Cryptochrome 2. Light-induced oligomerization of the optoFcR triggers ITAM phosphorylation and engulfment of unopsonized targets in a dose-dependent manner, suggesting that clustering is sufficient to activate native FcR signaling. To determine if macrophages have a molecular memory of prior sub-threshold FcR activation, we briefly activated the optoFcR, allowed the system to completely deactivate, then presented the macrophages with beads coated with a low-dose IgG signal. Prior FcR activation doubled phagocytosis, suggesting macrophages add up sequential signals. Increased phagocytosis occurs by two discrete mechanisms - short- and long-term memory. The short-term memory does not require new protein synthesis and peaks around 1 hour after FcR activation. A long-term memory,

requiring new protein synthesis, begins at 4 hours after FcR activation and persists for at least 72 hours. Further investigations will reveal the molecular mechanism behind FcR activation memory and determine if this memory occurs *in vivo.* This work may provide insights into innate immune memory and may suggest strategies to enhance antibody-dependent cellular phagocytosis or chimeric antigen receptor (CAR) macrophages.

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# *SPECIAL SYMPOSIUM*

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## 4DCP Symposium: Unraveling Multicellular Life: Exploring How Cells Cooperate to Form Tissues, Organs, and Organisms- Supported by Janelia

SS9

**Imaging the molecular processes of cell division across scales.**

**J. Ellenberg**; European Molecular Biology Laboratory, Heidelberg, Germany.

The recent rapid development of imaging technologies allows unprecedented insights into the molecular machinery inside living cells and organisms. For the first time, light and electron microscopy have molecular sensitivity and resolving power in situ, and can be correlated to connect the scales of structural detail and dynamics of single molecules to imaging a whole living cell and organism. Aided by machine learning driven image analysis and open sharing of image data, this provides unprecedented opportunities for new insights into the molecular mechanisms that drive life's core functions.
I will present the progress we have made in my research group to study the protein network and individual protein complexes that drive one of life's most fundamental functions, cell division, in human cells and early mammalian embryos. To this end, we have developed advanced microscopy and image analysis, ranging from live embryo light-sheet microscopy, quantitative live cell imaging using fluorescence correlation spectroscopy (FCS), to super-resolution and correlative light and electron microscopy.
The presentation will highlight how we can use advanced imaging technologies to study dynamic cellular signaling networks and the dynamic assembly of key individual protein complexes. By doing this in live dividing cells and developing embryos, this allow us to better understand how the molecular machinery functions to ensure faithful cell division and prevent errors, that occur very frequently in early mammalian development and underlie congenital disease and infertility. The exciting opportunities for open access to such cutting-edge imaging technologies provided by the EMBL Imaging Centre and support in image data archiving and sharing provided by the EMBL Bioimage Archive will also be discussed.

SS10

**Enabling Environmental-structural-cell Biology with Embl'S Advanced Mobile Laboratory: From Field Sampling to Volume Electron Microscopy of Marine Dinoflagellates.**

**Y. Schwab**; EMBL, Heidelberg, GERMANY.

In Spring 2023, EMBL and many partners have launched a sampling expedition called TREC (for Traversing the European Coastline). For almost 2 years, many groups will collect organisms across multiple scales and gradients, from land to sea, capturing the huge biodiversity of coastal ecosystems to study how they are impacted by changing environments. To enable cellular, structural and molecular

analyses on such complex specimens, EMBL has developed a dedicated service facility, the Advanced Mobile Laboratory, that is bringing cutting edge equipment directly to the coast to capture fresh specimens in their native environment. Amongst the embarked technologies are instruments that enable sample preparation for electron microscopy, including a plunge freezer and a high-pressure freezer, thus creating a unique opportunity to study the sub-cellular organization of specimens.This talk will focus on how such approaches are enabling a detailed analysis of dinoflagellates, an important group of unicellular plankton that, as primary producers, play a central role for global oxygen production and carbon capture. It will also illustrate how high-throughput electron microscopy, volume electron microscopy and multimodal correlative imaging techniques can be utilized to build subcellular atlases that are important to enhance our understanding of such environmental samples.

SS11

**Shocking Secrets of Cell Motility and Wound Healing.**

**J. Theriot**; University of Washington, Seattle, WA.

Many animal cell types are able to crawl in a specific direction based on the spatial distribution of environmental cues, such as growth factors or soluble chemoattractants. Motile cells of the immune system and epithelial cells are also able to respond directly to the presence and orientation of electric fields, a process known as “galvanotaxis” or “electrotaxis”.

The ability of cells to sense and respond to electric fields is particularly useful in the context of wound healing. Normal, intact epithelia typically maintain a significant disparity in ion concentration from one side to the other, giving rise to a transepithelial potential of a few millivolts. A break in the epithelium causes a short-circuit in this potential, resulting in rapid ion flow at the site of the wound and generation of an endogenous electric field. In larval zebrafish, we have found that laceration of the epidermis in the developing fin fold is sufficient to generate an electric field that contributes to the directional migration of multiple cell types, including basal epidermal keratocytes, neutrophils, and T-cells. Both keratocytes and neutrophils move toward the wound (i.e. toward the cathode of the endogenous electric field), while T-cells move in the opposite direction, away from the wound (i.e. toward the anode). The same directional galvanotactic movement of these three cell types can be observed in cultured adult skin explants in response to exogenously applied electric fields.

Biophysical characterization of galvanotaxis in isolated fish basal epidermal keratocytes has supported the hypothesis that electric fields are sensed by electrophoretic migration of highly negatively charged transmembrane proteins in the cell plasma membrane. Using a human neutrophil-like cell line, we have performed a genome-wide CRISPRi screen to identify genes required for normal cathode-directed galvanotaxis. In addition to a large number of factors generally involved in neutrophil actin-based motility, we have found a few leading candidates for the putative molecular electric field sensor, whose disruption strongly inhibit galvanotaxis with little or no effect on serum-stimulated cell motility.

Our work on galvanotaxis illustrates the importance of studying complex biological processes using an integrated approach that spans molecular, cellular, and tissue-level scales.

SS12

**Neuro-vascular interactions in the brain.**

**C. Gu**[1,2]; [1]Harvard Medical Sch, Boston, MA, [2]Howard Hughes Medical Institute, Chevy chase, MD.

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.

SS13

**How Mycobacteria Exploit Cell Signaling Networks to Cause Tuberculosis.**

**L. Ramakrishnan**[1,2]; [1]University of Cambridge, Cambridge, UNITED KINGDOM, [2]MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM.

Tuberculosis, the world's leading infectious killer, is characterized by infectious foci called granulomas, highly organized structures of macrophages and other immune cells. In most infected individuals the granuloma is successful in eradicating infection. Manifestation of tuberculosis in the minority of infected individuals represents a failed granuloma. I will discuss our discoveries about how pathogenic mycobacteria exploit myriad host genetic vulnerabilities to thwart and even exploit these generally host-protective cell communication networks to promote their expansion and transmission. These discoveries lead to new host-targeting approaches to treat TB while teaching us about new cellular pathways.

# *MINISYMPOSIA*

## Minisymp: Actin Dynamics and Cell Homeostasis

M1

**Calmin, a novel ER-actin tether on ER tubules that influences cell migration.**

**H. Merta**[1], T. Isogai[2], G. Danuser[2], W. M. Henne[1]; [1]Department of Cell Biology, UT Southwestern Medical Center, Dallas, TX, [2]Lyda Hill Department of Bioinformatics, UT Southwestern Medical Center, Dallas, TX.

Interactions between membrane-bound organelles and the cytoskeleton influence a variety of processes that are critical to cell physiology. The endoplasmic reticulum (ER) is an interconnected organelle characterized by a variety of membrane shapes, including sheets and tubules. Interactions between the ER and microtubule cytoskeleton are well-characterized, but an unresolved question is how the ER interacts with the actin cytoskeleton, which largely governs cell shape and movement. Using proximity-labeling comparative proteomics, here we identify a new ER tubule-specific protein, calmin (CLMN), and propose it mediates ER-actin tethering and regulates cell migration. Mechanistically, CLMN contains F-actin-binding calponin homology (CH) domains related to the CH domains of nesprin proteins. Live and fixed-cell imaging of overexpressed and endogenously-tagged CLMN reveals that CLMN localizes to ER tubules and basolateral punctae that are positive for actin and focal adhesion markers. Structure-function analysis reveals that the interaction between the CH1 and CH2 domains tunes the extent and specificity for CLMN binding to F-actin, while a C-terminal transmembrane domain mediates its ER

localization. Thus, we propose that CLMN is a new ER-to-actin tether. Using imaging-based and functional assays, we show that CLMN is required for proper actin organization. Overexpressed CLMN enriches at a subpopulation of focal adhesions under the cell, where adhesions are more likely to be disassembling. At the same time, CLMN-deficient cells display slowed migration and have more adhesions, further indicating that CLMN tethering of ER to actin mediates adhesion turnover. Collectively, we propose that CLMN tethering of the ER to actin stress fibers promotes adhesion disassembly to ensure proper cell migration. This work identifies a new ER tubule-localizing protein, reveals the first demonstration of an ER-actin molecular tether, and highlights how organelle-cytoskeleton interactions play a significant role in cell motility.

M2

**Different Types of Direct Talin-Actin Binding Play Specific Roles in Cell-ECM Adhesion during Mammalian Development.**

**W. Deng**[1], R. L. Carr[2], R. R. Kaul[2], M. Pavlova[3], A. Haage[4], P. Roca-Cusachs[3], G. Tanentzapf[1]; [1]Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, CANADA, [2]School of Biomedical Engineering, University of British Columbia, Vancouver, BC, CANADA, [3]Institute for Bioengineering of Catalonia, University of Barcelona, Barcelona, SPAIN, [4]Department of Biomedical Sciences, University of North Dakota, Grand Forks, ND.

During animal development, cell-ECM adhesion plays a key role in the assembly and maintenance of complex tissues and organs. The integrin family of transmembrane adhesion receptors is the main link between the ECM and the actin cytoskeleton, and their functions are precisely regulated. A large multiprotein complex inside the cell called the integrin adhesion complex (IAC) is required for the integrin-mediated adhesion. The cytoplasmic protein Talin, which links integrin to actin, is known to act as a molecular scaffold for the assembly of the IAC and is essential for mammalian development. Talin has three actin binding sites, ABS1, ABS2 and ABS3. Among which the best characterized one is the C-terminal ABS3 domain. It has been proposed that ABS3 is specifically required for transducing mechanical force across the adhesion after talin binds to integrin. As a result, Talin is stretched to expose cryptic protein-protein interaction sites including ABS2 domain. To study the role of ABS3 and ABS2 domains in mammals, in an *in vivo* context, we generated three different mouse models, one containing the KVK/DDD mutations that disrupt talin-actin binding through ABS3 (*Tln1*$^{KVK/DDD}$). Another mouse line contains six point mutations that reduce talin-actin binding via ABS2 (*Tln1*$^{ABS2mut}$), and finally, an ABS2/ABS3 trans-heterozygous mouse line (*Tln1*$^{ABS2/ABS3}$). We have observed that the *Tln1*$^{KVK/DDD}$ mutant embryos showed developmental defects starting at embryonic day (E)8.5 and became resorbed at around E12.5, whereas the *Tln1*$^{ABS2mut}$ mutant and *Tln1*$^{ABS2/ABS3}$ embryos were completely viable and fertile. By generating primary mouse embryonic fibroblasts (MEFs) from mutant embryos, we have found that *Tln1*$^{KVK/DDD}$ affected the ability of focal adhesions to grow and mature. It also disrupted integrin activation, cell morphology, cell-ECM attachment, actin dynamics & organization, and traction force generated by cells during early cell spreading, however, with time, cells exhibited substantial recovery from these defects. We further investigated the physiological role of ABS3 using a melanoblast-based *ex vivo* cell migration assay and found strong cell migration defects in mutant mice. In comparison, *Tln1*$^{ABS2mut}$ mutant and *Tln1*$^{ABS2/ABS3}$ cells only exhibited mild defects in integrin-mediated adhesion, cell spreading and actin organization pattern. Our study suggests that specific ways of linking talin to actin have unique roles in controlling cell-ECM adhesion during normal tissue development and maintenance.

M3

**Functionally distinct actin filament nanoscale layers control focal adhesion assembly and microtubule-dependent disassembly.**

**R. Kumari**[1], K. Ven[1], M. Chastney[2], J. Peränen[1], J. Aaron[3], L. Almeida- Souza[1], E. Kremneva[1], R. Poincloux[4], T. Chew[3], P. W. Gunning[5], J. Ivaska[2], P. Lappalainen[1]; [1]University of Helsinki, Helsinki, FINLAND, [2]University of Turku, Turku, FINLAND, [3]HHMI Janelia, Ashburn, VA, [4]Université de Toulouse, Toulouse, FRANCE, [5]School of Medical Sciences, UNSW, Sydney, AUSTRALIA.

Focal adhesions (FAs) form the physical connections between cells and their surrounding extracellular matrix (ECM). Being localized at the interface between a cell and its ECM, cell-matrix adhesions mediate cell's attachment to its substrate, cell spreading, mechanotransduction, cell migration, proliferation, and signaling. However, the cellular processes such as cell migration rely on complex molecular machineries that control FA turnover, which remained elusive. By using super-resolution iPALM microscopy, we identified two additional nanoscale layers within FAs, specified by actin filaments bound to tropomyosin isoforms Tpm1.6 and Tpm3.2. By applying super-resolution imaging along with CRISPR-cas9 knockout, we demonstrate that these layers have critical, but opposite functions in adhesion turnover. The Tpm1.6-actin filaments towards the top of FA beneath the previously identified 'actin-regulatory layer' are critical for FA maturation and controlled cell motility. In contrast, the Tpm3.2-actin filaments towards the bottom of FA facilitates adhesion disassembly and tail retraction during migration. Mechanistically, Tpm3.2 actin filaments stabilizes KANK-family proteins at adhesions, and hence targets microtubule plus-ends to FAs to catalyse their disassembly. Thus, FAs are composed of at least three distinct actin filament layers, each having specific roles in coupling adhesions to the cytoskeleton and in adhesion dynamics. These findings demonstrate how distinct actin filament populations can co-exist and regulate the functions of a defined cellular compartment.

M4

**Calcium influx mediated by a mechanosensitive ion channel TRPV4 controls actin architecture in lamellipodia to promote cell migration.**

**E. Iu**[1], A. Bogatch[1], W. Deng[2], J. Humphries[3], C. Yang[4], M. Humphries[5], G. Tanentzapf[2], T. Svitkina[4], S. Plotnikov[1]; [1]University of Toronto, Toronto, ON, CANADA, [2]University of British Columbia, Vancouver, BC, CANADA, [3]Manchester Metropolitan University, Manchester, UNITED KINGDOM, [4]University of Pennsylvania, Philadelphia, PA, [5]University of Manchester, Manchester, UNITED KINGDOM.

Cell migration underlies a wide range of physiological processes from embryonic development to tissue regeneration. Cell migration is driven by the extension of actin-based lamellipodia protrusion. This protrusive structure is powered by actin polymerization, which is tightly regulated by a complex of signaling pathways, including Rho GTPases and $Ca^{2+}$-dependent signaling. While the key role of $Ca^{2+}$ signaling in lamellipodia protrusions has been demonstrated, the molecular mechanisms by which $Ca^{2+}$ facilitates lamellipodia assembly are largely unknown.We first identified a mechanosensitive $Ca^{2+}$-permeant channel, TRPV4, as a positive regulator of lamellipodia protrusion by screening a panel of siRNAs targeting ion channels. We showed that suppression of TRPV4 reduces the $Ca^{2+}$ influx in protruding lamellipodia, which subsequently decreases the local density of F-actin. To understand how the TRPV4-mediated $Ca^{2+}$ influx facilitates the assembly of actin network in lamellipodia, we interrogated the involvement of small GTPases by using FRET biosensors. Suppression of TRPV4 was found to decrease RhoA activity in protruding lamellipodia while having a negligible effect on Rac1 and Cdc42. Suppression of RhoA or its downstream effectors, formins and cofilin, decreases F-actin density

to a similar level as TRPV4 inhibition, suggesting that TRPV4-mediated $Ca^{2+}$ influx promotes actin assembly through RhoA. Next, we identified the molecular link between TRPV4 and RhoA. We showed that suppression of TPRV4 reduces the activity of $Ca^{2+}$-calmodulin-dependent protein kinase II (CaMKII), a key $Ca^{2+}$ signaling hub, in lamellipodia. Furthermore, we showed that local inhibition of CaMKII halts cell protrusions, suppresses RhoA, and decreases F-actin density in lamellipodia, indicating that CaMKII transduces local $Ca^{2+}$ signals from TRPV4 to RhoA. To identify the RhoA regulator downstream of TRPV4 and CaMKII, we analyzed the phospho-proteomes of isolated lamellipodia fractions purified from TRPV4- and CaMKII-inhibited cells. We identified 14 potential RhoGEFs/GAPs, whose phosphorylation depends on TRPV4 and CaMKII, and tested their role in the regulation of RhoA-mediated actin assembly. We found that depleting RhoGEF TEM4 decreases density of F-actin and RhoA activity independent of TRPV4 or CaMKII, suggesting that TEM4 is a $Ca^{2+}$-regulated RhoGEF that regulates actin architecture and protrusive activity of lamellipodia. Finally, we showed that suppression of this signaling axis suppresses the protrusion and migration of melanoblasts in mouse skin explant. Together these data elucidate a novel $Ca^{2+}$ signaling axis composed of TRPV4/CaMKII/TEM4/RhoA that reinforces lamellipodial actin network, facilitates lamellipodial protrusions, and promotes cell migration.

M5

**Mitochondria and ER-associated actin are required for mitochondrial fusion.**

**P. Gatti**[1], C. Schiavon[2], U. Manor[3], M. Germain[1]; [1]Medical Biology, Université du Québec à Trois-Rivières, Trois Rivieres, QC, CANADA, [2]Molecular and Cell Biology, Salk Institute for Biological Studies, La Jolla, CA, [3]Department of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA.

Mitochondria play a crucial role in the regulation of cellular metabolism and signalling. Mitochondrial fission and fusion control these processes by balancing respiratory and metabolic functions, transferring material between mitochondria, and removing defective mitochondria. Mitochondrial fission occurs at sites of contact between mitochondria and the endoplasmic reticulum (ER). These sites serve as a platform to recruit the fission machinery, including the fission GTPase DRP1 responsible for the scission of the mitochondrial tubule. Importantly, when fission is experimentally stimulated, DRP1 recruitment and activation at these sites require the formation of actin filaments in a manner that depends on the ER-localised formin INF2. Similarly, actin was suggested to be present at sites of mitochondrial fusion, but its role in this context remains entirely unexplored. Here, using our recently characterized actin chromobody probes, we show that mitochondria-associated actin is recruited to both fission and fusion sites under unstimulated conditions, and that this precedes the recruitment of the ER and ER-associated actin. Importantly, selectively preventing the formation of actin filaments on either mitochondria or the ER with organelle-targeted deAct probes disrupts both mitochondrial fission and fusion. We also demonstrate that fusion but not fission is dependent on Arp2/3-dependent actin polymerization, whereas both fission and fusion are dependent on INF2-dependent actin polymerization. Finally, our work identifies two types of fusion events, with actin selectively required for tip-to-side fusion but not tip-to-tip fusion. Altogether, our work introduces a novel method for perturbing organelle-associated actin filaments and demonstrates a previously unknown role for actin in mitochondrial fusion.

M6

**Capping Protein contributes to the balance of actin assembly among different F-actin networks in the *C. elegans* zygote.**

**S. E. Yde**, R. S. Kadzik, E. Munro, D. R. Kovar; Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

The actin cytoskeleton consists of actin and actin-binding proteins, which organize into filamentous (F)-actin networks with diverse morphologies and dynamics to accomplish a diverse range of cellular tasks. These processes often occur simultaneously within a single cell—so how do cells self-organize such diverse structures from a single pool of proteins? The cortical actin cytoskeleton in the *C. elegans* zygote undergoes rapid changes during the first cell division of development. During late interphase this cell assembles filopodia, which are membrane-bound bundles of actin assembled by two different actin assembly factors: Formin, a processive elongator, at the tips, and Arp2/3 complex, a nucleator that makes branched F-actin networks, at the base. During mitosis, the cell transitions to assembling mini-comets, which are assembled by Arp2/3 complex. Both the filopodia and the mini-comets contain Capping Protein (CP). CP binds the barbed end of an actin filament and prevents further elongation and is thus a regulator of actin filament length. What is CP's role in the assembly of these two distinct networks? Using near-TIRF microscopy, we find that knocking down CP via RNAi in the zygote causes overassembly of filopodia and underassembly of mini-comets, indicating a role for CP in maintaining the balance of assembly among these distinct networks. Additionally, CP RNAi produces longer-lived filopodia, suggesting a role for CP in the turnover of filopodia, consistent with evidence that CP and Formin antagonize each other's residence on barbed ends. We hypothesize that overassembly of filopodia causes underassembly of mini-comets by mis-allocating the limiting pool of actin monomers toward filopodia instead of mini-comets. We are currently testing this hypothesis using a temperature-sensitive formin in cells treated with CP RNAi. We predict that perturbing formin, which is normally not involved in comet assembly, will depress the overassembly of filopodia and thus partially rescue comet assembly in CP knockdown conditions. Using single-molecule techniques to compare binding kinetics of CP *in vivo* in the zygote and *in vitro* with purified *C. elegans* proteins, we discovered a surprising paradox: The lifetime of CP-barbed end binding events *in vivo* is two orders of magnitude shorter than *in vitro*. This suggests the presence of CP regulators *in vivo,* which may change CP on- or off-rates in different networks to achieve different filament lengths. We are currently investigating the roles of putative CP regulators to understand how CP on-rates may be regulated *in vivo*, and how differential regulation of CP may contribute to the diversity of distinct F-actin networks.

## Minisymp: Biogenesis and Dynamics of Cellular Organelles

M7

**Structural Mechanism of Mitochondrial Membrane Remodeling by Human OPA1.**

**H. Aydin**; University of Colorado Boulder, Boulder, CO.

Distinct morphologies of the mitochondrial network support divergent metabolic and regulatory processes that determine cell function and fate. The mechano-chemical GTPase, Optic Atrophy 1 (OPA1), influences cristae architecture and catalyzes the fusion of the mitochondrial inner membrane (IM). Despite its fundamental importance, the molecular mechanisms by which OPA1 modulates mitochondrial morphology have proven elusive. Here, using a combination of cellular and structural analyses, we illuminate the molecular mechanisms key to OPA1-dependent membrane remodeling and

fusion. Human OPA1 embeds itself into cardiolipin-containing membranes via a lipid-binding paddle domain (PD). A conserved loop within the PD inserts deeply into the bilayer, further stabilizing the interactions with cardiolipin-enriched membranes. OPA1 dimerization via the PD promotes the helical assembly of a flexible OPA1 lattice on the membrane, which drives mitochondrial fusion in cells. Moreover, the membrane-bending OPA1 oligomer undergoes conformational changes that pull the membrane-inserting loop out of the outer leaflet and contribute to the mechanics of membrane remodeling. Our findings provide a structural framework for understanding how human OPA1 shapes mitochondrial morphology and show us how human disease mutations compromise OPA1 functions.

M8

**Peroxisomes import proteins by a nuclear pore-like mechanism.**

**M. L. Skowyra**, Y. Gao, P. Feng, T. A. Rapoport; Cell Biology, Harvard Medical School / HHMI, Boston, MA.

Peroxisomes are ubiquitous metabolic organelles whose dysfunction causes fatal human diseases. Without exception, peroxisomal enzymes are synthesized in the cytosol and imported into the organelle. Surprisingly, peroxisomes can import folded proteins and even preformed complexes, but the underlying mechanism has been a longstanding mystery. We show that peroxisomal proteins are imported by mechanism similar to nuclear transport. The cargo is recognized in the cytosol by mobile receptors, which then shuttle the bound cargo across the membrane through an aqueous permeability barrier. This selective phase is formed by the conserved tyrosine (Y)- and glycine (G)-rich YG domain of the peroxisomal membrane protein PEX13, and resembles the dense meshwork formed by nucleoporin FG repeats inside nuclear pores. In vivo disulfide-mediated crosslinking and protease-protection experiments reveal that the YG phase is suspended in the peroxisomal membrane by multiple PEX13 molecules arranged in alternating orientations, an unusual property among membrane proteins. Crucially, the peroxisomal phase can be reconstituted in vitro as a hydrogel formed from the purified YG domain. Peroxisomal import receptors selectively partition into the YG hydrogel and can bring bound cargo along, whereas other proteins including cargo itself are impeded. Partitioning into the hydrogel relies on conserved aromatic motifs in the import receptors that transiently melt the cohesive interactions holding the meshwork together. We thus propose that peroxisomal import receptors shuttle folded proteins into peroxisomes through a nuclear pore-like conduit assembled from PEX13. Our findings lead to the first comprehensive model of peroxisomal protein import, and reveal a previously unrecognized mechanism by which proteins are translocated across membranes.

M9

**Lysosome related organelles contain an expansion compartment that mediates zinc transporter delivery to promote zinc homeostasis in *C.elegans*.**

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Zinc is a transition metal that is essential for all life. The Kornfeld lab uncovered conserved, homeostatic mechanisms respond to zinc excess and zinc deficiency in *C. elegans*. Lysosomes are a site of zinc storage and release. Zinc mobilization from the lysosome is mediated by zinc transporters CDF-2 and ZIPT-2.3 that are reciprocally regulated at the mRNA and protein levels. Super-resolution microscopy experiments showed that in addition to housingCDF-2 and ZIPT-2.3, lysosomes alter their morphology in

response to cytosolic zinc and form-acidified and expansion compartments. ZIPT-2.3 and CDF-2 surround the acidified compartment, while CDF-2 surrounds the expansion compartment. The acidified compartment is comprised of LysoTracker and zinc regions. This configuration is significant because it allows the lysosome to participate in canonical degradation activities while conducting zinc metabolic activities. The expansion compartment is highly dynamic and deviates greatly in size. Our model proposes that lysosome restructuring facilitates the rapid turnover of transporters needed to store or release zinc. We, therefore, predicted that the expansion compartment grows and shrinks to accommodate zinc. Time course super-resolution microscopy with worms expressing CDF-2::GFP and ZIPT-2.3 mCherry in excess zinc shows a time-dependent increase of the expansion compartment, suggesting the expansion compartment continually stores zinc. Preliminary genetic analysis with *hlh-30* and *hizr-1 lof* mutants shows that these genes are necessary for the assembly of compartments. Labile zinc occupies the acidified compartment. However, CDF-2 presence on the expansion compartment membrane suggests total zinc is present in the lumen. Preliminary X-Ray Fluorescence Microscopy (XFM) on isolated lysosomes shows elevated total zinc levels in lysosomes from worms grown in zinc excess conditions, and the presence of total zinc in the expansion compartment, suggesting that CDF-2 is mobilizing zinc into the lysosome. Our work with *C. elegans* shows that lysosomes are a critical site of zinc trafficking and metabolism by utilizing zinc transporters and by altering morphology to compensate for perturbations in zinc levels. Future studies will involve elucidating how the structure facilitates zinc homeostasis in *C. elegans* and human model systems.

M10

**Endoplasmic Reticulum moves by hitchhiking on Golgi-derived vesicles.**

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Peripheral endoplasmic reticulum (ER) tubules extend, retract, and rearrange to form membrane contact sites with most organelles. To maintain membrane contact sites and perform a variety of core functions, ER tubules travel on microtubules by two canonical mechanisms called sliding and tip attachment complexes (TAC) and a newly described mechanism called hitchhiking. In hitchhiking, ER tips are pulled along microtubules by interacting with motile vesicles. Previously, ER tubules have been shown to hitchhike by forming contact sites with endolysosomal vesicles (marked by Rab5 and Rab7), but it is unclear if other Rab-vesicles are capable of this function. We systematically screened several GFP-Rabs in U2OS cells for their ability to cotransport with ER tubules and found that ER hitchhikes on Golgi-derived vesicles marked by two Rab6 isoforms (a and b). ER hitchhiking on Golgi-derived vesicles occurs independently of (1) ER-endolysosome contacts, (2) TAC-mediated ER movement and (3) mitochondrial motility. Disrupting Rab6-vesicle motility using a dominant negative construct, as well as a bioengineering heterodimerization approach, demonstrates that Rab6-vesicle motility is required for a subset of ER movements. Furthermore, ectopically re-localizing Rab6-vesicles to the cell periphery leads to a dramatic accumulation of the ER at peripheral sites suggesting that Rab6-vesicle motility is sufficient to drive peripheral ER movements. We propose that upstream Golgi-derived vesicles are capable of ER hitchhiking. In support of this, inhibiting ADP ribosylation factor 1 (Arf1), a regulator of Golgi-derived vesicle budding, disrupts ER movement. In contrast, downstream post-Golgi vesicles including those marked by Rab8, Rab10, and Rab14 are dispensable for ER movement. Our current work provides valuable insight for the understudied molecular mechanisms of ER hitchhiking. We are now in the process of identifying ER-vesicle tethering molecules at the Rab6-ER contact site and are investigating the biological function of Rab6-ER hitchhiking.

M11

**Mechanism of insulin granule biogenesis.**

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Type-2 diabetes (T2D) and other chronic metabolic disorders pose significant clinical challenges worldwide. The molecular mechanisms governing proinsulin routing to insulin granules within beta-cells have remained mysterious, as no cargo receptor in the trans-Golgi Network (TGN) has been identified. Our research reveals that the TGN's acidic environment leads to forming biomolecular chromogranin B condensates, enabling proinsulin and its processing enzymes (clients) to segregate independently of cargo receptors. Electrostatic interactions in the intrinsically disordered domain are the primary driving force behind this chromogranin B condensation. Notably, cargo selectivity relies on client molecules' charge, size, and concentration rather than specific sequences or structural elements. Chromogranin B condensates interact with the TGN membrane upon cargo recruitment, generating immature secretory granules. Mutations disrupting Chromogranin B condensation or client recruitment lead to impaired granule biogenesis in INS1 and human pancreatic beta-cells. Based on these intriguing findings, we suggest that phase-separated chromogranin B condensates act as "cargo sponges" in the TGN lumen, gathering soluble client proteins without relying on specific sequences or structural elements. This enables receptor-independent sorting of cargo molecules, challenging traditional views on TGN sorting. Our study provides fresh molecular insights into insulin granule biogenesis, potentially paving the way for innovative therapeutic approaches to address insulin dysregulation in T2D and related metabolic disorders, alleviating their global clinical burden.

M12

**Understanding membrane dynamics in autophagy - biogenesis of omegasomes and autophagosomes.**

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Autophagy is a major cytoplasmic recycling pathway that degrades unwanted or damaged cytoplasmic components, including protein aggregates and damaged organelles. Autophagic cargo is sequestered by a phagophore membrane, which expands to enclose cargo, giving rise to an autophagosome. Cytotoxic stress and starvation induce nonselective autophagy in which bulk cytoplasm is degraded. This ambivalent selectivity is a hallmark of autophagy and involves different regulation of the pathway by the autophagy machinery. Our understanding of molecular membrane remodeling events in autophagy has revealed many fundamental principles. However, how nonselective phagophores are formed, which membranes contribute lipids for their biogenesis and what molecular mechanisms are driving the reaction remain largely elusive. Using a combination of in vitro reconstitutions, in which we rebuild autophagosomes from purified components on model membranes, and in vivo reconstitutions in which we target parts of the autophagy machinery to non-autophagic membranes, we were able to reveal so far unprecedented insights into membrane dynamic in autophagy. We revealed how omegasomes, the membrane platform form which autophagosomes emerge, are generated. We identified lipid sources and membrane transport pathways involved in the generation of these compartments. We also reconstituted the formation of cup shaped phagophores and identified key components that drive this unique membrane remodeling reaction. Our most recent and unpublished data allow us to propose an integrated model of phagophore biogenesis.

## Minisymp: Current Concepts in Cellular Signaling and Metabolism

M13

**A Chemical Genetic Screen Implicates *O*-GlcNAc Transferase (OGT) in Maintaining Cellular Homeostasis through Mitochondrial Proteostatic Mechanisms.**

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*O*-GlcNAc transferase (OGT) is an essential and well-conserved metazoan protein that is implicated in numerous cellular processes including metabolism, gene regulation, and signal transduction. It is the only glycosyltransferase that acts in the nucleus and the cytoplasm rather than the secretory pathway. Because OGT glycosylates thousands of cellular proteins, it has been difficult to define which processes OGT is most important for. We hypothesized that a CRISPR interference (CRISPRi) growth screen using a small-molecule inhibitor of OGT would reveal processes that become essential or dispensable when glycosylation is inhibited. Therefore, we conducted a genome-wide CRISPRi screen with OSMI-4, a cell-permeable inhibitor developed in our lab. We found that several genes involved in mitochondrial translation and all four genes involved in mitochondrial one-carbon metabolism were enriched hits. We also found that *CLPB,* a gene that codes for a mitochondrial disaggregase, and several mitochondrial inner membrane proteins were depleted. Taken together our results imply that *O*-GlcNAc plays a crucial role in mitochondrial function. Consistent with this notion we have found that cells treated with the OSMI-4 have striking defects in mitochondrial morphology. We are actively investigating the impact of O-GlcNAc loss on myriad mitochondrial phenotypes toward elucidating the role of OGT in compartmentalized cellular homeostasis.

M14

**The balance of mitochondrial biogenesis and degradation in neurons is impaired by ALS-linked mutation of the ER protein VapB.**

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In neurons, mitochondrial population maintenance in the distal axon is important for neural circuit health and function. Mitochondrial maintenance requires a balance between biogenesis of new and degradation of damaged organelles. The ER integral membrane protein VapB (VAMP-associated protein B) is poised to play a role in regulating this balance. VapB is involved in both mitochondrial DNA replication at ER-mitochondrial contact sites and autophagosome formation at the ER. Therefore, we hypothesized that VapB acts as a homeostatic regulator of the mitochondrial population in neurons. First, we asked if VapB controls mitochondrial density. Using live imaging in zebrafish larvae, we observed that both VapB overexpression and expression of the P56S VapB mutant (proline to serine mutation-ALS8 associated) increased mitochondrial density in the axon terminal. We reasoned that the changes in mitochondrial density could be due to an abnormal balance of mitochondrial biogenesis and mitophagy. We found that VapB and $VapB^{P56S}$ overexpression upregulate key regulators of mitochondrial biogenesis. Strikingly, while VapB overexpression also increases mitophagy in the axon terminal, $VapB^{P56S}$ does not. Therefore, $VapB^{P56S}$ mutation retains function in regulating biogenesis but fails to show compensatory upregulation in degradation. Together, our data demonstrate that VapB coordinates mitochondrial biogenesis and mitophagy through distinct mechanisms, and it is the role of this protein in mitophagy that is specifically impaired by the P56S ALS-linked mutation.

M15

**Oncogenic BRAF Activates the ATF5-Dependent Mitochondrial Unfolded Protein Response to Restrict Mitochondrial Function and Senescence in Early Disease.**

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The mitochondrial unfolded protein response (mtUPR) is an organelle quality control program that facilitates proteostasis within the mitochondrial network. Upon stress, Activating Transcription Factor 5 (ATF5) induces the canonical mtUPR via mitochondrial-nuclear crosstalk to upregulate chaperones, redox enzymes, and proteases to restore organelle function. The mtUPR pathway is most often studied using model systems and transformed mammalian cell lines, therefore, direct relationships between the mtUPR and cancer-initiating programs remain speculative. Here, the relationships, contributions, and consequences of oncogenic mitogen activated protein kinase (MAPK) and the mtUPR were investigated throughout the melanomagenesis process. We observe that oncogenic MAPK signaling (*e.g.,* BRAFV600E) induces a unique ATF5-dependent mtUPR pathway in primary human melanocytes, and this induction is sustained throughout melanomagenesis. BRAFV600E incites broad macromolecular stress within the mitochondrial network resulting in ATF5-dependent biogenesis, bioenergetics, and delayed senescence. Melanoma models of early disease exhibit dependency on the mtUPR pathway to restrict mitochondrial function and sustain proliferation, and patients with early BRAFV600E disease display constitutive mtUPR signatures with prognostic value to stratify low-risk and high-risk melanoma.

M16

**Metabolic rewiring promotes metastatic potential in STK11-null KRAS-driven non-small cell lung adenocarcinoma.**

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Non-small cell lung adenocarcinomas (LUAD) with concurrent oncogenic KRAS and STK11 loss-of-function mutations define an aggressive subtype characterized, in part, by enhanced metastasis. STK11 is a tumor suppressor that acts as a nutrient sensor to regulate a multitude of cellular processes, including metabolism. We therefore hypothesize that metabolic rewiring in STK11-null KRAS-driven LUAD cells increases metastatic potential. To test this, parental and ΔSTK11 KRAS-driven LUAD cells, generated via CRISPR-Cas9, underwent the Seahorse mitochondrial stress test in both full and glutamine-deficient media to obtain parameters of mitochondrial respiration. Overall, ΔSTK11 cells had a hypermetabolic phenotype associated with enhanced glutamine utilization. Upon exogenous glutamine deprivation, we observed a significant increase in live, detached ΔSTK11 cells as compared to the parental line. Detached ΔSTK11 cells maintained the ability to re-adhere and proliferate in full media while detached parental cells did not. Additionally, expression of pro-survival, anti-apoptotic and EMT markers was increased. To determine the potential mechanism(s) underlying the ability of ΔSTK11 cells to survive upon glutamine deprivation, we employed RNA sequencing and heavy nitrogen labeling. Results revealed an upregulation of the hexosamine biosynthetic pathway (HBP), an offshoot of glycolysis/gluconeogenesis that serves as a central hub to regulate many cancer fitness pathways via O-GlcNAcylation. Further assessment of O-GlcNAcylation levels via western blot and far western analysis revealed a decrease in HBP flux concordant with decreasing glutamine concentrations in parental LUAD cells. Conversely, O-GlcNAcylation was enhanced in ΔSTK11 cells upon decreasing glutamine, suggestive of an intracellular shunt to protect against nutrient stress. To this extent, we also observed an increase in the integrated stress response (ISR) pathway upon glutamine deprivation that coincided with the shutdown of mitochondrial respiration and enhanced flux of the HBP. Future directions aim to establish

the impact of enhanced HBP flux on metastatic potential in STK11-null KRAS-driven LUAD cells. Overall, this work reveals novel insight into the molecular mechanisms altered downstream of STK11-loss that link glutamine metabolism with metastatic properties in KRAS-driven LUAD.

M17

**Small amino acids that accumulate in tumors enable cancer cells to overcome lethal stresses.**

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In well-functioning tissues, healthy vascular systems carefully keep cells fed to prevent cellular stress and enable organ function. However, in pathological situations, such as tumor development, tissue vascularity is compromised and cells consequently experience multiple stresses, including nutrient scarcity and metabolic waste accumulation, requiring cancer cell adaptations to survive and cope with these stresses. To explore how pancreatic ductal adenocarcinoma (PDAC) cells survive in their hypovascularized tumor environment, we have isolated PDAC tumor interstitial fluid, the local perfusate of tumors, and performed quantitative metabolomics on this fluid to measure nutrient levels in the tumor microenvironment. We then developed a novel cell culture medium, called tumor interstitial fluid medium (TIFM), that replicates the physiological levels of over 115 metabolites found in the PDAC microenvironment. This allows us to culture PDAC cells under physiological nutrient stress and study the adaptations that enable survival under this stress. We found that, compared to PDAC cells in nutrient-replete conditions, PDAC cells cultured in TIFM were dramatically more resistant to apoptotic stimuli including DNA damage and chemotherapy. This suggests that nutrient stress in the tumor microenvironment, rather than challenging PDAC cell viability, surprisingly endows PDAC cells with the ability to resist otherwise lethal stresses. We then explored what TIFM nutrients are responsible for this phenotype. We found that glycine and alanine, two amino acids that accumulate to high concentrations in PDAC tumor interstitial fluid, drive resistance to DNA damage and chemotherapy. We further found that addition or removal of glycine/alanine instantaneously affects the viability of PDAC cells challenged with DNA damaging chemotherapies, suggesting tumor interstitial glycine/alanine protects PDAC cells by a fast-acting mechanism. Our results show that the metabolic environment of tumors not only challenges cancer cells but can be beneficial and improve the ability of cancer cells to withstand stress. In the context of PDAC, these results are crucial for the understanding the molecular underpinnings of chemoresistance prevalent in this disease and suggest dietary approaches to target nutrient:cancer cell interactions to improve therapy efficacy.

M18

**Exercise-induced senescence-like gene signature in fibro/adipogenic progenitors is impaired in diabetes muscle.**

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Exercise is a well-established intervention for obesity and diabetes and is known to confer various health benefits. However, it has been observed that 15-20% of individuals with diabetes fail to obtain the benefits of exercise. Inflammation, an adaptive response to exercise, is beneficial for myogenesis; however, exercise-induced inflammatory responses differ between individuals with normal glucose tolerance (NGT) and those with T2D. Additionally, exercise-induced inflammation associated with

senescence, known as the senescence-associated secretory phenotype (SASP), is necessary to promote muscle regeneration.Cellular senescence is an adaptive response triggered by various cellular stresses, resulting in irreversible cell cycle arrest and the secretion of factors, including cytokines, chemokines, and growth factors, as part of the SASP. The role of senescence in skeletal muscles remains a topic of ongoing debate; however, its importance in understanding the pathological mechanisms and biology of muscles is gaining consensus.In this study, we aimed to investigate the differences in senescence responses to exercise between individuals with T2D and those with NGT to advance effective exercise interventions for T2D. First, we performed a Gene Set Enrichment Analysis (GSEA) using the Gene Expression Omnibus dataset GSE59363 and a meta-analysis of senescence-related genes in NGT and T2D skeletal muscle before and after exercise by using MetaMEx. We found that exercise increased senescence-related gene expression in human skeletal muscle in NGT, but this was less likely to occur in T2D. Next, to verify which cell types cause senescence in response to exercise, we analyzed single-cell RNA-sequencing data of normal diet and high-fat diet (HFD) mice during exercise (GSE183288). We found that fibro/adipogenic progenitors (FAPs) were prone to senescence in response to exercise, an adaptive response that was attenuated in HFD mice. Additionally, mechanical stress to TIG-2M, a FAPs-like cell line, increased senescence-related gene expression, but this effect was decreased in an in vitro model of T2D with high glucose concentration and TNF-α stimulation.This study provides potential insights into the attenuation of senescence induction in the skeletal muscles of T2D subjects, especially in FAPs, which contributes to the reduced benefit of exercise.

## Minisymp: Dynamics of Proteins and Organelles in Human Diseases

M19

**Regulation of functional heterogeneity of insulin secretion hot spots in pancreatic beta cells.**

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Pancreatic beta cells secrete insulin to maintain normal blood glucose levels and prevent diabetes. Glucose-stimulated insulin secretion is clustered in hot spots at the beta cell/vascular interface, to assure targeting of insulin into the bloodstream. Hot spots occur at and require cortical accumulations of ELKS (ELKS patches), a known component of presynaptic active zones. However, mechanistic regulation of secretion at hot spots is largely unclear. In this study, we directly detect secretion events in intact GFP-ELKS-expressing mouse islets toward the vascular extracellular matrix (ECM) by TIRF microscopy, utilizing a sensor dye for Zn2+ co-secreted with insulin (FluoZin-3). Surprisingly, only ~19% of ELKS patches secrete, indicating that an additional mechanism underlies the secretory capacity of each patch. We aim to determine this mechanism.One possibility is that this capacity is defined by molecular dynamics of hot-spot machinery, which could influence insulin granule docking and exocytosis. TIRF-FRAP studies assessing the turnover between membrane-associated and cytoplasmic ELKS pools reveal a striking variability in molecular exchange rates between individual ELKS patches, which likely contributes to their functional heterogeneity.The second possibility is that differences in secretory activity between individual ELKS patches reflect differential availability of insulin granules at the cell periphery, which is regulated by microtubules (MTs). While MT plus end-directed transport is needed for the delivery of insulin for secretion, we have shown that MT arrays parallel to the plasma membrane promote withdrawal of excessive insulin granules, therefore negatively regulating secretion. Importantly, MT depolymerization specifically affects clustered, hot-spot secretion. Here, we test whether MTs associated with ELKS patches have a configuration that promotes secretion over granule withdrawal or vice versa. TIRF and VAEM (variable angle epifluorescence microscopy) analyses in islets

co-expressing GFP-ELKS and Halo-Tubulin show two types of MT association with ELKS patches (parallel or end-on), or no MT association at all, indicative of variable insulin granule delivery to these locations.Our working model predicts that secretion hot-spot heterogeneity relies on the interplay of variable molecular dynamics of cortical machinery and local MT morphology.

M20

**Depleting the Golgi ATPase SPCA1 in human keratinocytes and organotypic epidermis reveals defective cell-cell adhesion, ER stress, and actin disorganization as drivers of Hailey-Hailey disease.**

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Mutations in *ATP2C1*, which encodes the calcium ATPase SPCA1, were linked to Hailey-Hailey disease (HHD) more than 20 years ago, yet there are no FDA-approved therapies for this skin blistering disorder. While it is known that SPCA1 resides in the Golgi and supports its secretory function, our limited understanding of exactly how SPCA1 haploinsufficiency compromises epidermal tissue integrity has prohibited development of rational molecular treatments for HHD. Since *Atp2c1* knockout mice did not replicate the disease phenotype, we built human cellular and tissue models of HHD to delineate its pathogenesis and find new therapeutic targets. Leveraging CRISPR/Cas9 to ablate *ATP2C1* in human epidermal keratinocytes, we generated heterozygous (HET) cell lines to mirror the genetics of HHD. Using a mechanical dissociation assay, we confirmed that SPCA1-deficient monolayers exhibit defective cell-cell adhesion. When grown in an organotypic model of epidermis, HET cells replicated the key pathologic feature of HHD, tissue splitting between keratinocytes. We hypothesized that loss of epidermal integrity in HHD could be due to faulty expression or trafficking of adhesive proteins. While HET cells expressed comparable levels of desmosome components, they had reduced localization of desmosomal cadherins at cell-cell junctions, which could explain the loss of cell cohesion. To identify other candidate pathogenic drivers downstream of SPCA1 deficiency in an unbiased manner, we performed RNA sequencing of HET cells, which revealed elevated ER stress indicators and abnormal expression of actin regulators. Treatment of control keratinocytes with ER stress inducers weakened intercellular adhesive strength, suggesting proteotoxic stress may contribute to blistering in HHD. Interestingly, immunostaining actin in tissue cross-sections of epidermal cultures demonstrated highly irregular shapes of SPCA1-depleted cells compared to control cells. To determine if cytoskeletal dynamics were altered in live SPCA1-deficient keratinocytes, we stably expressed LifeAct-GFP and tubulin-mCherry in HET cell monolayers. While microtubule organization appeared unchanged, SPCA1-deficient cells showed marked changes in actin with an increase in filipodia, loss of stress fibers, and diminished cortical bundling. Based on these data, we propose that SPCA1 haploinsufficiency perturbs cadherin trafficking, increases ER stress, and disrupts actin organization, which together drive the loss of tissue integrity and impaired epidermal wound healing seen in HHD. In sum, our model of HHD replicated key features of the disease phenotype, elucidated its cellular pathogenesis, and uncovered potential pathways to target for treating this rare skin blistering disorder.

M21

**Single-cell, live-dynamic studies reveal AKT-driven drug resistance mechanism in colorectal cancer.**

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Colorectal Cancer (CRC) is the third most common malignancy in the US, and, despite its prevalence, treatment options are limited. The current gold standard is surgery with adjuvant chemotherapy - typically a cocktail of traditional chemotherapy agents, which are not curative and carry substantial side effect profiles. Additionally, drug resistance often leads to recurrence and metastasis, which are top causes of mortality in CRC. Thus, new treatments and a more comprehensive understanding of the treatment resistance mechanisms are much needed.

We aim to define molecular mechanisms and phenotypic signatures associated with drug resistance in CRC. Using our high-throughput, high-content quantitative imaging techniques and a collection of patient derived organoid (PDO) models, we have characterized phenotypic cellular heterogeneity present in CRC across Consensus Molecular Subtypes (CMS). Due to our 384-well organoid culturing format, our PDO profiling platform is highly amenable to multifactorial system perturbations and even large-scale screening. We have characterized chemo-induced phenotypic changes that demonstrate the incredible plasticity of tumors, a phenomenon that contributes to drug resistance in CRC. Additionally, we find that tumoroids with similar phenotypes respond similarly to drugs, suggesting that cell-level phenotypic data may succeed in predicting differential drug response where genomic and even transcriptomic data have failed.

Through our characterization of PDO cells that survive treatment with first-line chemotherapies 5-FU and oxaliplatin, we have found that upregulation of the AKT pathway is required in many resistant cell subpopulations. As these tumors do not display AKT upregulation or AKT inhibitor sensitivity at baseline, we hypothesize that the treatment induces a cell-survival response driven by AKT signaling. To test this, we have engineered our PDOs with a live-cell AKT reporter that allows us to observe AKT signaling dynamics in real-time. This, coupled with our high-dimensional imaging pipeline, provides us with a spatiotemporal understanding of the phenotypic and morphological changes induced in treatment-resistant tumor subpopulations and the precise dynamic involvement of AKT in these changes. Interestingly, not all tumoroids respond to chemo with immediate AKT upregulation, so an understanding of AKT dynamics, not just static levels, is required to predict if AKT inhibitors are efficacious when added as combinatorial treatment with 5-FU and oxaliplatin. Our work has shown that AKT is an important driver of drug resistance in CRC and that live-cell dynamic characterization in PDOs may be important for defining and predicting better combinatorial treatments for the clinic.

M22

**1-deoxysphingolipids dysregulate the secretory pathway by altering membrane properties of the endoplasmic reticulum.**

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Aberrant lipid metabolism can disrupt normal cellular processes, leading to the onset and progression of various diseases. 1-deoxysphingolipids (1-deoxySLs) are an unusual lipid class in which alanine replaces serine in the sphingoid backbone during sphingolipid (SL) biosynthesis. Accumulation of 1-deoxySLs has been linked to diseases such as macular telangiectasia (MacTel) and hereditary sensory and autonomic

neuropathy type 1 (HSAN-1). It is known that mutations in the enzyme that catalyzes the first step of SL biosynthesis, serine-palmitoyl transferase (SPT), or imbalances in plasma serine/alanine ratio, cause 1-deoxySL accumulation. However, the cellular mechanisms of 1-deoxySL toxicity are still unknown. In MacTel patients, retinal pigment epithelium (RPE) cells exhibit a loss of displayed apical phagocytic receptors, indicating a potential dysregulation in the secretory pathway. To understand how 1-deoxySLs are trafficked from the endoplasmic reticulum (ER), where it is synthesized, we applied an organelle-specific 'click' chemistry technique to visualize the distribution of metabolically-tagged lipids in the early secretory pathway. Unlike canonical SLs, we find that 1-deoxySL products become retained in the ER and specifically accumulate at ER exit sites (ERES). To investigate the cellular effects of 1-deoxySL accumulation in the ER, we generated a RPE1 cell line in which induced expression of a disease-associated SPT variant ($SPT^{C133W}$) drives 1-deoxySL synthesis. Using this system, we found that 1-deoxySLs metabolism causes a dramatic increase in membrane ordering of the ER, as measured by the solvatochromic dye Laurdan, and a concomitant morphological change to ERES structure. We then analyzed how these perturbations to the ER membrane affect the anterograde trafficking of cargo proteins by utilizing the RUSH system (retention using selective hooks) to image synchronized cargo release. Interestingly, we found that 1-deoxySL synthesis has a bimodal effect on cargo exit from the ER to Golgi, accelerating for cargoes that prefer disordered membrane environments, like the transferrin receptor, while decelerating for those that localize to ordered membrane domains, like GPI-anchored proteins. We propose that this modulation of secretory trafficking based on membrane properties could be relevant for the physiopathology of RPE cells in MacTel patients and that of other cell types in diseases characterized by 1-deoxySL metabolism.

M23

**Homotypic fibrillization of TMEM106B across diverse neurodegenerative diseases.**

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Misfolding and aggregation of disease-specific proteins, resulting in the formation of filamentous cellular inclusions, is a hallmark of neurodegenerative disease with characteristic filament structures, or conformers, defining each proteinopathy. Here we show that a previously unsolved amyloid fibril composed of a 135 amino acid C-terminal fragment of TMEM106B is a common finding in distinct human neurodegenerative diseases, including cases characterized by abnormal aggregation of TDP-43, tau, or alpha-synuclein protein. A combination of cryoelectron microscopy and mass spectrometry was used to solve the structures of TMEM106B fibrils at a resolution of 2.7 Å from postmortem human brain tissue afflicted with frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP, n = 8), progressive supranuclear palsy (PSP, n = 2), or dementia with Lewy bodies (DLB, n = 1). The commonality of abundant amyloid fibrils composed of TMEM106B, a lysosomal/endosomal protein, to a broad range of debilitating human disorders indicates a shared fibrillization pathway that may initiate or accelerate neurodegeneration.

M24

**Seeding primary neurons with Alzheimer's brain-derived pathological tau results in the progressive formation of pathogenic conformations of tau linked to neuron dysfunction.**

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A hallmark of Alzheimer's disease (AD) is the pathological aggregation of tau that spreads throughout the brain in a stereotypical pattern. The tau seeding hypothesis offers a potential mechanism underlying this spread. Tau can seed aggregation in cells; however, the extent to which disease-associated pathogenic conformations can be effectively seeded remains an area of active investigation. To address this gap, we seeded aggregation in primary neurons and assessed known pathogenic tau conformations. We are particularly interested in the conformational exposure of an N-terminal motif in tau known as the phosphatase-activating domain (PAD) that occurs early in AD and other tauopathies and aberrant exposure of this motif disrupts axonal transport. Pathological tau was purified from AD-brain and used to treat primary hippocampal neurons from human tau knock-in (hTau-KI) mice. The same purification protocol was used with non-demented human brain tissue as a control (CO). The AD-tau and CO samples were characterized by transmission electron microscopy and biochemical assays that measure total tau and pathological modifications. AD-derived material showed morphologies consistent with small pieces of the characteristic paired helical filaments of AD, phosphorylation at the PHF1 site, and the presence of oligomeric tau and PAD-exposed tau species. Neurons were treated at DIV5 and maintained for up to 28 days post-treatment. Treatment with AD-tau resulted in robust and progressive formation of PAD-exposed tau inclusions in the neurons from DIV9 to DIV31. Oligomeric and AD-associated phospho-tau species (PHF1 and pS422) were also present in the seeded inclusions. To assess the effect of tau aggregation on cell viability, we utilized CellTiter-Glo and ApoTox-Glo assays, as well as performed neuron and astrocyte counts using ICF. No significant effect of tau seeding on cell viability was observed using these approaches. The lack of overt neurodegeneration is consistent with the vast majority of tau seeding models that currently exist. Our ongoing studies are aimed at assessing the function (e.g., axonal transport and synaptic dysfunction) of seeded neurons. Determining the effects of known AD-associated tau species on neuron function using this model could provide critical insight into the disease pathogenesis and guide development of disease-modifying therapies.

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## Minisymp: Higher-order Microtubule Assemblies

M25

**A Near-Micrometer-Scale Self-Assembled Platform around a Human Centriole: How Does It Form, and What to Make of It After All?.**

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Proper organization of intracellular assemblies is fundamental for optimal function and the efficient promotion of biochemical processes. As a membraneless multiprotein organelle and the main microtubule-organizing center in animal cells, the centrosome plays a pivotal role in the orderly progression of the cell cycle. Improper function of the centrosome results in abnormal cell division that

can lead to the development of various human disorders. How pericentriolar materials (PCM) are architecturally organized around a barrel-shaped centriole remains poorly understood. We reported that two long coiled-coil PCM scaffolds, Cep63 and Cep152, interact with each other to form a heterotetrameric complex that self-assembles into a micrometer-scale cylindrical architecture in vitro, resembling their localization morphologies in vivo. Thus, unlike intermediate filament-forming long coiled-coil proteins, such as keratin, lamin, and vimentin, which construct a long cable-like structure in an open cytosolic environment, Cep63 and Cep152 have an inherent capacity to cooperatively generate a PCM architecture by self-assembling them around a centriole. Through size-exclusion chromatography, sedimentation equilibrium ultracentrifugation, interferometric scattering mass spectrometry, and X-ray crystallography, here we showed that the Cep63•Cep152 complex generates a higher-order self-assembly by undergoing a concentration-dependent and reversible tetramer-octamer-hexadecamer transition. A Cep152 mutant arrested at the octameric state misorganized the Cep63•Cep152 platform around a centriole and failed to promote polo-like kinase 4 (Plk4)-dependent centriole duplication. Thus, the organizational properties of the Cep63•Cep152 platform determine the functionality of Cep152-bound Plk4. Remarkably, the self-assembly of the Cep63•Cep152 architecture was driven by the cooperative action of two short (approximately 25-residue-long) fragments (named self-assembly modules [SAMs]) from each Cep63 and Cep152. Analyses with chemically synthesized SAMs revealed that they cophase-separate and self-organize into a cylindrical assembly, whose constituent SAMs undergo internal rearrangement within the assembly while freely exchanging with other SAMs in the surroundings. As expected, if the SAM-driven assembly and dynamics are important, mutations in the SAM disrupted the organization and function of the Cep63•Cep152 platform and crippled the Plk4-dependent downstream event. Given that the organization of PCM is conserved throughout evolution, this work may serve as a paradigm for studying the assembly and function of centrosomal scaffolds in various organisms.

M26

**ZYG-1-mediated phosphorylation of SAS-5 positively and negatively controls centriole assembly in *Caenorhabditis elegans*.**

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Centrioles are microtubule-based cylindrical structures which act as the major Microtubule-Organizing Center (MTOC). In dividing cells, centriole duplication is tightly controlled. Mis-regulation of the process is linked to cancer and microcephaly. In *C. elegans*, centrioles are built by the sequential addition of the proteins SAS-7, SPD-2, ZYG-1, SAS-5 & SAS-6 and SAS-4. While, the homologs and the general theme of assembly are conserved, the molecular mechanism of centriole biogenesis is not completely understood. In particular, how the kinase ZYG-1 regulates the process is unknown. We addressed this question using *in vitro* refolded full-length recombinant centriolar proteins. We found that ZYG-1 interacts and phosphorylates SAS-5 *in vitro*. Using mass spectrometry, we identified residues that were phosphorylated *in vitro*. Mutational scanning of the residues using CRISPR/Cas9 genome editing revealed that Ser10 and Ser331/338/340 are indispensable for proper SAS-5 function *in vivo*. Embryos expressing either SAS-5$^{S10A}$ or SAS-5$^{S10E}$ exhibited monopolar spindles during the early embryonic divisions. Unexpectedly, we found that SAS-5$^{S331/338/340A}$ (3A) embryos exhibit multipolar spindles. Consistent with an overactive centriole assembly pathway in this mutant, we found that the level of SAS-5$^{3A}$ protein was significantly elevated. In contrast, SAS-5$^{S10A/S10E}$ levels were unaffected. Live imaging of early SAS-5$^{S10A}$ embryos that express SAS-6::GFP show that SAS-6 is initially recruited to centrioles.

However, SAS-6 is rapidly lost, resulting in centriole duplication failure. Thus, phosphorylation at Ser10 is required for stable incorporation of SAS-6 into the nascent centriole. Overall, our results suggest that ZYG-1 both positively and negatively regulates SAS-5 through phosphorylation to stringently control centriole biogenesis.

M27

**Poc1 is a triplet microtubule inner junction protein that promotes triplet microtubule integrity and interconnections to resist ciliary forces.**

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Basal bodies (BBs) are ancient eukaryotic structures that organize motile and primary cilia formation and function. BBs are comprised of nine triplet microtubules (TMTs) arranged in a cylinder shape. These TMTs are connected to each other by inter-TMT linkages that maintain BB organization and structure. During ciliary beating, forces are transmitted to the BB and must be resisted by the BB to prevent BB disassembly. Poc1 is a conserved BB protein important for BBs to resist ciliary forces. To understand how Poc1 confers BB stability during ciliary beating, we used cryo-electron tomography and super-resolution light microscopy to identify the precise position of Poc1 binding in the BB of the ciliate *Tetrahymena* and the effect of Poc1 loss on BB structure. The subnanometer cryo-ET BB structure shows that Poc1 binds at the TMT inner junctions, stabilizing the TMT structure directly. Poc1 also stabilizes inter-TMT linkages throughout the BB, including the inner scaffold, pinhead, and A-C linkers. Thus, while not essential for the assembly of the BB itself, Poc1 promotes BB interconnections that establish an architecture that is competent to resist ciliary forces. We establish the first high-resolution structure-function relationship to explain the mechanics of BB stability conferred by a conserved BB protein.

M28

**Cryo-electron tomography reveals the step-wise process of cilia assembly.**

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Motile cilia are built of a highly ordered microtubule array called the axoneme. Axonemal microtubules are decorated by repeating protein complexes which produce and regulate the ciliary beat. We now know the molecular details of how axonemal complexes are arranged on microtubules but do not yet understand how these intricate structures self-assemble. Since cilia grow exclusively from their distal-most region, we set out to investigate cilia assembly by performing cryo-electron tomography of Chlamydomonas reinhardtii cilia tips. Using subtomogram averaging, we show how microtubule inner proteins (MIPs) are hierarchically added to nascent microtubules. We identify a group of pioneering MIPs that bind microtubules first, thus setting up the axonemal repeat, and show which MIPs are required for the recruitment of motile components. In addition, our data shed light on tip-specific structures whose role is not well understood. We describe numerous distinct capping structures at the microtubule ends, including a novel structure which spans the ciliary membrane to bridge the intra- and extra-cellular environment. Finally, we generated a strain lacking a tip-resident protein called FAP256 (Cep104 in mammals) which was previously shown to remain localized to the tip during cilia growth and disassembly. Cells lacking FAP256 have a defect in initiation of cilia growth. By cryo-electron tomography, we find the incorporation of axonemal components is drastically altered and they lack all

visible microtubule-end capping structures. Together our results provide crucial insight into the process of cilia assembly and the role of tip-specific proteins in cilia function.

M29

**In vitro reconstitution of the protein complex controlling ciliary tip dynamics.**

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Cilia are essential sensory and motile organelles found on many eukaryotic cells, and defects in cilia can lead to various human diseases, called ciliopathies. Cilia structure and function rely on a sophisticated core of microtubules, the axoneme. Unlike cytoplasmic microtubules, ciliary microtubules exhibit much slower tip dynamics, and therefore likely have different mechanisms of regulation. In contrast to our understanding of proteins that regulate cytoplasmic microtubules, very little is known about the biochemistry of ciliary microtubule regulators. Here, we focus on the so-called ciliary tip module, a group of five proteins known to interact with each other, and mutations in which perturb cilia growth and stability and cause a human brain development disorder known as Joubert syndrome. Despite multiple studies in vivo characterizing loss-of-function mutations where defects in cilia growth and stability were seen, we know little about how these proteins affect microtubule dynamics. Using *in vitro* reconstitution experiments, we are elucidating how these proteins function together to influence microtubule stability and structure. Two of the five studied proteins, TOGARAM-1 and CEP104, have evolutionary conserved tubulin-binding TOG domains that are expected to bind to the outer microtubule surface. Surprisingly, these two proteins have very different effects on microtubule dynamics: TOGARAM-1 acts as a rescue factor, whereas CEP104 blocks microtubule growth. Another ciliary tip module protein, CSPP1, binds to the microtubule lumen and triggers microtubule pausing events. The last two members of the ciliary tip module, CCDC66 and ARMC9, do not affect microtubule dynamics directly but appear to act as scaffolds for other members of the module. Excitingly, the combination of all five proteins leads to very slow and persistent microtubule polymerization, which recapitulates the slow growth dynamics of axonemal microtubules in cells. Our data show that the combined effect of all five ciliary tip module members, acting from both sides of the microtubule wall, leads to a unique dynamic state of the microtubule tip characterized by very slow but processive elongation.

M30

**A genome-wide CRISPRa screen reveals a pathway for primary cilium disassembly linked to neurodevelopmental disease.**

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The primary cilium is an organizing center for signal transduction, and mutations that compromise ciliary function cause disorders known as ciliopathies. While much is known about the process of cilium assembly and the consequences of defects in ciliogenesis, much less is known about cilia disassembly and the disease phenotypes associated with errors in this process. To gain new insights into cilia disassembly and negative regulators of cilia dynamics, we conducted a genome-wide CRISPR activation (CRISPRa) screen for genes whose over-expression inhibits cilium-dependent Hedgehog (Hh) signaling. From follow-up studies on a number of newly identified hit genes, we define a novel pathway that

enacts cilia disassembly in response to relevant cues, including F2R GPCR signaling and serum mitogens, a major physiologic regulator of cilia resorption. This pathway includes the neurodegeneration-linked NADase SARM1, the second messenger cyclic-ADP-ribose (cADPR) that is produced by SARM1, ER calcium release triggered by cADPR action on Ryanodine Receptor channels, and RhoA activation of Rock kinases. We show that hyperactivation of this pathway induces cilia disassembly. Conversely, pharmacologic inhibition of this pathway in wildtype cells blocks cilia disassembly and promotes ciliation under non-permissive conditions. Thus, this pathway is a key endogenous mediator of cilia disassembly. Lastly, we find that several pathway components are mutated in a neurodevelopmental disorder, suggesting aberrant cilia disassembly as a shared pathomechanism in this neurological disease. Consistent with this model, we find that a disease-associated mutation in SARM1 potentiates cilia loss. Together, our studies illuminate the poorly understood process of cilia disassembly and suggest a new type of ciliopathy caused by aberrant activation of cilia disassembly, forming a complement to canonical ciliopathies caused by inactivation of cilium assembly. CRISPRa-based over-expression screening thus forms a valuable counterpart to CRISPR knockout screens, revealing distinct mechanistic insights and distinct contributions of cilia to disease.

## Minisymp: Mechanobiology across Scales

M31

**Regulating the material properties of focal adhesions through protein phase separation.**

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Focal adhesions (FAs) are supramolecular complexes that are critical regulators of mechanotransduction at the plasma membrane. Recently, studies have found that certain FA proteins can undergo phase separation to cluster integrins and promote nascent adhesion formation. In other biological contexts, phase separation drives formation of biomolecular condensates that possess distinct material properties such as specific viscosity and surface tension. Talin is a large focal adhesion adaptor protein that is important for FA maturation, it binds to both actin cytoskeleton in the cytoplasm and integrin at the plasma membrane. Upon mechanical stretch generated by actin force, talin reveals up to 11 multivalent vinculin binding sites (VBSs), recruiting actin-binding vinculin to physically reinforce the talin-actin linkage. Together, vinculin, talin and actin represent a mechanosensitive, multivalent binding system. Since multivalent interactions between proteins can drive phase separation, we hypothesized that talin, vinculin, and actin could undergo phase separation. To test this, we designed chimeric talin with different numbers of exposed VBSs to reconstitute this system *in vitro*. The chimeric talins enable us to tune VBS valency in the absence of force. We found that chimeric talin and vinculin form micron-sized assemblies in the presence of filamentous actin. These assemblies contain a talin-vinculin core with actin bundles extending from the surface and are morphologically similar to cytoplasmic puncta induced in cells by the expression of constitutively active vinculin. Our preliminary *in vitro* experiments demonstrate that multivalent interactions between talin, vinculin, and F-actin are sufficient to promote phase separation. The stoichiometry between vinculin and VBS as well as talin valency both regulate phase separation. We are currently probing the material properties of *in vitro* talin-vinculin condensates to determine how talin VBS valency regulates condensate viscosity. In cells, talin VBS valency is tuned by mechanical force. By characterizing the relationship between talin VBS valency, phase separation, and condensate viscosity, we will learn how specific protein interactions may tune the material properties of focal adhesions. Future experiments will investigate the role of VBS valency in FA mechanotransduction and cell migration.

M32

**Curved crease origami at cellular scales enables hyper-extensibility of *Lacrymaria olor*.**

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Eukaryotic cells can undergo dramatic morphological changes over many different length and time scales. Some of these morpho-dynamic processes, such as cell division, phagocytosis or cellular motility are well-studied. However the fundamental limits of cellular morpho-dynamics, such as how fast or how much cellular shapes can change without harm to a living cell, remain poorly understood. Here we show that hyper-extensibility of the single-celled protist *Lacrymaria olor*, a 40 μm cell capable of repeatable extensions of a neck-like protrusion up to 1500 μm in 20 seconds, are enabled by unique cortical cytoskeleton and membrane architectures. Using multi-modal imaging techniques, we map the exact cortical cytoskeleton of *Lacrymaria olor* and find a multi-layer spooled microtubule architecture. We further discover a new motif in cellular membrane folding, a curve-crease origami structure in a living cell, that precisely enables *L. olor* to rapidly deploy and retrieve a neck-like protrusion. The deployment of this unique helical curved crease origami in a cell is controlled by a fold singularity that creates a transition zone between folded and unfolded states of the membrane. These folds are supported by the cortical cytoskeleton, enabling the cell to achieve reversible spooling and unspooling of microtubules along the neck. Our work demonstrates a direct link between form and function of cortical cytoskeleton in a unicellular protist, emphasizing the role of cytoskeleton geometry and cell architecture in controlling single cell behavior.

M33

**Dynamic Microtubule Acetylation Drives Actomyosin Contractility in Directional Cell Migration.**

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One of the most evolutionarily conserved microtubule post-translational modification: acetylation of Lysine-40 in α-Tubulin is critical for mechano-sensing and cell migration. However, the molecular mechanisms by which microtubule acetylation mediates directional migration is not well understood. Here we report that mouse embryonic fibroblast (MEF) cells from α-TAT1 knock-out (KO) mice show severe defects in chemotaxis, but not in overall motility, compared to wild-type (WT) MEF cells. KO MEFs also show defects in front-and-back cell polarity, actin cytoskeleton, Myosin-II activity and focal adhesions. Using a FRET-based Vinculin Tension-Sensor, we further show that focal adhesions in α-TAT1 KO MEF cells experience reduced tension. We have previously shown that cytoplasmic localization of α-TAT1 is critical for its function, and based on this model of spatial regulation, we developed a molecular actuator to rapidly and reversibly induce microtubule acetylation with blue light stimulation in live cells. This tool, named optoTAT, was predominantly nuclear in dark and was rapidly shuttled to the cytoplasm upon blue light stimulation in a reversible manner. Hela or KO MEF cells expressing optoTAT exposed to blue light showed a robust increase in microtubule acetylation compared to those kept in dark, validating the functionality of the tool. Stimulating optoTAT induced rapid activation of actomyosin contractility as evidenced by increased actin stress fibers, Myosin-Light chain accumulation and maturation of focal adhesions. These effects were dependent on its catalytic activity as well as ROCK signaling, thus establishing an acute and causal relationship between microtubule acetylation and ROCK-mediated actomyosin contractility. Finally, we demonstrate that microtubule acetylation releases the RhoA-activator GEF-H1 from being sequestered on microtubules, thus identifying a molecular basis for

the crosstalk between microtubule acetylation and actomyosin contractility. To summarize, we have developed a molecular actuator, optoTAT, to specifically induce microtubule acetylation, and have demonstrated a causal role of microtubule acetylation in mediating actomyosin contractility in directional cell migration.

M34

**Probing mechanotransduction pathways in breast cancer metastasis through visualization and control of vinculin conformation.**

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Cancer cells have been shown to invade from the primary tumor along aligned collagen fibers. This phenomenon is dependent on cell mechanosensation through focal adhesions. Vinculin is a mechanosensitive adhesion protein that facilitates force transmission between integrins and actin by stretching under tension to open protein-binding sites. We hypothesize that localized vinculin stretching and activation in focal adhesions facilitates metastatic cell invasion along aligned collagen fibers. To test this hypothesis, we have developed vinculin analogs for use in living cells to 1) report vinculin conformational state, and 2) control vinculin activity with light. To visualize vinculin conformation, we inserted a short peptide that is exposed only in the open, "stretched" conformation of vinculin. When exposed, the tag can be accessed by a small fluorescently labeled binder protein. Using this approach, vinculin conformation can be quantified with simple colocalization of the binder to vinculin. To control vinculin-based force transmission, we sought to allosterically modulate the vinculin-actin interaction using an optogenetic approach. Previous studies have shown that protein activity can be controlled by inserting the LOV2 domain, which undergoes conformational changes when irradiated. We made photo-inhibitable and photo-activatable vinculin analogs by inserting the LOV domain in surface loops where LOV conformation allosterically modulates vinculin activity in response to blue light. We apply these analogs and fluorescent biosensors breast cancer cells migrating on micropatterned substrates that mimic aligned collagen found in the tumor microenvironment.

M35

**Role of epigenetic modifications in the establishment of mechanical memory.**

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Cells are known to sense and respond to mechanical cues by undergoing various phenotypic changes: on softer substrates cells tend to exert less forces and present slower migration speeds. Surprisingly, these phenotypic changes have been shown to persist for some time after the mechanical cues subside. This ability to retain information and the effects of past mechanical cues has been named "mechanical memory". Little is known of the mechanisms involved in mechanical memory set-up although epigenetic modifications have recently been proposed to serve as a means to store this information. Our project consists in testing this hypothesis using two cell lines: murine osteoblast cells and mammary cancer cells (HS 578T).To assess cells' ability to establish mechanical memory, they were mechanically primed on either a soft or stiff primary [B1] substrate for a defined period of time (3days) before being transferred to a stiff secondary substrate. We observed that both cell lines establish a mechanical memory as their migration speed on the stiff secondary substrate was affected by the priming rigidity: soft-primed cells presented a lower migration speed than stiff-primed cells. Interestingly, transcriptomic analysis revealed

an increase in matrix protein expression, such as fibronectin (FN), in soft-primed cells suggesting that increased adhesion capacities in these cells may impede cell migration. Indeed, depletion of FN during mechanical priming in both cell lines affected the establishment of mechanical memory in both cell lines as the migration speed of soft-primed FN depleted cells was increased compared to that of the soft-primed control cells. We also identified through the transcriptomic analysis a histone demethylase (KDM7a) up-regulated in soft-primed cells. Depletion of KDM7a during mechanical priming impaired the cells ability to establish a mechanical memory: cells' migration speed was no longer dependant on the priming rigidity but solely on the rigidity of their immediate substrate. This suggested that KDM7a was involved in mechanical memory set-up. Interestingly, we observed that depletion of KDM7a also affected FN expression levels hinting at a possible regulation of FN by the demethylase. We are currently investigating this potential regulation of FN by KDM7a and its involvement in the establishment of mechanical memory.

M36

**Mechanical Control of Actin Polymerization is an Essential Regulator of Nuclear Mechanotransduction and Cell Programming.**

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Cells are exposed to a variety of mechanical cues, which they respond to by engaging the mechanisms of mechanotransduction leading to changes in cellular behavior including proliferation, migration, and differentiation. These mechanical cues include stiffness, viscosity, and alignment of the extracellular matrix (ECM) as well as forces exerted on or generated within the cell. Many studies have shown that forces, transmitted through the actin cytoskeleton to the nucleus, alter nuclear mechanics and the genetic landscape. There is mounting evidence that forces also tune the organization and dynamics of the actin cytoskeleton. Force-sensitive actin polymerization by formin Dia1 controls tension on the cytoskeleton protecting stress fibers upon acute mechanical damage, but whether these rapid changes in cytoskeleton mechanics modulate the long term cellular responses mediated by nuclear mechanotransduction is unknown. To test the role of force sensitive actin polymerization in nuclear mechanotransduction, we generated a force insensitive Dia1 mutant and assessed its effects on cell programing induced by mechanical cues. We showed that under permissive conditions, ECM stiffness drives fibroblast to myofibroblast (MFs) differentiation and this change in cell phenotype requires nuclear mechanotransduction. Replacement of Dia1 with force insensitive Dia1 abolishes MF differentiation induced by stiff ECM. We showed that the defects in cell programming are not caused by changes in the activity of the TGFβ pathway, which regulates MF differentiation. Instead, they are due to a failure in nuclear translocation of SMAD transcription factors. Such effect on the transcription factor localization was correlated with an increased nuclear height and a reduction in perinuclear stress fibers, suggesting that force sensitive actin polymerization controls SMAD nuclear shuttling through nuclear deformation mediated by stress fibers. Accordingly, compression of nuclei of cells expressing force insensitive Dia1 rescues nuclear localization of SMAD and MF differentiation. To assess the physiological role of force sensitive actin polymerization, we analyzed MF differentiation on ECMs with a range of stiffness characteristic of healthy and diseased tissues. We found that force insensitive Dia1 decreases the permissive stiffness for differentiation - while the differentiation of control fibroblasts is induced by ECM stiffnesses of fibrotic tissues (>30kPa), cells expressing force-insensitive Dia1 differentiate on ECM stiffnesses of healthy tissues (5-8kPa). Together these findings demonstrate that force sensitive actin

polymerization establishes the range of ECM stiffness that is translated into long term cellular responses by nuclear mechanotransduction.

## Minisymp: Signaling and metabolic interactions between cells and the environment

M37

**Tuning Epidermal Growth Factor Receptor (EGFR) Spatiotemporal Organization Through Adaptor Protein Valency and Competition.**

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Receptors at the plasma membrane receive and transmit environmental cues to guide a cell's behavior. The EGFR family of receptors are critical in regulating cellular growth and survival by coordinating localization of cytosolic adaptor and signaling molecules to direct activation of pathways like MAPK and PI3K signaling. However, organizing the spatiotemporal recruitment of downstream adaptor molecules to these EGFR signaling clusters during both physiological and oncogenic signaling remains poorly understood. Recently, it has been demonstrated that many transmembrane receptors, including EGFR, undergo phase separation to form micron-sized biomolecular condensates on the plasma membrane. These condensates concentrate multivalent adaptor proteins and signaling molecules and can alter signaling dynamics. Whether phase separation influences signaling downstream of EGFR condensate formation remains unclear. We hypothesize that different combinations of EGFRs and adaptor proteins GRB2, SOS1, GAB1, and N-WASP will yield distinct condensate kinetics and characteristics to guide signal activation. SOS1 promotes Ras and MAPK activation, GAB1 promotes PI3K recruitment and Akt activation, and N-WASP promotes actin polymerization. Thus, recruitment of these different adaptor proteins helps determine downstream signal pathway activation in cells. To systematically determine how specific adaptor proteins influence EGFR condensate kinetics and characteristics, we use *in vitro* biochemical reconstitution and total internal reflection fluorescence (TIRF) microscopy. Using purified and fluorescently labelled recombinant proteins, we attached the fully phosphorylated intracellular C-terminal tail of EGFR to a supported lipid bilayer and monitored protein clustering by TIRF microscopy. Upon the addition of SH2-SH3-containing adaptor Grb2 and Proline-rich protein N-WASP, receptors immediately phase separated through spinodal decomposition. However, replacing N-WASP with different Proline-rich proteins (Gab1 or SOS1) changed condensate properties. Strikingly, pre-formed EGFR condensates spiked with Gab1 dissolved and nucleated new puncta clusters. Moreover, within the same condensate, adaptor proteins exhibit different dwell times. Together, our data suggests that EGFR phase separation and downstream signaling may be modulated by the recruitment of different adaptor proteins. We predict that condensates with unique adaptor protein composition help discern downstream signal pathway activation in cells.

M38

**PDGFRα/β Heterodimers Have Distinct Internalization and Signaling Dynamics from PDGFR Homodimers.**

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Signaling through the platelet-derived growth factor receptor (PDGFR) family of receptor tyrosine kinases plays critical roles in multiple cellular processes during both development and adult life. The PDGFR family consists of two receptors, PDGFRα and PDGFRβ, that dimerize to form PDGFRα

homodimers, PDGFRα/β heterodimers and PDGFRβ homodimers. It has previously been impossible to study dimer-specific dynamics for the PDGFRs because commonly used antibody-based approaches do not allow for the visualization and purification of individual PDGFR dimers. Here, we overcome these previous limitations in studying PDGFRα/β heterodimers using bimolecular fluorescence complementation (BiFC). We generated and analyzed a cell line stably expressing C-terminal fusions of PDGFRα and PDGFRβ with BiFC fragments corresponding to the N-terminal (V1) and C-terminal (V2) regions of the Venus fluorescent protein, respectively. We confirmed heterodimerization of the receptors and bimolecular fluorescence complementation upon PDGF-BB ligand stimulation of these cells via fluorescence microscopy and immunoprecipitation with a nanobody (GFP-Trap) that recognizes an epitope spanning the V1/V2 interface. We found that these receptors heterodimerize and are activated relatively quickly in response to PDGF-BB ligand, with a peak of receptor autophosphorylation following 5 minutes of ligand stimulation. Moreover, we demonstrated that PDGFRα/β heterodimers are rapidly internalized into early endosomes, particularly signaling endosomes, where they dwell for extended lengths of time. Interestingly, we found that PDGFRα/β heterodimer activation does not induce downstream phosphorylation of ERK1/2 and significantly inhibits cell proliferation, unlike PDGFR homodimer activation. Together, these findings indicate that PDGFRα/β heterodimers may serve as sinks that bind PDGF-BB and prevent the ligand from activating PDGFR homodimers. Finally, we combined BiFC with quantitative mass spectrometry to identify proteins that differentially interact with the various PDGFR dimers at the time of internalization, including a subset involved in trafficking that specifically interact with PDGFRα/β heterodimers. Collectively, our findings impart valuable insight into the molecular mechanisms by which specificity is introduced to generate unique responses downstream of PDGFR activation.

M39

**DGS-1 and FIG-1 allow glia to detect and counter defects in ensheathed dendrite structure.**

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Cell structure is critical for cell function. An important question is whether and how cell structure is monitored by interacting cells. We found that in the *C. elegans* amphid sensory organ, glia detect and respond to defects neuron structure. In the amphid, the amphid sheath (AMsh) glia secretes an extracellular matrix that surrounds dendrite cilia. We found that in mutants defective in dendrite cilia structure and sensory neuron function, excess matrix accumulates in the extracellular space surrounding the dendrite cilia, and an increased number of matrix-laden vesicles appear within AMsh glia. Inducible cilia disruption shows that AMsh glia respond within hours to cilia loss. Additionally, we found that AMsh glia alter their gene expression in cilia mutant animals, which correlates with increased activity of the conserved glial transcription factor PROS-1. We conclude that glia monitor the structure or activity of the dendrites they ensheath and respond by altering their secretion and transcription. To search for molecules involved in signaling from dendrite cilia to glia, we performed candidate-gene and forward genetic screens seeking mutants with normal cilia but aberrant accumulation of AMsh glia-secreted matrix. One mutant we identified carries a causative lesion in a gene encoding a predicted 7-transmembrane domain protein, which we named Dendrite-Glia Signaling-1 (DGS-1). DGS-1 functions in a subset of sensory neurons whose cilia are ensheathed by AMsh glia and localizes to these cilia. Another mutant we isolated perturbs the gene *fig-1*, which encodes a multifunctional extracellular protein that senses cilia integrity and regulates other neuronal properties. FIG-1 functions in AMsh glia and localizes to the AMsh glia secreted matrix surrounding dendrite cilia. DGS-1 and FIG-1 regulate each

other's abundance and localization. Importantly, mutations in *dgs-1* protect cilia in our inducible cilia-destruction paradigm. As *dgs-1* mutants display the glial responses to cilia disruption without any discernible defects in cilia structure or neuron function, this finding indicates that the AMsh glial response is protective to associated neurons. Our studies reveal that glia sense dendrite cilia integrity, and uncover a previously uncharacterized signaling pathway mediating a protective response involving glial secretion of extracellular matrix. Given the evolutionary conservation of sensory organ organization, we propose that similar dendrite-glia interactions may be conserved.

M40

**Reactivation of TORC1 by pheromone signaling during starvation.**

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Target of rapamycin (TOR) is a critical regulation of cell growth and proliferation, which forms two distinct TOR complexes 1 and 2 across eukaryotes. TOR complex 1 (TORC1) senses and integrates several environmental signals, including nitrogen availability, to promote cell growth. Fission yeast cells proliferate mitotically when grown in rich conditions and reproduce sexually, upon activation of pheromone-MAPK signaling, when compatible mating types are starved for nitrogen. It is well established that TORC1 is essential for cell proliferation, and that mutations in the catalytic subunit mimic nitrogen starvation, leading to sexual differentiation. To gain insight into the signaling of sexual reproduction, we developed assays to synchronize yeast cells during the mating and fusion process and conducted phospho-proteomic time course analyses, revealing hundreds of phosphorylation site changes. Proteins with significant phosphorylation decrease and increase displayed highly divergent functions in transporter activity and cell polarization respectively, consistent with preparation for cell-cell fusion. Surprisingly, among the proteins with most dramatic phosphorylation increase were the two ribosomal protein S6 (Rps6) paralogs, which are known targets of the S6 kinase Psk1 downstream of TORC1. We confirmed that Rps6 phosphorylation depends on Psk1, itself also phosphorylated during mating, and that both depend on TORC1. Indeed, these phosphorylations are abrogated by rapamycin treatment, indicating that TORC1 is active during mating. Furthermore, rapamycin reduces mating success in a manner dependent on binding the TORC1 kinase. We conclude that, although nitrogen starvation inactivates TORC1 to promote sexual differentiation, efficient mating requires TORC1 reactivation. We are probing the specific role of the pheromone-MAPK signaling in this reactivation.

M41

**A Comprehensive Lipotoxicity Profile for Podocytes Reveals Distinct Cellular Susceptibilities.**

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**Objective:** Maladaptive cellular utilization of excess circulating lipids such as free fatty acids (FFAs) can impair cell function, a phenomenon known as lipotoxicity. In the kidney, podocyte lipotoxicity has been implicated in many diseases including diabetic kidney disease, the most common cause of kidney failure worldwide. As the only postmitotic cells in the kidney, podocytes are indispensable for kidney filter function. However, to date, podocyte lipotoxicity has only been studied in the context of a limited number of FFAs. Here, using FALCON (Fatty Acid Library for Comprehensive ONtologies; Wieder, Coraor-Fried et al. 2023), we investigated the effects of podocyte exposure to a comprehensive library of >60 FFAs. **Methods:** Through a live cell image-based FALCON screen, we assayed immortalized mouse

podocytes for cell morphology, cell viability, and production of reactive oxygen species (ROS) at eleven time points. These data were complemented by a fixed cell image-based FALCON screen, characterizing detailed cytoskeletal structures after 24h of FFA treatment. Live cell screening results were compared to a similarly acquired lipotoxicity profile from a human immortalized tubular epithelial cell (TEC) line. To further investigate the FFA most cytotoxic to podocytes, we probed cellular signaling pathways and FFA-mediated changes to membrane fluidity. We validated the cytotoxicity of this FFA in isolated mouse glomeruli (kidney filtering units). **Results:** Screening a wide spectrum of structurally diverse FFAs and aggregating live cell image-based morphological features into podocyte phenotypic profiles revealed distinct morphological states of FFA treated cells. Grouping FFAs by their effects on cell viability and ROS production resulted in a functional profile distinct from that obtained in TECs. In particular, in TECs but not in podocytes did a subset of mono- and polyunsaturated FFAs induce large amounts of ROS while also decreasing cell viability. Mechanistically, the most podocytotoxic FFA was found to induce membrane rigidification, ER stress, and cytoskeletal rearrangements. **Conclusion:** We established a comprehensive lipotoxicity profile for podocytes which informs our understanding of biologically relevant insults to podocytes in health and lipotoxicity-driven kidney diseases.

M42

**Immune signaling subprograms invoked by macronutrients adapt antiviral defenses.**

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Interferons upregulate hundreds of interferon-stimulated genes early in infection to activate the frontline of antiviral defenses. Studies of interferon have revealed general principles of cell signaling and served as a prototype for transcriptional induction mediated by signal transduction. Still, characterization of interferon responses has largely been limited to a number of cell types and tissues with a holistic view lacking. Analyzing transcriptomes from fifty human tissues derived from tens to hundreds of individuals, we find widespread yet stratified heterogeneity in interferon-stimulated gene expression under homeostatic conditions. In contrast to the view as a singular inducible class of genes, these data suggest ill-defined interferon-stimulated gene subclasses that are differentially regulated across cell types. Given recently discovered viral-encoded mimics of mitochondrial metabolic machinery, we hypothesized macronutrients in the local microenvironment might contribute to the observed expression architecture of interferon responses. To explore the functional relevance of these putative interferon-stimulated subprograms, we infected cells primed with different interferons under conditions that favor either glycolysis or oxidative phosphorylation. Using a model poxvirus, we find that human cells favoring glycolysis and primed with interferon-gamma but not interferon-alpha or untreated, potently inhibit viral replication by one hundred-fold. Notably, cells favoring oxidative phosphorylation showed no differences in poxvirus replication between untreated and interferon-primed. The glycolytic/interferon-gamma response is broad acting as it also markedly restricts the unrelated herpes simplex virus-1. We uncover that macronutrients act as secondary cues to rewire the combination of interferon-stimulated genes at the protein but not RNA level in human, mouse, and cat cells. Mechanistically, macronutrients trigger selective protein degradation of some but not all induced interferon-stimulated gene products. Deletion of a single interferon-stimulated gene that is differentially stabilized in response to macronutrient cues is sufficient to rescue attenuated poxvirus replication. These data reveal unappreciated interferon-stimulated gene subprograms conserved for nearly ~100 million years of evolution that, in principle, could rapidly adapt immune responses by sensing changing macronutrient levels consumed during viral replication and cell proliferation.

## Education Minisymposium: Narrowing the Gap: Inclusive Teaching Strategies to Engage and Retain Students

M49

**Modernizing graduate and postdoctoral education to support development of a diverse biomedical workforce.**

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National policymakers have called for the transformation of graduate education to better prepare a diverse body of early career scientists for transitions into the biomedical workforce. Simultaneously, both NIH and NSF raised expectations that training programs incorporate evidence-based, inclusive practices for doing so. However, training programs are challenged with developing and evaluating programs de novo, which is resource-intensive and increases the potential for ineffective or surface-level approaches. Career development processes are individualistic and complex, and inequities further exacerbate students' and postdocs' experiences. We have developed and are testing a central framework that provides training programs access to inclusive, effective practices and builds capacity for dissemination, implementation, and rigorous evaluation. This framework-the NIH-funded pd|hub Collections-was envisioned collectively by leaders in the biomedical research training community via the initiative Professional Development Hub (pd|hub). The pd|hub Collections framework incentivizes and supports educators in disseminating their work; partners with DEI scholars to maximize inclusive, identity-conscious practices; and provides PhD training programs seeking to adopt these practices with detailed lesson plans, training, and consultations for implementation and evaluation. Here, we present our first pd|hub Collection, featuring five distinct program models for supporting graduate students and postdocs in exploring career options. Sixteen institutions across the U.S. are currently adopting these models as part of a cross-site national study to develop deeper understanding of effective practices. We will describe these models—three courses and two workshops, each selected via peer review-and themes that have emerged from their content analysis: self-assessment, identity-sharing, career investigation, career fit, and goal setting. We will then highlight strategies defined through this project to enhance inclusive and identity-conscious practices. Finally, we will introduce the evaluation strategy being used to test outcomes and share preliminary data emerging this Fall from the cross-site study. This talk is designed to connect scientists to data and resources that will support evolution of evidence-based, inclusive practices in graduate and postdoctoral education.

M50

**An Inclusive Summer Research Experience Promotes Minority Student Engagement in STEM.**

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Lane College is a Historically Black College with a mission to educate underserved minority students. As part of a primarily undergraduate teaching institution, the Division of Natural and Physical Sciences provides students with a variety of hands-on experiences, including an eight-week summer research experience. Prior to the implementation of the Lane College summer research experience, only a small number of students participated in summer research or internships at other institutions. The Lane

College summer undergraduate research experience aims to be more inclusive by eliminating GPA requirements, encouraging first- and second-year students to apply, and allowing students to select any of the available research projects in the areas of biology, chemistry, computer science, mathematics, or physics, regardless of major. Each year, twelve to fifteen students participate in mentored research in the areas of biology, chemistry, computer science, mathematics, and physics. The students participate in a professional development course twice per week where they learn about career opportunities in science and mathematics, preparing personal statements, scientific writing, and practice on how to effectively present their research findings. The students conduct their research in small groups with a faculty mentor. At the end of the summer, students present their overall results at the Lane Summer Science Symposium. Evaluation of student attitudes towards the research experience during the first iteration in summer 2021 indicates students internalized STEM community values, and developed a sense of self-efficacy for research, a strong sense of project ownership, and a sense of belonging to the science research community. Students participating in the evaluation believe that the experience made science more interesting and that they have better clarity of career opportunities in STEM. Similar levels of engagement were observed in the summers of 2022 and 2023. Students participating in the program are encouraged to submit abstracts to both regional and national conferences. This has resulted in 14 students presenting annually at discipline-specific conferences and one publication co-authored by two summer research students.
This work is supported by grants NSF EES 2011938 and EDU 1833960.

M51

**Undergraduates' science identity and science literacy is enhanced by a new curriculum on molecular biology preprint peer review.**

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Science education focuses on how experiments are carried out and the resulting literature, but rarely engages students in the peer review process that translates one into the other. Peer review is integral to science and so we envision a paradigm shift that makes teaching peer review integral to science education (McDowell et al., MBoC, 2021). Few training opportunities exist in peer review and none are designed for undergraduates (McDowell et al., eLife, 2019). Teaching students how to peer review manuscripts would unmask part of STEM's hidden curriculum, the untaught knowledge required for success in STEM which disproportionately hinders minoritized students. with NSF support, we created and are evaluating a curriculum in which biology undergraduates learn about peer review then write and publish their own reviews of preprints. Preprints are freely available online and their use in molecular biology has exploded, making this curriculum broadly applicable. By authentically reviewing works-in-progress created by practicing scientists, students meaningfully contribute to the scientific literature and community. As a result, we hypothesize that our preprint peer review curriculum enhances students' scientific identity and scientific literacy (McDowell et al., Learned Publishing, 2022). To test this hypothesis, our mixed-methods approach uses validated pre-post surveys and thematic analysis to measure changes in literacy and identity formation as a result of the curriculum. Last year at CellBio2022, we presented preliminary results that the curriculum increases students' scientific literacy (now published as Otto et al., JMBE, 2023). Here, we report unpublished data showing that students' sense of identity as a scientist also increases significantly as a result of the curriculum ($p < 0.0001$ by RM ANOVA), with a particular impact on enhancing scientific identity prominence and self-efficacy. New comparisons between diverse educational settings (a liberal arts college, a land grant university, and an

R1 veterinary school) will also be shared. Gains in science identity formation are most significant in students with minoritized racial and ethnic identities (e.g. BIPOC, Hispanic students). Therefore, using this curriculum to enhance students' scientific identity and literacy has the potential to broaden participation in STEM and prepare students for success in the science classroom and workforce.

M52

**Investigations into undergraduate biology syllabi: instructor omissions and recommendations.**

**S. Ewell**[1], A. Harvey[2], M. Maloney[2], A. Clark[3], L. Stevison[2], C. Ballen[2]; [1]University of Mississippi, Oxford, MS, [2]Auburn University, Auburn, AL, [3]University of Alabama at Birmingham, BIrmingham, AL.

Undergraduate biology students report limited knowledge of effective learning and metacognitive strategies (Ewell et al., 2023; Walck-Shannon et al., 2021; Hora & Oleson, 2017; Sebesta & Speth, 2017, Stanton et al., 2015). Given the importance of these skills in engaging and retaining students, multiple calls suggest instructors incorporate training in their courses and even recommend stand-alone "learning to learn" courses (Hensley et al., 2021; Lang, 2016; Pressley et al., 1989; Zimmerman, 2002; Dignath & Veenman, 2020). However, these efforts require significant time investment from faculty and students (Bernacki & Utz, 2020). There is a clear need for alternative methods of learning strategy and metacognition training that is accessible to all students and does not require a significant time investment. One such alternative is the inclusion of information in course syllabi. Course syllabi are a form of written communication that contain information related to the course (e.g., course and institutional policies) and are typically viewed as contracts between instructors and students (Donnelly and Winkleman, 2021; Calhoon and Becker, 2008; Parkes and Harris, 2002). Syllabi can also communicate to students how to be effective learners (Wheeler et al., 2019; Palmer et al., 2016; Donnelly & Winkelman, 2021; Parkes and Harris, 2002). In this exploratory study, we collected a national sample of Introductory Biology course syllabi from Fall 2019 to Fall 2021 (n=117 syllabi from 94 institutions) and used deductive coding to determine the presence and quality of explicit communication related to learning behaviors and metacognition. We further assessed how well syllabi communicated this information using a validated rubric (Palmer et al., 2014). We found that ~60% of syllabi provided learning strategy or metacognition recommendations to students focused on (1) study behaviors, (2) academic help-seeking, and (3) metacognition. First, most syllabi recommended that students use a mixture of ineffective and effective study behaviors. Second, syllabi frequently provided a list of academic help-seeking resources with no encouragement or explanation on how to use them. Finally, we found that syllabi were not constructed in a way that required that students interact with the document beyond the first day of class. These findings highlight that while biology syllabi incorporate learning strategy and/or metacognition recommendations, instructors should consider promoting student interaction and incorporate complete, accurate information regarding study behaviors and academic help-seeking.

M53

**Differences in the perceptions of trust by undergraduate students from marginalized backgrounds and their faculty research mentors in STEM.**

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California Louis Stokes Alliance for Minority Participation (CAMP) is a program that aims to support and increase the number of STEM graduate students and professionals from marginalized backgrounds. An

active program at all nine undergraduate University of California (UC) campuses, CAMP support of students is multifaceted, including research opportunities, financial support, academic advising, career counseling, professional development workshops, graduate school admission and preparation, and social activities. A key component of the CAMP program is getting undergraduate students involved in faculty-mentored research. Trust in faculty research mentors is associated positively with increased motivation and career expectations in students (Ream et al., 2014). Three major factors of trust include ability, benevolence, and integrity (Mayer et al., 1995). Across all nine undergraduate UC campuses, we investigated how CAMP students and faculty research mentors perceived factors of trust regarding the faculty research mentor. CAMP students and mentors were surveyed and a subset of participants were interviewed. While the ability of mentors was rated very highly by both CAMP students and mentors, students rated the mentors' ability significantly higher compared to the mentors. In comparison, students rated the mentors' benevolence/integrity significantly lower compared to the mentors. Interviews revealed that there may be greater variation in how students interpreted their mentor's behavior as acts of benevolence. This study suggests that there may be a difference in how students from marginalized backgrounds and faculty perceive benevolence in mentor-mentee relationships. It may be beneficial for faculty research mentors to reconsider how they communicate benevolence to better support students from marginalized backgrounds.

M54

**Developing Scholars: a Bilateral Approach to Building a Community of Researchers.**

**J. Moore**; University of Colorado Anschutz Medical Campus, Aurora, CO.

Similar to chemical reactions, training paths are confronted with activation barriers that separate young trainees from careers in biomedicine. These barriers are particularly steep for trainees from backgrounds that are underrepresented in science, and who may have limited opportunities for research training. Effective mentoring relationships represent a catalyst for lowering barriers and promoting progress along the training path. At many biomedical research institutions, establishing effective mentoring relationships for undergraduate students is a significant challenge. These institutions often lack dedicated populations of and support structures for undergraduates; however, they are hubs for training graduate students, many of whom require mentoring skills for success in their future careers. We sought to address both of these challenges by establishing a program that provides research training opportunities for undergraduates, and structured mentor training for Ph.D. students. The Developing Scholars Program brings a cohort of undergraduate students ("scholars") to the University of Colorado Anschutz Medical Campus each summer for a 9-week, paid internship, during which each scholar works under the mentorship of a Ph.D. student. Scholars gain research training in fundamental techniques in cell and molecular biology and work on projects related to developmental disease. In addition, scholars gain professional development training through weekly seminars that inform and excite them about biomedical careers and promote access to subsequent graduate training. In parallel, Ph.D. student mentors receive training in project design, interviewing and evaluating applicants, establishing communication, assessing understanding, and other mentoring skills, and implement these skills in through mentoring a scholar in their lab. We discuss successes and challenges in implementing this program, and how we have adapted the program across years.

M43

**The molecular mechanism of the core planar cell polarity complex elucidated with single-molecule imaging techniques in live Drosophila wing cells.**

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The planar cell polarity (PCP) signaling pathway polarizes epithelial cells along an axis parallel to the epithelial sheet to generate front-back asymmetry at the tissue level. Failures in PCP signaling underlie developmental defects and diseases such as neural tube and heart defects, deafness, and contribute to cancer. Six core PCP proteins assemble into large, asymmetric clusters at cell-cell junctions. In the canonical picture of this pathway, Van Gogh (Vang) and Prickle (Pk) recruited to the proximal side of the cell-cell junction while Frizzled (Fz), Diego (Dgo), and Dishevelled (Dsh) are recruited to the distal side. Flamingo (Fmi) forms intercellular bridges connecting polarity between adjacent cells. Neither the potential requirement for clusters nor their detailed organization are understood. To explore these questions, we used total internal reflection fluorescence (TIRF) microscopy to image live GFP-tagged PCP proteins in the Drosophila pupal wing disc. Quantification of these data allowed us to quantify the number of PCP proteins in individual complexes. Using this approach, we find that the molecular size distribution of PCP complexes follows an exponential function, suggestive of a single underlying growth mechanism. As expected, for Fmi, these data reveal equivalent size distributions and numbers of clusters on the proximal and distal sides of individual cells. In contrast, Fz and Pk are highly polarized, being almost absent on the proximal and distal sides, respectively. On the distal size, we find Vang is present in numbers equal to Fz, Fmi, Dgo, and Dsh, a finding not anticipated based on previous confocal imaging. On the proximal side, Vang exists in 3x higher numbers relative to other components. Finally, we show that mutations that block Dsh oligomerization decrease average cluster sizes and result in PCP phenotypic defects, consistent with a requirement for large clusters in polarization. These findings provide quantitative insight into how the PCP mechanism generates stable polarization during early embryonic development.

M44

**Pre-patterning of an actomyosin network by a cell adhesion gradient to control tissue flow.**

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The movements that give rise to the structure of the body are powered by motility and shape change at the cellular level, but these events must be coordinated at larger spatial scales to enable morphogenesis. Adjacent cell populations often exhibit distinct and complementary behaviors, such as a group of cells stretching or moving to allow a neighboring tissue to constrict or fold. How such coordination arises, and whether it is in response to embryonic patterning or mechanical cues, is still unclear.

Here, we report tissue-wide patterns of cell adhesion and actomyosin within the ectoderm of the *Drosophila* embryo that support the folding of the mesoderm during gastrulation. Using quantitative imaging, we find a graded pattern of adherens junction (AJ) components in the ectoderm prior to the onset of gastrulation: levels of E-Cadherin, B-Catenin, and p120-Catenin are enriched at sub-apical cell junctions in the dorsal and ventrolateral ectoderm and reduced in the lateral ectoderm. This AJ pattern

corresponds closely to the pattern of EGFR-Ras-Raf-MAPK signaling activity downstream of the dorsal-ventral patterning axis in the early blastoderm embryo. Genetic and pharmacological perturbations of EGFR activity inhibit tissue flow resulting in gastrulation defects. We find that the underlying mechanism is an AJ-dependent recruitment of actomyosin to lateral cell junctions and show that both EGF signaling perturbations and AJ-independent inhibition of lateral myosin activity impede tissue flow in a similar manner. Computational simulations of gastrulation support a model where myosin-dependent tissue stiffness in the ectoderm modulates tissue flow. Together, these results suggest a role for AJs in scaffolding cytoskeletal activity at multicellular scales and provide a mechanistic link between embryo-scale axial patterning and cell-scale mechanics.

M45

**Spatial and temporal regulation of cell-cell fusion in human placenta.**

**V. Farmer**, M. Keenen, A. Gladfelter; Duke University, Durham, NC.

The placenta is essential for human life. Central to placenta function is the syncytiotrophoblast (STB); a multinucleate, terminally differentiated, gigantic cell that separates mother and fetal vasculature. The STB forms a single, continuous, polarized epithelial-like cell that functions as multiple organs, acting as an endothelium, immune system, and endocrine system. The development, maintenance, and multifaceted functions of the STB are required for successful pregnancy. While simultaneously carrying out its myriad of essential functions, the STB must increase in size as the fetus develops to ultimately achieve 12-14 $m^2$ of surface area at term. The STB is formed and expands through the fusion of mononucleate cytotrophoblasts (CTBs), a proliferating stem cell population that resides beneath the STB in the placenta. CTB fusion is essential for STB formation and function, however **it is unknown how CTB fusion is regulated through space and time**.

Here we use a trophoblast organoid system to **test the hypothesis that CTB fusion to the STB is spatially and temporally directed by the STB**. Primary trophoblast stem cells, which have features of CTBs, are derived from full-term placentas obtained via cesarian-section. These CTB-like-cells are mononucleate and spontaneously fuse to form syncytia, providing a model for the STB and when embedded in Matrigel droplets form 3D organoid cultures that contain mononucleate and multinucleate cells. These multinucleate cells express and secrete human chorionic gonadotrophin (hCG), a pro-fusion signal and readout for healthy STB formation. We genetically edit this organoid system using CRISPR-Cas9 to fluorescently-tag proteins, enabling live-cell imaging of cell-cell fusion over time. The membrane protein CAAX and nuclear protein H2B are used to determine locations of cell-cell fusion. We are testing the ability for pro-fusion factors, such as hCG and Syncytin-1, to drive fusion at specific locations and times to support STB growth. Future work is focused on understanding how fusion mechanisms differ in the context of STB repair compared to normal growth. Our studies will reveal how CTB fusion is regulated through space and time to drive STB growth and repair.

M46

**Surround yourself with the finest: Organs, basement membranes, and morphogenesis.**

**H. Ku**; University of California, Berkeley, Berkeley, CA.

Morphogenesis is an intricate process whose precision is vital to optimize the organism's overall fitness. Growing evidence demonstrates that the basement membrane (BM), an extracellular matrix that enwraps all epithelia, can actively instruct organ shape. BMs are carefully created in time and space during development, but the mechanisms by which they do so remain largely unknown. We will discuss

recent advances into how the Drosophila egg chamber creates a finely patterned BM, and actively tunes it by dynamically integrating global mechanical information to ensure proper morphogenesis. Patterning signals from each pole of the organ activate ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) enzymes, whose activity regulates both BM production and intracellular secretion to create a matrix whose pattern is symmetrically graded along the A-P axis. In parallel, a single pair of specialized cells at the anterior pole use integrins to sense both local and remote BM mechanical properties. These cells respond by producing a matrix metalloproteinase (MMP) that does not degrade BM components; instead it modulates BM mechanics that determine organ elongation. Our work reveals two tightly regulated mechanisms, one establishing patterned BM levels and the other dynamically tuning its mechanical properties, that ensure the fidelity of shape.

M47

**A morphogenetic wave in the chick embryo lateral mesoderm generates mesenchymal-epithelial transition through a 3D-rosette intermediate.**

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Formation of epithelia through mesenchymal-epithelial transition (MET) is essential for embryonic development and for many physiological and pathological processes. This study investigates MET *in vivo* in the chick embryo lateral mesoderm, where a multilayered mesenchyme transforms into two parallel epithelial sheets that constitute the coelomic lining of the embryonic body cavity. Prior to MET initiation, mesenchymal cells exhibit non-polarized distribution of multiple polarity markers, albeit not aPKC. We identified an epithelializing wave that sweeps across the lateral mesoderm, the wavefront of which is characterized by the accumulation of basal fibronectin and a network of 3D rosettes composed of polarized, wedge-shaped cells surrounding a central focus of apical markers, now including aPKC. Initiation of the MET process is dependent on extracellular matrix-integrin signaling acting through focal adhesion kinase and talin, whereas progression through the rosette phase requires aPKC function. We present a stepwise model for MET, comprising polarization, 3D-rosette, and epithelialization stages.

M48

**Coordination of cell cycle and morphogenesis during organ formation.**

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Organ formation requires precise regulation of cell cycle and morphogenetic events. Using the Drosophila embryonic salivary gland (SG) as a model, we uncover the role of the SP1/KLF transcription factor Huckebein (Hkb) in coordinating cell cycle regulation and morphogenesis. The *hkb* mutant SG exhibits defects in invagination positioning and organ size due to abnormal death of SG cells. Normal SG development involves distal-to-proximal progression of endoreplication (endocycle), whereas *hkb* mutant SG cells undergo abnormal cell division, leading to cell death. Hkb represses the expression of key cell cycle and pro-apoptotic genes in the SG. Knockdown of cyclin E or cyclin-dependent kinase 1, or overexpression of fizzy-related rescues most of the morphogenetic defects observed in the *hkb* mutant SG. These results indicate that Hkb plays a critical role in controlling endoreplication by regulating the transcription of key cell cycle effectors to ensure proper organ formation.

## Minisymp: Evolutionary mechanisms in non-model organisms

M55

**AID governs plasma cell fate decision by co-operation with TET2 to demethylate *irf4* locus in germinal center B cells.**

**R. D'aulerio**, M. He, M. M. S. Oliveira, L. S. Westerberg; Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, SWEDEN.

Activation-induced cytidine deaminase (AID) is essential for antibody affinity maturation by initiating Immunoglobulin (Ig) class switch recombination (CSR) and somatic hypermutation (SHM) in germinal center B cells. AID deaminates cytosine into uracil in single-strand DNA to generate U:G mismatches, which in turn triggers error-prone DNA repair to mediate the secondary diversification of Ig gene. As a potent genome mutator, dis-regulated AID activity leads to an increased risk of B cell lymphomagenesis. On the other hand, AID deficiency leads to a primary immunodeficiency disease named type II hyper-IgM syndrome. In addition to the abolishment of SHM and CSR, both AID deficient patients and mice possess lymphadenopathy caused by giant and persistent germinal centers. Here, we investigated why AID deficiency gives rise to unresolved germinal centers. Immunization of $AID^{R112H}$ mice, devoid of AID activity, led to dramatically increased GC response when compared to WT mice with the accumulation of GC B cells predominantly in the light zone. $AID^{R112H}$ GC B cells showed reduced capacity to up-regulate IRF4 to initiate plasma cell differentiation, leading to the accumulation of a transitional GC B cell population with reduced GL7 expression. Intriguingly, the genetic introduction of high-affinity BCR was unable to restore plasma cell differentiation in $AID^{R112H}$ B cells. We found that R112H mutation in AID disrupted its interaction with Ten-eleven Translocation 2 (TET2), a dioxygenase that mediates DNA demethylation. This led to failure of DNA demethylation and reduced active histone marks H3K4me3 at the *irf4* enhancer/promoter region, therefore impeding the *irf4* expression in the $AID^{R112H}$ GC B cells. This is the first evidence demonstrating that AID is directly involved in governing the plasma cell fate decision by co-operation with epigenetic machinery.

M56

**Single cell RNA sequencing reveals the intracellular developmental program of a highly divergent eukaryotic parasite.**

**K. McCarty**[1], P. Jaroenlak[2], D. Ekiert[1], G. Bhabha[1]; [1]New York University Grossman School of Medicine, New York, NY, [2]Chulalongkorn University, Bangkok, THAILAND.

Microsporidia are an early-diverging group of fungal pathogens comprising over 1500 species that infect a wide variety of hosts ranging from insects to humans. The infectious form of all microsporidian species exists in the environment as highly resistant spores with a thick, two-layer spore wall which require a host cell to replicate. To gain entry into the host cell, microsporidia utilize a unique and specialized organelle called the polar tube which is conserved among all species. Infection is initiated when the polar tube is activated and transfers the infectious material, called the sporoplasm, into the host cell where replication and development occur. As an obligate, intracellular parasite, microsporidia have evolved highly reduced genomes resulting in the loss of many regulatory and metabolic pathways, including the biosynthesis of amino acids, nucleotides, and lipids, driving these parasites to rely solely on their hosts for metabolites. Our understanding of how these parasites acquire nutrients and avoid host defense mechanisms to sustain successful development remains limited. Using single cell RNA

sequencing (scRNA-seq), we have investigated the transcriptional changes in both the host cell and the parasite during infection. We performed scRNAseq on macrophages infected with the human-infecting microsporidian parasite *Encephalitozoon intestinalis* to explore how the host responds to infection as well as the developmental programs of the parasite. Our scRNAseq analysis of the parasite transcriptome reveals a large transcriptional change as the parasite matures. of importance, we observed a vast increase in the transcription of secreted proteins which may be important for spore wall formation, polar tube development, and exit from the host cell. On the host side, our results reveal that most of the macrophages fail to detect invading parasites and are transcriptionally indistinguishable from uninfected cells. This suggests that *E. intestinalis* invades macrophages without being detected allowing for successful parasite development. Overall, our data provide insight into the transcriptional dynamics of parasite development and the host cell response.

M57

**Topological damping in an ultrafast giant cell.**

**R. Chang**, M. Prakash; Bioengineering, Stanford University, Stanford, CA.

Cellular systems are known to exhibit some of the fastest movements in biology, but little is known as to how single cells can dissipate this energy rapidly and adapt to such large accelerations without disrupting internal architecture. To address this, we study *Spirostomum ambiguum* - a giant cell (1-4 mm in length) well-known for its ultrafast contractions (50% of body length) within 5 msec with a peak acceleration of 15*g*. Utilizing transmitted electron microscopy (TEM), confocal imaging, and serial block-face scanning electron microscopy (SBF-SEM), we discover a novel association of rough endoplasmic reticulum (RER) and vacuoles throughout the cell, forming a contiguous fenestrated membrane architecture that topologically entangles these two organelles. A nearly uniform inter-organelle spacing of 60 nm is observed between RER and vacuoles, closely packing the entire cell. Inspired by this entangled organelle structure, we study the mechanical properties of entangled deformable particles using a vertex-based model, with all simulation parameters matching 10 dimensionless numbers to ensure dynamic similarity. We demonstrate how entangled deformable particles respond to external loads by an increased viscosity against squeezing and help preserve spatial relationships. As this enhanced damping arises from the entanglement of two networks incurring a strain-induced jamming transition at subcritical volume fractions, which is demonstrated through the spatial correlation of velocity direction, we term this phenomenon "topological damping". Our findings suggest a new mechanical role of RER-vacuolar meshwork as a metamaterial capable of damping an ultra-fast contraction event.

M58

**Ruptoblasts act as surveillance for hormonal dysregulation in planarian.**

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Hormones are crucial regulatory molecules of various physiological processes. In mammals, many key hormone precursors are recognized by T-cells as auto-immune antigens, providing a surveillance mechanism to prevent overproduction of hormones and eliminate hypersecreting clones. However, it remains unknown whether such immunological regulation of hormones is conserved in other animals, especially those lacking adaptive immunity. Here, we report that surgical stitching of tissues from two

distinct genotypes of the planarian flatworm, *Schmidtea mediterranea*, induces aberrant activation of activin signaling, which causes tissue degeneration mediated by p38. We identify a novel, specialized cell type responsible for this inflammatory response to activin, which we term 'ruptoblast'. Upon activin stimulation, ruptoblasts rapidly undergo a cell death process reminiscent of NETosis, where they explosively release their granular cytoplasmic contents, killing adjacent cells. These ruptoblasts are found in close vicinity to activin-secreting intestinal cells and stem cells, which can generate more intestinal cells. This special form of cell death can therefore maintain stringent control over activin levels and remove secreting cells if the local activin concentration becomes too high. Given that activin is a key hormone regulating regeneration, homeostasis, and reproduction in planarians, our results demonstrate that even animals without adaptive immunity can evolve a strategy parallel to T-cell surveillance to monitor hormone levels.

M59

**Learning and Memory without a Brain.**

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Although learning and memory are often viewed as a unique features of organisms with complex nervous systems, single-celled organisms also demonstrate basic forms of learning and memory. The giant ciliate *Stentor coeruleus* responds to mechanical stimuli by contracting into a compact shape, presumably as a defense mechanism. When a *Stentor* cell is repeatedly stimulated at a constant level of force, it will learn to ignore that stimulus but will still respond to stronger stimuli. Prior studies of habituation in *Stentor* reported a graded response, suggesting that cells transition through a continuous range of response probabilities. By analyzing single cells using an automated apparatus to deliver calibrated stimuli, we find that habituation occurs via a single step-like switch in contraction probability within each cell, with the graded response in a population arising from the random distribution of switching times in individual cells. This step-like response allows *Stentor* behavior to be represented by a simple two-state model whose parameters can be estimated from experimental measurements. We find that transition rates depend on both the stimulus force and on the time between stimuli. The ability to measure the behavior of the same cell to the same stimulus allowed us to quantify the functional heterogeneity among single cells. Together, our results suggest that the behavior of *Stentor* is governed by a two-state stochastic machine whose transition rates are sensitive to the time series properties of the input stimuli.

We have also characterized the timescale of memory retention in *Stentor* following habituation training. After short-term training (one hour), *Stentor* forget their habituation within 15 minutes. However, after long-term training (overnight), *Stentor* can retain their habituated state for hours. Surprisingly, although most metazoans require protein synthesis for long-term memory formation, we find that inhibiting protein synthesis enhances memory retention in *Stentor*. This suggests that forgetting in *Stentor* is an active process that may require new proteins.

Cellular habituation mechanisms hold clinical relevance for conditions such as ADHD and Tourette's Syndrome, in which habituation is impaired. Studying *Stentor* habituation might also shed light on the origins of intelligence by unveiling non-synaptic learning paradigms independent of complex cellular circuitry.

M60

**Single-cell RNAseq in a sponge sheds light on the origin of animal cell types.**

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Sponges are the sister group to all other animals, and diverged prior to the evolution of a nervous system, musculature, or gut. Yet, sponge genomes have been shown to contain genes encoding key synaptic and muscle proteins. To determine how sponges use these genes, and to shed light on the origin of animal cell types, we generated a comprehensive molecular and morphological atlas of cells in *Spongilla lacustris* via whole-body single-cell RNAseq. We identify many specialized cell types bearing functional and regulatory signatures similar to those of other animals. This includes muscle-like contractile cells, which we demonstrate experimentally are responsive to nitric oxide signaling, phagocytes involved in innate immunity, and digestive cells that express a nearly complete set of postsynaptic genes. Remarkably, we also find secretory immune cells expressing presynaptic genes and which send long projections that directly contact and enwrap 'postsynaptic' digestive cells. This reveals new evidence linking the origin of neuronal and immune cells, suggests a primordial neuro-immune system cleared intruders and controlled ciliary beating for feeding.

## Minisymp: Genome Architecture in Space and Time

M61

**Exportin-mediated docking of transcription factors to the nuclear pore complex mediates localization of chromatin to the nuclear periphery.**

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In yeast, flies, worms and mammals, the nuclear pore complex physically interacts with hundreds of chromosomal loci. These interactions promote either epigenetic silencing or transcriptional activation. In budding yeast, nuclear pore basket proteins such as Nup2 interact strongly upstream of highly active genes and mutations that disrupt the interaction between these genes and the NPC lead to strong defects in transcription. Previous work has shown that localization to the nuclear periphery requires both nuclear pore proteins and transcription factors. Furthermore, tethering a majority of yeast transcription factors to chromatin is sufficient to mediate both repositioning to the nuclear periphery and physical interaction with Nup2. To better define the molecular basis of this mechanism, we have exploited a mutation in the Gcn4 transcription factor that blocks peripheral targeting without altering its DNA binding or activation domains. SILAC mass spectrometry revealed that this mutation reduces the interaction of Gcn4 with Crm1/Xpo1, the highly conserved Exportin-1 nuclear export factor. Crm1 and Nup2 interact with highly overlapping genes. Inhibition of Crm1 using leptomycin B both disrupts peripheral localization of active genes and leads to a global loss of Nup2 association with the genome. Recombinant Crm1 binds directly to Gcn4 and these proteins form a complex with Nup2. Importantly, this interaction does not require Ran-GTP and does not compete with nuclear export sequences, suggesting that it represents a distinct function of Crm1. Finally, the interaction between Gcn4 and Crm1 strongly stimulates DNA binding by Gcn4. These results suggest that allosteric coupling between Crm1 binding and DNA binding leads to docking of transcription factor-bound enhancers at the NPC, rather than nuclear export.

M62

**Profiling the interactions of epigenetic regulators and their control of various T cells states.**

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Characterizing the gene regulatory mechanisms that define distinct T cell states is essential to understanding T cell biology in health and disease. Despite advances in identifying transcriptional and epigenetic differences across T cell states, the regulators of these changes remain poorly defined. Here, we describe a system-level proteomic and genomic study that identifies transcription factor (TF) and chromatin modifying cofactor (COF) complexes that regulate cell state in resting, activated, and exhausted T cells. To rapidly and thoroughly profile TF-COF complexes present in cells, we introduce a high-throughput, protein-binding microarray (PBM)-based approach called CoRec (Cofactor Recruitment). We used CoRec to profile numerous COFs in resting and stimulated Jurkat T cells, as well as in acutely stimulated and exhausted CD4+ primary cells. We identify unique TF-COF recruitment networks for these T cell states and examine the patterns of these interactions. We demonstrate that within the same cell state, COFs are recruited to distinct classes of TFs, and that this can happen when comparing close COF paralogs, such as the histone acetyltransferases (HATs) P300 and CBP. We further highlight how some COFs are recruited specifically to TFs known to drive T cell state changes. For example, we have identified several COFs, including histone deacetylases (HDACs), that were only recruited to the T-box TF family (EOMES, T-bet, etc.) during T cell exhaustion. This TF family has been implicated in the exhausted state and its exhaustion-specific recruitment of COFs suggests a possible mechanism by which these TFs act to establish the exhausted state. During establishment of exhaustion, this TF family appears to recruit chromatin modifying cofactors that can then place or remove epigenetic marks that alter expression of exhaustion-related genes and help create a functional change in the T cell state. Additionally, by integrating our TF-COF interaction data with mass spectrometry and ChIP-seq data we examine how these TF-COF networks relate to protein abundance levels, COF recruitment across the genome, and genome-wide chromatin marks. The CoRec method and its integration with other methods provides an exciting new approach for profiling cell-specific TF-COF complexes and allows us to better understand and identify the epigenetic regulators of T cell states.

M63

**SUMO as a glue promotes ALT telomere maintenance via PML-dependent and independent phase separation.**

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SUMOylation often holds proteins together like glue by promoting SUMO-SIM (SUMO interaction motif) interactions between proteins. In cancer cells that employ an alternative lengthening of telomeres (ALT) pathway for telomere maintenance, SUMOylation is crucial for the formation of APBs, which are PML nuclear bodies mislocalized to ALT telomeres. APBs are diagnostic biomarkers that contain DNA repair factors and telomere clusters to promote homology-directed telomere DNA synthesis in ALT cells. Previously, we reported that mimicking SUMOylation on ALT telomeres drives phase separation to form APBs and cluster telomeres. Here we show that SUMOylation-mediated phase separation contributes to ALT beyond APB formation. First, we found SUMOylation, but not PML, was required for ALT telomere

clustering and telomere DNA synthesis. In addition, artificial enrichment of SUMO on telomeres in PML knockout cells induced phase separation, telomere clustering, and telomere DNA synthesis, suggesting SUMOylation can drive PML-independent phase separation for ALT telomere maintenance. Furthermore, SUMO/SIM containing DNA repair factors, including Rad51AP1, BLM, and Rad52 drove SUMO-dependent phase separation without PML. Consequentially, the fusion of these SUMO-enriched telomeres with existing PML bodies led to APB formation and was more frequently seen than de novo nucleation of PML bodies on SUMOylated telomeres via the recruitment of PML protein. The formation of APBs in turn enhanced SUMO-mediated phase separation by helping create SUMO hotspots on telomeres, enable access to PML body components, and increase telomere clustering efficiency. Supporting the essential role of SUMO in the ALT pathway, inhibiting SUMOylation with a small molecule led to more telomere shortening and less colony formation in ALT cells but not non-ALT cells. Together these data suggest that SUMOylation promotes protein phase separation in a manner that is independent of, but benefits from, the PML body for telomere maintenance in ALT cells. Using ALT telomeres as an example, our work highlights the regulatory role of SUMO in protein phase separation, which may be applicable to other cellular processes where SUMO has been observed to act as glue.

M64

**Centromere size variations: new genetic determinants of mitotic fidelity.**

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Centromeres in humans comprise repeats of alpha-satellite DNA, epigenetically marked by the histone H3 variant CENP-A. Owing to their repetitive nature human centromeres on the same chromosome have the potential to display large variations in sizes across the human population. Every human exhibits a unique complement of centromere compositions by virtue of inheriting one set of chromosomes from Mom and one from Dad. Despite the important function of centromeres in ensuring mitotic fidelity, little is known about how the underlying centromeric DNA is organized to accommodate these variations while maintaining the highly conserved process of mitosis. We used structured illumination microscopy coupled with single particle averaging to probe the structure of centromeres on all human chromosomes. This showed a highly conserved core of the centromere comprising CENP-A containing chromatin. Excess centromeric DNA that does not participate in kinetochore-building is distinct from this core. We also found that the centromeric chromatin organizer cohesin is spatially separated from the core centromere and enriched at the peri-centromeres. We further developed and employed quantitative fluorescence *in-situ* hybridization (FISH)-based single-cell assays to distinguish homologs of chromosomes displaying larger and smaller centromeres, based on their fluorescence intensities. Our simple hypothesis is that among homologous pairs, the smaller centromere is weaker in function compared to the larger centromere. As one readout of function, we assessed sister chromatid cohesion at centromeres, necessary for chromosome biorientation during metaphase, where failures often lead to chromosome mis-segregation and aneuploidy. We found that within a pair the smaller centromere is more prone to spontaneous loss of cohesion compared to the larger centromere, consistent with our hypothesis. As a second readout of centromere function, we assessed if smaller centromeres predispose a chromosome to mis-segregate and form micronuclei when mitosis is compromised. For several homologous pairs of chromosomes, we found that the chromosome with the smaller centromere is more likely to mis-segregate, again consistent with the hypothesis. Our study suggests that centromeres can act in cis as genetic determinants of chromosome transmission fidelity. This research is significant as it emphasizes the importance of a distinct genetic composition of centromeric loci as determinants of

individual's specific susceptibility to chromosomal abnormalities, ultimately influencing their predisposition to aneuploidy-related disorders.

M65

**Coilin Mediates m6A RNA Methylation through the Regulated Expression of METTL3 and hnRNPA2B1.**

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Coilin is a nuclear protein canonically known for its role as the marker for Cajal bodies, which are subnuclear domains involved in the modification and assembly of ribonucleoproteins. However, a variety of noncanonical functions have been associated with coilin in recent years, including a regulatory role in the biogenesis of microRNAs by which coilin knockdown results in a decrease in the maturation of microRNAs. While studies have shown that coilin regulates the post-translational modification and gene expression of DGCR8, a key protein in the microRNA biogenesis pathway, we were interested in a potential regulatory effect of coilin on $m^6A$ RNA methylation, an RNA modification that - when downregulated - reduces the maturation of microRNAs. Specifically, we hypothesized that suppressing coilin would result in a decrease in $m^6A$ RNA methylation, thereby contributing to the dysregulation of microRNA biogenesis. To test this hypothesis, we applied a series of siRNA knockdowns, methylated RNA immunoprecipitations, RNA dot blots, western blots, and qRT-PCR in HeLa cells. Our results show that coilin knockdown results in a reduction of global m6A RNA methylation, as well as reduction in the $m^6A$ modification of the primary Let7a microRNA. We also found that coilin knockdown results in dysregulated RNA expression and reduced protein expression of the $m^6A$ *writer* METTL3 and $m^6A$ *reader* hnRNPA2B1. The discrepancy in RNA versus protein expression of METTL3 upon coilin knockdown prompted us to assess phosphorylation, a post-translational modification that influences METTL3 stability. We found that coilin knockdown also reduced the phosphorylation of Serine 43 of METTL3, as well as specifically reducing METTL3 levels within the nucleoplasm, but not the cytoplasm. Additionally, upon ectopic expression and immunoprecipitation of a FLAG-tagged METTL3 plasmid, we found that METTL3 and coilin interact. Collectively, we have uncovered a novel role that coilin plays in the $m^6A$ RNA methylation network by influencing the gene expression and post-translational modification of notable $m^6A$ regulatory proteins, hnRNPA2B1 and METTL3.

M66

**PRC1 Composition and Chromatin Interaction Determine Degree of Chromatin Condensation.**

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Precisely maintaining gene expression levels is essential to define cellular identity and to prevent a wide range of diseases, including developmental disorders and cancers. The Polycomb repressive complex 1 (PRC1) family proteins play a key role in maintaining gene repression. Canonical PRC1 forms condensates *in vitro* and in cells and the ability of PRC1 to undergo liquid-liquid phase separation has been proposed to contribute to maintenance of repression. However, how chromatin and the various subunits of PRC1 contribute to condensation is poorly understood. To address this, we developed a TIRF microscopy-based approach using reconstituted protein complexes to observe condensate formation at the single-molecule level in real time. Our findings reveal that nucleosomal arrays lower the critical concentration required for PRC1 condensate formation from the micromolar to nanomolar regime, indicating a synergistic effect between chromatin and PRC1 in condensate formation. Additionally, we

identified that a sufficient length of chromatin and a stoichiometric excess of PRC1 to nucleosomes are required for condensation. Using single-molecule and live cell imaging, we determined that condensate initiation depends on the properties of the CBX subunit of PRC1, while the PHC subunit alters condensate morphology and is essential for the stability of PRC1 condensates. Moreover, we found that the polymerization activity of PHC2 is critical for generating distinct, isolated, and long-lived domains. In summary, our data suggest that PRC1 binding to chromatin establishes an initial scaffold which is crucial for efficient condensate formation. This dual-component scaffolding mechanism ensures that condensates only form at specific loci where PRC1 molecules can bind nucleosomes and create an initial scaffold. Furthermore, our results suggest that the composition of PRC1 can fine-tune the extent of condensate formation, which might offer important flexibility of regulatory function during various stages of development.

## Minisymp: Integration of signaling and metabolism in cell physiology

M67

**High throughput identification of calcium-regulated proteins across diverse proteomes.**

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Calcium-regulated proteins play important roles in nearly every biological process. Given their integral role, the characterization of novel calcium-regulated proteins is an important area of biological research. Current methods to identify calcium-regulated proteins are limited to computational prediction of well-established motifs (e.g. EF-hand), or biochemical testing of calcium binding to individual proteins. However, only 10% of calcium-binding proteins have an easily identifiable motif, and biochemical characterization is low throughput. As a result, the calcium-regulated proteome is likely to be underreported and understudied. For example, yeast only has 56 proteins that are annotated to be calcium-regulated (0.8% of its proteome), whereas this number is ~5% for mouse and human proteomes.We hypothesized that many calcium targets remain unidentified due to lack of methods for their unbiased and high throughout identification. To overcome this challenge, we first adapted a protein thermostability assay for rapid, unbiased, and high throughput identification of calcium-regulated proteins. Application of this method to three different proteomes (yeast, human and mouse) identified 199 previously annotated and 2825 putative novel calcium regulated proteins across these proteomes. Analysis of human data suggests that spliceosome is a calcium-regulated process (6-fold enrichment, -log2 FDR< 10). In yeast, we identified 796 novel calcium-regulated proteins. 14 of these hits were also reported to show differential expression in response to calcium depletion previously. In addition, we show direct calcium binding to Decr1 protein that was not associated with calcium signaling before but appeared as a hit in our screen. Decr1 is a rate limiting enzyme in mitochondrial fatty acid oxidation pathway, suggesting that this pathway is a novel calcium target in the mitochondria.Analysis and verification of our data suggest important roles for calcium signaling in regulation of splicing, significantly expands calcium regulated proteome in yeast, and finds that calcium signaling is a novel regulator of mitochondrial fatty acid oxidation. Moreover, our results establish a new workflow and database for unbiased and high throughput identification of calcium-regulated proteins and provide a unique resource for the community.

M68

**Dynamics of ATP metabolism generates two heritable stable states in yeast.**

**P. Nanda**, A. W. Murray; Molecular and Cellular Biology, Harvard University, Cambridge, MA.

Abruptly shifting exponentially growing yeast cells on glucose to ethanol depletes the cellular ATP pool within a few seconds and reveals two epigenetically distinct subpopulations: arrestors and recoverers. Arrestors (75% of the population) halt their cell division, and recoverers (25%) switch their metabolism to respiring ethanol and restart their cell cycle after a lag phase. The individual epigenetic states are heritable for several generations forming a dynamically stable system where the proportions of two states are maintained throughout growth by two-way switching. Altering the concentration or quality of carbon source prior to the nutrient shift modulates both the fraction of recoverers and their lag times. Modulating the activity of the Ras/PKA pathway or perturbing glucose sensing through the Gpr1/Gpa2 G-Protein Coupled Receptor alters the fraction of recoverers. Perturbing the rate of translation phenocopies Ras/PKA modulation of recoverer fraction. During exponential growth on glucose, arrestors show high glycolytic flux and low ATP:ADP (energy charge) compared to recoverers. Recoverers show low glycolytic flux and are respiring as well as fermenting glucose. A thermodynamically constrained metabolic model of glycolysis, translation and respiration predicts a conflict between ATP producing reactions (glycolysis) and ATP consuming reactions (translation) in the same compartment. The model suggests that a high ATP:ADP ratio, while necessary for driving ATP consuming reactions, is inhibitory to glycolysis creating a metastable state i.e., arrestors. Compartmentalization of ATP producing reactions (respiration in mitochondria) and consuming reactions (translation in cytosol) resolves the conflict by allowing the two compartments to maintain different ATP:ADP ratios in recoverers. Compartmentalization theoretically resolves the conflict, but respiration operates during low glycolic flux penalizing ATP production rates for stability. Ectopically changing ATP:ADP ratios by expressing ATPases alters the fraction of recoverers in predictable ways, establishing the role of ATP:ADP ratio in controlling the switch. Instabilities in the nature of chemical reactions in cells automatically generates two heritable stable states in yeast which maximizes growth during favorable environments through arrestors and maximizes the rate of adaptation during environmental shifts through recoverers. We argue that yeast cells use active sensing and computation based on the current nutritional environment to hedge their bets against uncertain environments by altering the fractions of recovers and arrestors.

M69

**PPTC7 maintains mitochondrial protein content by suppressing receptor-mediated mitophagy.**

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PPTC7 is a resident mitochondrial phosphatase essential for maintaining proper mitochondrial content and function. Newborn mice lacking *Pptc7* exhibit aberrant mitochondrial protein phosphorylation, suffer from a range of metabolic defects, and fail to survive beyond one day after birth. Using an inducible knockout model, we reveal that loss of *Pptc7* in adult mice causes marked reduction in mitochondrial mass and metabolic rewiring with elevated hepatic triglyceride accumulation. *Pptc7* knockout animals exhibit increased expression of the mitophagy receptors BNIP3 and NIX, and *Pptc7*$^{-/-}$ mouse embryonic fibroblasts (MEFs) display a major increase in mitophagy that is reversed upon deletion of these receptors. Our phosphoproteomics analyses reveal a common set of elevated phosphosites between perinatal tissues, adult liver, and MEFs, including multiple sites on BNIP3 and NIX. Follow up studies demonstrate that PPTC7 can directly interact with and dephosphorylate these mitophagy receptors, and that loss of PPTC7 significantly decreases BNIP3 and NIX turnover rates. These

data suggest that *Pptc7* deletion causes mitochondrial dysfunction via dysregulation of several metabolic pathways and that PPTC7 may directly regulate mitophagy receptor stability. Overall, our work reveals a significant role for PPTC7 in the mitophagic response and furthers the growing notion that management of mitochondrial protein phosphorylation is essential for ensuring proper organelle content and function.

M70

**Metabolic Regulation of Cell Fate and Function in Mammalian Skin Implications for Tissue Homeostasis and Aging.**

**M. Forni**, Y. Xu, W. Krause, V. Horsley; Yale University, New haven, CT.

The precise mechanisms by which metabolism governs cell fate and function in mammals remain largely unknown. This knowledge gap is particularly significant for tissue regeneration, where cells face the challenge of maintaining homeostasis by efficiently producing biomass and meeting high energy demands. In this study, we investigated the intricate relationship between metabolism and cell fate during skin wound healing, a process that involves a timely inflammatory response and differentiation of blood-derived circulating monocytes into macrophages within the wound bed. We employed state-of-the-art techniques, including single-cell RNA sequencing and single-cell secretomics, to dissect the wound microenvironment Secretomics. Our comprehensive analysis revealed that macrophage-secreted molecules played a pivotal role in establishing the metabolic milieu of the wound, as evidenced by metabolomics and respirometry analyses. Importantly, these secreted molecules profoundly influenced cell fate and polarization. Notably, impaired secretion in aged mice resulted in chronic non-healing wounds, but intriguingly, restoration of the levels of secreted molecules rescued macrophage differentiation. Cell differentiation is an energy-intensive process, and the metabolic substrates present in the wound niche significantly impact the outcome of wound healing. We also found that dermal adipocytes as essential providers of fatty acids, which play a critical role in meeting the metabolic demands necessary for immune cell differentiation within the skin wound bed. We showed that monocytes actively uptake extracellular vesicles Evs filled with lipids. Furthermore, real-time respirometry and metabolomics analyses elucidated that these fatty acids activate the β-oxidation pathway in monocytes, and inhibition of this pathway abolishes macrophage differentiation. Our results further highlighted that aging compromises the production and efficacy of adipocyte-derived EVs in rewiring monocyte metabolism toward oxidative phosphorylation, which is essential for successful differentiation. These compelling findings underscore the fundamental importance of metabolic control in governing cell fate and function. Moreover, our discoveries provide a ground for exploring promising interventions that hold the potential to impact longevity and health span.

M71

**Mitochondrial Chaperonin CLPB: A New Regulator of Mitochondrial Cristae Structure and Functions.**

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Caseinolytic peptidase B (CLPB) belongs to the Hsp104 and Hsp78 chaperone family and is the only known chaperonin/disaggregase localized in the mitochondrial inter-membrane space. Loss-of-function mutations in CLPB have been associated with prenatal encephalopathy, progressive brain atrophy, movement disorders, cataracts, and neutropenia, highlighting its critical role in mitochondrial physiology. To investigate the role of CLPB in mitochondrial physiology, we utilized CLPB knockout (*Clpb*$^{-}$

$^{/-}$) and overexpressing (CLPB-OE) mouse embryonic fibroblasts, along with a range of cell and molecular biology techniques. These included mitochondrial bioenergetics assays, imaging for mitochondrial structure and functions, calcium flux assays, biotinylation-based proximity labeling, LC-MS proteomics, co-immunoprecipitation, and NanoBiT protein-protein interaction assays. Our results demonstrate that loss of CLPB leads to increased oxygen consumption rate, elevated mitochondrial ATP and NADH levels, activation of mitochondrial unfolded protein response, and upregulation of the mitochondrial biogenesis factor TFAM. *Clpb*$^{-/-}$ cells exhibited comparable calcium exchange, calcium retention capacity, basal ROS, mitochondrial membrane potential, mtDNA content, and cytochrome c release with control cells. Notably, we identified several components of the mitochondrial contact site and cristae organizing system (MICOS), including the core component MIC60, as top interactors of CLPB. Furthermore, CLPB was found to regulate the protein quality control of MIC60 and other MICOS components, with *Clpb*$^{-/-}$ cells displaying increased insoluble protein aggregates. We also discovered that CLPB directly modulates mitochondrial ultrastructure and cristae organization. Furthermore, specific protein domains and regions of CLPB and MIC60 responsible for their interaction were identified. This study provides valuable molecular insights into CLPB-MIC60 interactions and their significance in mitochondrial physiology, enhancing our understanding of CLPB's role in protein quality control. These findings have implications for the development of therapeutics targeting mitochondrial dysfunction-related diseases.

M72

**Signal integration by a bHLH transcription factor network enables neural fate choice.**

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**Signal integration by a bHLH transcription factor network enables neural fate choice**

A central feature of stem cells is their ability to integrate multiple extracellular signals to decide fate. For example, neural stem cells choose to become astrocytes upon combined treatment with two signals, BMP and LIF, but not either signal alone. This decision is accompanied by suppression of alternative fates. How is signal integration achieved and simultaneously coupled to the coordinated regulation of appropriate fate programs? We addressed this question by analyzing a network of basic helix-loop-helix (bHLH) transcription factors, which are known to act as dimers to regulate fate in neural stem cells (NSCs). Multiplexed single-molecule RNA-FISH of 14 bHLH factors in cultured mouse NSCs revealed how each bHLH is regulated upon individual and combined BMP and LIF treatments. Complementary analysis of ectopically expressed bHLHs revealed their roles in regulating fate. In parallel, a quantitative yeast-based assay of protein-level dimerization showed that bHLHs interact promiscuously, but with tiered binding affinities. Together with mathematical modeling, these analyses showed that individually, BMP and LIF can each regulate a subset of bHLHs in the network, but this is insufficient to fully relieve the suppression of astrocyte genes. However, combined treatment with both BMP and LIF leads to strong inactivation of suppressive bHLHs both transcriptionally and through heterodimerization with other bHLHs in the network, thus resulting in synergistic astrocyte differentiation. Notably, since bHLHs that suppress astrocyte fate are also known to be responsible for driving alternative neural fates such as neurogenesis, signal integration by the bHLH network enables tight coupling with fate choice. bHLH factors are co-expressed and utilized in fate control in most stem cell types. This work therefore has broad relevance to understanding signal integration and fate choice during development and in adult tissues. More generally, it reveals principles of computation by transcription factor networks that characterized by a combination of transcriptional and protein-level interactions.

## Minisymp: Organization during Development

M73

***De novo* generation of spatially organized cytoplasm.**

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Functional cytoplasm is spatially organized. How such organization may be generated *de novo* remains an open question. Cell-free *Xenopus laevis* frog egg extracts, essentially a scrambled pool of cytoplasmic components, self-organize into cell-like units ~300 micrometers in size in a one-pot reaction. Thus, egg extracts carry out *de novo* generation spontaneously and serve as a powerful model for identifying the underlying mechanisms. Here we investigate what breaks the spatial homogeneity in extracts at the beginning of self-organization. We found that symmetry-breaking occurs through the formation of an unconventional type of microtubule aster. The microtubule organizing center (MTOC) focuses the plus ends of microtubules, forming a "plus end inside, minus end outside" radial array that has the opposite polarity of a conventional centrosome aster. The MTOC shows properties of biomolecular condensates. In extracts, the MTOCs localize to the edge of a plane previously occupied by the metaphase plate, where they accumulate pre-formed F-actin, a phenomenon typically seen near the cytokinesis furrow in cells. Thus, these polarity-reversed asters promote spatial organization of the cytoplasm and might be involved in cytokinesis signaling.

M74

**Centrosome-induced plasma membrane infoldings initiate growth of a cortical actin domain.**

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Regulated cell shape changes involve local inductions of specialized cortical domains. Folded plasma membrane (PM) topographies have been linked to such inductions. Additionally, microtubule motors can pull PM tubules toward the centrosome, a major organizer of the cell cortex. To test whether centrosome-induced PM infoldings contribute to cell shape change, we examined the syncytial Drosophila embryo, in which centrosomes induce Arp2/3 network growth and membrane exocytosis to form actin caps that grow into dome-like compartments for mitotic nuclei. We found that the nascent cap is a collection of PM infoldings and tubules positioned over the centrosome. Further investigation showed that embryo surface PM topography is patterned by a combination of forces. Our data suggest that PM infoldings of the nascent cap depend on Dynein motor activity and astral centrosomal microtubules, and that PM folding is prevented elsewhere by myosin-based surface tension. An Arp2/3 induction pathway (Sponge, Rac-GTP, SCAR, Arp3, and F-actin) and an exocytosis pathway (Rab8 and Sec5) are recruited to infoldings of the nascent cap with centrosome dependence. However, ectopic PM folds that arise when myosin is depleted were not sufficient for the same degree of recruitment, indicating specific centrosome effects. To test the effects of the induced actin polymerization on the nascent cap, we depleted Arp3 and injected an F-actin inhibitor. These experiments indicated that Arp2/3 network growth between infoldings counterbalances pulling forces of the centrosome to separate and maintain the infoldings. This Arp2/3-dependent maintenance of infoldings correlates with Arp2/3-dependent exocytic vesicle accumulation at the base of the folds, further implicating PM folding in the pathways of actin cap growth. Overall, centrosome-dependent PM infoldings seem to coordinate Arp2/3 network growth and exocytosis to assemble a new cortical domain.

M75

**Inducing cortical polarity by designed proteins reveals the molecular mechanism of central spindle symmetry breaking in unpolarized mammalian cells.**

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Polarized cells rely on a polarized cytoskeleton to drive key processes such as polarized trafficking and asymmetric cell division. Asymmetric cell division promotes the generation of two daughter cells with different fates, as they inherit distinct fate determinants. During this process polarized microtubule networks promote the asymmetric partitioning of fate determinants. Yet, how the cytoskeleton is polarized, in particular, how polarity cues present at the cell cortex regulate cytoskeleton symmetry breaking remains elusive.

Here, we tackle this question using a synthetic biology approach, artificially polarizing normally unpolarized mammalian cells in culture. Capitalizing on recently established designed 2D proteins arrays allowed us to ectopically induce cortical polarity of virtually any protein of interest during mitosis in various cell types. Using this assay, we dissected the logic of the Par complex pathway, a key regulator of cytoskeleton polarity during asymmetric cell division. We show that cortical clustering of any Par complex subunit (Par6/Par3/aPKC) is sufficient to trigger complex assembly, and that inducing cortical Par complex polarity is sufficient to induce two hallmarks of asymmetric cell division in unpolarized mammalian cells: spindle orientation, occurring via Par3, and central spindle asymmetry, depending on aPKC.

We further establish that, molecularly, the formation of an asymmetric central spindle, where one side contains a higher microtubule density as opposed to the other, requires an asymmetric kinase activity of aPKC at the cortex. Downstream of aPKC, Camsap3 (microtubule minus-end stabilizer), and the three kinesin-13 homologs Kif2A, Kif2B, and Kif2C/MCAK (minus-end depolymerases) promote central spindle symmetry breaking. This suggests that these central spindle microtubule regulators, are targets of aPKC's kinase activity originating from cortex. aPKC may shape their activity into a gradient and in turn, promote central spindle symmetry breaking.

M76

**Germ fate mediates protection of germ precursor cell division.**

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Cytokinesis is the physical division of one cell into two, driven by constriction of an actomyosin contractile ring at the cell division plane. The textbook view of cytokinesis is that all animal cells divide using the same molecular machinery. Yet, growing evidence supports both cell type-specific regulation of cytokinesis and cell type-specific consequences for cytokinesis failure. The 4-cell *C. elegans* embryo is a powerful model for studying cell type-specific differences in cytokinesis as the cells are already programmed to form distinct cell linages, and previously, we identified cell type-specific regulation of cytokinesis at the 4-cell stage. We weakened the contractile ring using a temperature sensitive (ts) diaphanous formin/CYK-1 mutant. Under this condition, the two anterior cells (ABa and ABp) always failed in cytokinesis, whereas the two posterior cells (EMS and P2) divided successfully at a high

frequency, even without detectable F-actin in the cell division plane. Here we focus on the cell type-specific protection of cytokinesis in the P2 germ precursor cell, required to produce all gametes in the adult worm. Using a candidate-based RNAi mini-screen to identify genes required for protection of P2 cytokinesis in the *formin(ts)* embryos, we identified members of the CCCH $Zn^{2+}$-finger protein family that are enriched in P2 and required for proper germ cell fate specification. Depletion of either MEX-1, PIE-1, or POS-1 led to loss of cytokinetic protection and P2 cytokinesis failure in *formin(ts)* mutants, but not in control embryos. While depletion of MEX-1 effected multiple cell types, PIE-1 and POS-1 acted exclusively in the P2 cell. Further analysis revealed these germ fate regulators protect cytokinesis by preventing excessive accumulation of septin/UNC-59 and its binding partner, anillin/ANI-1, on the cell cortex in the P2 cell division plane, both negative regulators of actomyosin constriction during cytokinesis in many contexts. We further found that co-depletion of septin and PIE-1 was sufficient to both reduce anillin levels at the P2 division plane and restore cytokinetic protection of P2 in *formin(ts)* mutant embryos. Thus, germ fate specification protects the P2 germ precursor cell from cytokinesis failure upon damage to the actin cytoskeleton at least in part by controlling the levels of septin and anillin at the division plane.

M77

**miR-31-mediated local translation at the mitotic spindle is important for early development.**

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miR-31 is a conserved microRNA that plays critical roles in cell proliferation, migration, and differentiation. Using the sea urchin embryo as a model, we discovered that miR-31 and its validated targets have a cell cycle-dependent dynamic distribution: they are enriched on the mitotic spindles in dividing cells and in the perinuclear region of non-dividing cells. Localization of miR-31 and its targets at the mitotic spindles is evolutionarily conserved, as we also observe this in mammalian cells. To test its function, we inhibited miR-31 and observed that miR-31 inhibition led to developmental delay that correlated with longer microtubules and increased filamentous actin, as well chromosomal segregation defects. To understand the mechanism of these defects, we identified miR-31 to directly target several actin remodeling transcripts, such as *β-actin, Gelsolin*, *Rab35*, and *Fascin*. Translational initiation factors, newly translated proteins, and newly synthesized Fascin are observed at the spindle, leading to our hypothesis that miR-31 regulates local translation of transcripts encoding cytoskeletal remodeling proteins to ensure proper cell division. In support of this hypothesis, miR-31 inhibition results in increased Fascin and Rab35 proteins at the mitotic spindle midzone and perinuclearly in non-dividing blastomeres. Further, forced ectopic localization and translation of *Fascin* transcripts at the cell cortex leads to delayed development and significantly increased lethality rate at the blastula stage, indicating local translation of *Fascin* at the mitotic spindle is essential for development. This study revealed the novel and evolutionarily conserved role of miR-31 in mediating local translation of cytoskeletal modifying transcripts that impact mitosis and early development. Overall this work contributes to the fundamental understanding of early cell division, birth defects, and predisposition to cancer.

M78

**Nuclear envelope buds traffic large trancripts in muscle cells.**

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For decades, the nuclear pore complex (NPC) has been considered the sole route to transport molecules across the NE. However, in recent years, NE budding has emerged as an alternative non-canonical route

for nuclear export of cargo with a size above the limit of the NPC, such as viral nucleocapsids, as well as large ribonucleoprotein (RNPs). Yet, the significance of this unconventional export pathway in mammalian cells remains largely unexplored. Here, we show that NE budding is an active process in differentiating muscle cells, and we hypothesize that it can serve as an alternative export mechanism for uniquely long sarcomeric transcripts (including Titin, the longest transcript found in mammalian cells). Using a combination of electron and fluorescence microscopy, we demonstrated that NE budding events are present in C2C12 mouse myoblasts only upon differentiation to myotubes, concomitant with the expression of long sarcomeric transcripts. Furthermore, we showed that NE buds are single-membrane structures originating from the INM and contain several INM markers. Single-molecule fluorescence in situ hybridization (smFISH) experiments revealed that long transcripts, such as Titin and Nebulin, are preferentially localized within NE buds of differentiated C2C12 cells. To gain insight into the underlying mechanism of mRNA targeting to NE buds, protein proximity labeling using TurboID was performed. Notably, proteomic analysis and validation experiments revealed the enrichment of several nucleoporins within NE buds. Together, these results suggest that, during muscle differentiation, uniquely long sarcomeric transcripts are unable to be efficiently exported across the NE through the canonical NPC route, leading to the activation of NE budding as an alternative exit route. We are currently testing the hypothesis that long transcripts can cause NPC clogging, leading to NE budding activation, and resulting in long mRNAs and associated nucleoporins getting packaged into the buds.

## Minisymp: Subcellular Dynamics and Interorganelle Crosstalk

M79

**Cell-cycle regulated tug-of-war between microtubule motors positions major trafficking organelles.**

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Rapidly dividing cell populations must maintain efficient membrane trafficking while constantly remodeling their interior in preparation for cell division. Efficient protein processing and sorting in the mammalian Golgi apparatus relies on the integrity of this organelle. The Golgi exists in a constant membrane exchange with the Endoplasmic Reticulum (ER) through ER exit sites (ERES). Both ERES and the Golgi are moved by microtubule-dependent molecular motors, and cytoplasmic dynein is thought to assemble these organelles around the centrosome to provide Golgi integrity. However, the Golgi must dissociate from the centrosome to allow for unperturbed centrosome separation in mitosis. We have previously shown that this dissociation occurs as early as the G1/S transition, but cell-cycle signaling and molecular mechanisms that drive this process still need to be understood. Here, we apply live cell imaging, photokinetics, and loss-of-function approaches to show that cell-cycle signaling tunes tug-of-war between cytoplasmic dynein and kinesin-1, resulting in differential positioning of ERES and the Golgi in interphase sub-stages. Specifically, in G1, both membrane structures are brought to the centrosome by the minus-end-directed action of dynein. On the onset of the S-phase, CDK1 activity rises sufficiently to inhibit BICD2/dynein-dependent transport of the ERES, resulting in partial spreading of ERES throughout the cytoplasm. At the same time, dynein association with the Golgi reduces so that kinesin-1 overpowers dynein and drives it away from the centrosome. CDK1 inhibition in S-phase reverses the association of both ERES and Golgi with dynein and leads to a compact ERES/Golgi configuration around the centrosome, similar to G1. Interestingly, while dynein is incapable of transporting ERES in S/G2, it remains associated with the Golgi: at this stage, acute kinesin-1 inhibition causes rapid dynein-dependent transport of the Golgi toward the centrosome. Since ERES are not associated with dynein in S/G2, kinesin inhibition does not affect ERES positions, which leads to a dramatic dissociation of the

Golgi from ERES. We conclude that cell cycle signaling regulates Golgi and ERES positioning differentially, likely reflecting that these two organelles are associated with different sets of cell-cycle-sensitive dynein adaptors.

M80

**Zika virus NS3 drives the assembly of a viroplasm-like structure with MTOC activity.**

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Zika virus (ZIKV) is a mosquito-transmitted flavivirus that caused an epidemic outbreak in 2015 in the Americas and raised serious global health concerns due to its association with congenital defects resulting from infected mothers. The ZIKV genome encodes three structural proteins (envelope, prM, and capsid) forming virus components and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) involved in virus replication, packaging, and host manipulation. ZIKV replicates its genome in the cytosol and assembles virus particles in a virus-regulated compartment called the replication factory or viroplasm in part by remodeling the endoplasmic reticulum (ER). Consequently, the nucleus is often deformed, taking on a concave 'bean-like' shape, imposed by the viroplasm. The prevailing shape of the mature viroplasm is toroidal, while the spherical shape occurs less frequently. We show that NS3 is sufficient for the formation of toroidal-shaped and spherical Viroplasm-Like Structures (VLS) when NS3 is overexpressed in human cells. Like the ZIKV-induced viroplasm, the VLS assembles around the centrosome into a toroid structure, recruits ER, Golgi, dsRNA, and G3BP1, organizes microtubules (MTs) at its surface, and deforms the nucleus. The recruitment of ER, however, is inefficient and slow at the VLS compared to the virus-generated viroplasm. The viroplasm requires MTs for efficient assembly and becomes a microtubule organizing center (MTOC) with MTs organized on its surface. We show that MTs are organized at the VLS which appears to function as a non-centrosomal MTOC through recruitment of γ-tubulin and a select cadre of centrosome PCM proteins. Moreover, we show that a pre-formed NS3-induced VLS facilitates ZIKV replication upon virus infection. NS3 is a multidomain protein containing an N-terminal protease and C-terminal helicase domain. We will present the domain and functionality requirements to discern the molecular mechanisms of NS3 for the formation of VLSs. Overall, these findings advance our knowledge of how a virus can manipulate cellular mechanisms to facilitate its replication, opening new possibilities for intervention to regulate its spread and potentially prevent human infection.

M81

**Dysregulation of the ER blocks recruitment of centrosome associated proteins resulting in mitotic failure.**

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The endoplasmic reticulum (ER) is a large membranous network that maintains contacts and functional interactions with various other organelles within the cell. Recent work has shown that the ER is an active participant in other processes besides its canonical roles of protein synthesis and calcium storage. The mitotic ER displays massive morphological changes that coordinate with the cell cycle but whether these transitions in behavior are important for modulating other mitotic events is less clear. Here, we use the cleavage divisions that take place in the early *Drosophila* embryo as a potent system for understanding the dynamic changes facilitate successful mitoses and elucidate the distinct challenges that exist during

the rapid division phase in animal embryos. Here, we show that as the mitotic spindle begins to form, the ER retracts and forms a coated structure surrounding the spindle, with enriched areas at each spindle pole with further reorganization events occurring as the spindle elongates. We identified Rab1 in a screen for Rab GTPases that display dynamic behaviors at this stage. Depletion of *Rab1* led to an excess build-up of ER membranes surrounding the spindle poles and interestingly, mitotic failures occurred. Mitotic spindle formation appeared to be disrupted in *Rab1* deficient embryos with the presence of a "mini-spindle" phenotype. Importantly, key centrosomal proteins fail to localize at mitosis, leading to weakened gamma-tubulin function and suggesting a lack of centrosome maturation. Finally, we were able to rescue these phenotypes with Dynein disruption, suggesting that Dynein is a regulator of ER morphologies during mitosis. These results indicate that ER levels must be carefully tuned during mitosis to ensure proper stability of division machinery and promote genomic stability.

M82

**Single-molecule tracking of protein transport from the ER to lipid droplets.**

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Lipid homeostasis is maintained by localization of key lipid synthesis and lipolysis enzymes to lipid droplets (LDs). Several of these proteins originate in the ER and subsequently translocate to the phospholipid monolayer surface of LDs. The prevailing model, based on studies of ER-to-LD cargoes in Drosophila cells, is that such ER proteins are membrane-embedded via short hairpins and traffic to LDs via membrane bridges. However, this model has not been tested in mammalian cells due to the critical barrier of ER to LD trafficking occurring fast (seconds), but over long period (hours) of LD formation. Current imaging methods lack spatial and temporal resolution required to determine trafficking pathways. Thus, whether or how ER proteins target to LDs after their formation remains unknown.To investigate the mechanism of ER-LD protein targeting, we employ a synchronous trafficking system using LiveDrop as a model LD protein, combined with single molecule tracking in live cells. Upon release from the ER, LiveDrop diffuses throughout the ER and rapidly accumulates on LDs. Using a machine learning approach, we identified single molecule tracks traveling from the ER to LDs, revealing entry points to individual LDs where protein movement is occasionally bidirectional. In seipin-deficient cells, LiveDrop accessed some but not all LDs, consistent with the hypothesis that LDs detach from the ER in the absence of seipin. Together, our results demonstrate that proteins access LDs via membrane continuities maintained by the seipin complex and provide an innovative imaging system to study how other proteins access the LDs.

M83

**Quantitative mapping of autophagic cargo during nutrient stress reveals membrane receptors for Golgiphagy.**

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In response to nutrient availability, mammalian cells can remodel the proteome through protein production, transport, and degradative mechanisms. During nutrient stress, one such mechanism called macroautophagy degrades cellular macromolecules, thereby recycling biosynthetic building blocks while

simultaneously remodeling the proteome. The current macroautophagy paradigm suggests non-specific capture of bulk cytoplasmic contents within autophagosomes. However, the extent to which individual proteins, protein complexes, and organelles are selected for autophagic degradation, and the underlying targeting mechanisms is limited. To uncover the landscape of autophagy cargo during nutrient stress, we used a dual proteomics approach that combined ATG8 proximity biotinylation proteomics with whole cell proteomics under starvation conditions. Using autophagic cargo profiling, we deconvoluted starvation-based cellular responses to find that Endoplasmic Reticulum and Golgi Apparatus proteins are the predominate cargo for selective autophagy during nutrient stress. We were particularly interested in the Golgi Apparatus, as membrane receptors mediating its selective autophagy (Golgiphagy) are not well understood. From our data, we identified a membrane-embedded protein complex, YIPF3 and YIPF4, as receptors for Golgiphagy. During nutrient stress, YIPF4 is mobilized into ATG8-positive autophagosomes that traffic to lysosomes as measured via Golgiphagy flux reporters in a process that requires the VPS34 and ULK1-FIP200 arms of the autophagy system. Cells lacking YIPF3 or YIPF4 are selectively defective in elimination of Golgi membrane proteins during nutrient stress. In addition, we find that remodeling of the Golgi during conversion of human embryonic stem cells (hESC) to induced neurons (iNeurons) in vitro requires YIPF4 mediated Golgiphagy, highlighting the broader role of YIPF3/4 as autophagy-based Golgi remodelers in response to both nutrient stress and cell state changes.

M84

**Cytoplasmic ribosomes hitchhike on mitochondria to dendrites *in vivo*.**

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Extra-somatic protein synthesis in neurons, also known as "local translation," plays diverse roles in dendrite physiology. For local translation to occur, the translation machinery must first be transported to the neurite from the cell body. Although ribosomes have been observed in dendrites for over half a century, how ribosomes are transported to distant neuronal compartments *in vivo* remains poorly understood. Here, using live cell imaging and electron microscopy in *C. elegans*, we present evidence that cytoplasmic ribosomes “hitchhike” from neuronal soma to dendrites on mitochondria. Overexpression of a fluorescent ribosome reporter in chemosensory neurons reveals punctate clusters of ribosomes in the dendrite. An unbiased forward genetic screen on this reporter yielded hits in the genes *trak-1*/TRAK1 and *drp-1*/DNM1L, implicating a role for mitochondria in ribosome transport. Next, we found that fluorescent reporters for ribosomes and mitochondria colocalize in the dendrite, which was further confirmed by electron microscopy. Mutant alleles for components of the mitochondrial transport machinery, including *dli-1*/DYNC1LI2, *trak-1*/TRAK1, *miro-1*/RHOT1, and *mtx-2*/MTX2, are sufficient to block the transport of both mitochondria and ribosomes into the dendrite. Furthermore, ectopic reintroduction of mitochondria back into the dendrites of *miro-1; mtx-2* double mutants using an artificial “MitoTruck” tethering construct also rescues the dendritic localization of ribosomes, demonstrating that mitochondria are both necessary and sufficient for dendritic ribosome localization in this system. Finally, using time-lapse imaging we demonstrate that ribosomes cotransport with mitochondria in space and time, and that mitochondria movements account for >95% of all detectable ribosome movement events. These results uncover a novel role for mitochondria in neuronal ribosome transport, and are consistent with recent reports of cotransport between mitochondria and mRNA.

## Minisymp: The Healthy Cell: Homeostatic Mechanisms Controlling Bioresilience

M85

**The differential proteostasis mechanisms in response to acute and persistent stresses.**

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Organisms invariably encounter diverse environmental stresses throughout their lifespan, which can manifest as either acute shocks or long-term stressors extending over generations, such as climate change. In this study, we explore the contrasting cellular strategies invoked in response to these acute and chronic environmental stresses.Acute stress commonly instigates protein misfolding and aggregation. Our results show that misfolded cytosolic proteins do not autonomously aggregate in vivo; instead, they are recruited to the aggregation sites initiated by Tom70's substrates (nascent mitochondrial proteins) on the mitochondrial membrane using multivalent hydrophobic interactions. This Tom70 and nascent mitochondrial proteins-based initiation of protein aggregation explains the localized condensation of otherwise ubiquitously distributed misfolded proteins on the mitochondria.Conversely, in the face of persistent stress, cells demonstrate the capacity to adapt without forming protein aggregates. Using a combination of high-content imaging-based screening, machine learning, and a novel proteomics-structure screening pipeline, we observed that instead of activating the classic heat shock response, cells reduce thermolabile proteins and relocate many proteins to different subcellular compartments as they adapt to persistent thermal stress. Additionally, our investigation uncovered that this thermal adaptation triggers many proteins to assume alternative stable conformations, facilitating the emergence of novel protein-protein interactions and cellular functions. This context-dependent alternative folding extends the structural plasticity observed in prion proteins to more soluble proteins, implying that a single polypeptide can adopt multiple three-dimensional conformations depending on the context. Consequently, we propose that beyond the recognized mechanisms of adaptation, alternative protein folding allows the same polypeptide to acquire new biophysical properties and functions in response to enduring environmental change.

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M86

**An obligate intracellular bacterial pathogen forms a direct, interkingdom membrane contact site.**

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Interorganelle communication regulates cellular homeostasis through the formation of tightly-associated membrane contact sites (MCSs). Prior work has identified these sites as regions vulnerable to vacuolar pathogen manipulation to enable pathogen survival. These pathogens either alter existing membrane contact sites or establish new membrane contact sites between the host endoplasmic reticulum (ER) and the pathogen-containing, host-derived membrane. However, there is no existing evidence for membrane contact sites directly spanning eukaryotic and prokaryotic membranes. Here,

using a combination of live-cell microscopy and transmission and focused-ion-beam scanning electron microscopy, we demonstrate that the intracellular bacterial pathogen *Rickettsia parkeri* forms a direct membrane contact site with the ER. These sites were approximately 55 nm apart, with visible tethers linking the bacterial outer membrane to the ER. These interactions were not present with *Listeria monocytogenes* or *Shigella flexneri*, suggesting this is a unique adaption by *R. parkeri*. Interestingly, we found that an immobile *R. parkeri* mutant displays increased interactions with the ER that are stable over several minutes, resembling the stability displayed by eukaryotic membrane contact sites. Depletion of the ER-specific tethers VAPA and VAPB reduced the frequency of rickettsia-ER contacts, suggesting these interactions mimic organelle-ER contacts. Overall, our findings illuminate a direct, interkingdom membrane contact site uniquely mediated by rickettsia that appears to mimic traditional host MCSs. Moreover, our findings uncover a new example of microbial mimicry of host cell processes, which presents an exciting avenue to gain deeper insights into host cell biology.

M87

**Parkinson Sac domain mutation in Synaptojanin 1 affects ciliary properties in dopaminergic neurons.**

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Synaptojanin-1(SJ1, PARK20) is a major neuronal PI(4,5)$P_2$ phosphatase implicated inthe shedding of endocytic factors during clathrin-mediated endocytosis. A homozygousmutation (R258Q) that impairs the activity of its Sac 4-phosphatase domain causesearly-onset Parkinsonism in humans and neurological defects in knock-in mice (SJ1$^{RQ}$ mice) harboring the same mutation. Studies ofthese mice showed, in addition to an abnormal assembly state of endocyticfactors at synapses, the presence of dystrophic changes in a subset of dopamine(DA)-ergicaxons in the dorsal striatum, suggesting a special lability of DA neurons tothe impairment of SJ1 function. Here we have used WT and SJ1KO or SJ1$^{RQ}$iPSC-derived DA neurons as a model system to study the impact of SJ1 on theirproperties. We confirmed the clustering of endocytic factors in nerve terminalsof the SJ1 mutant neurons and we observed a link of SJ1 function to cilia. Wefound that a pool of SJ1 localizes to the base of primary cilia in theseneurons. In both SJ1 KO and SJ1$^{RQ}$DA neurons, cilia were longer than in control neurons. Additionally, in SJ1 KOneurons, a small proportion of cilia appeared abnormal in shape withsome cells having more than one cilium emerging from the same site. Further analysis of the ciliary shaft of SJ1$^{RQ}$DA neurons revealed a major increasein immunoreactivity for ubiquitin chains (FK1 and FK2 antibodies), suggesting animpaired regulated exit from cilia of ubiquitinated membrane proteins, whichmay be a consequence of an endocytic defect at the ciliary base. One protein that massively accumulatedthroughout the ciliary shaft in SJ1$^{RQ}$ DA neurons was the L-typevoltage-gated calcium channel (VGCC) $Ca_v1.3$, which is known toparticipate in the pacemaker function of DA neurons. In control DA neurons, $Ca_v1.3$ wasconcentrated at the ciliary base, but was not detectable in the cilia shafts. We suggest that SJ1 may contribute with otherinositol 5-phosphatases in the control of cilia dynamics, and that it may alsobe required for the regulated turnover of ciliary proteins, most likely viaclathrin-mediated endocytosis at the cilia base, with implications on cilia-mediatedsignaling.

M88

**SQST-1/p62 and SKN-1/NRF2 promote phagocytosis under stress by promoting lysosomal trafficking.**

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Programmed cell death and autophagy are among conserved quality control processes that regulate homeostasis and development. Autophagy and cell death have been linked, with autophagy shown to be involved in cell clearance. Our understanding of autophagy and programmed cell death in morphologically complex cells is incomplete owing to unique features of such cells such as vastly different environments between different domains and differences in subcellular architecture.We present a novel in vivo system to decipher the mechanisms of autophagy and programmed cell death and the link between them in complex cells. In this “tri-partite” killing program, Compartmentalized Cell Elimination, or CCE, three segments of the *C. elegans* tail-spike cell and the sex-specific CEM neurons die in different ways-the soma rounds as in apoptotic death, the proximal part of the process beads and fragments and the distal process undergoes a bidirectional retraction. We asked whether there is a link between CCE and autophagy. We subjected wild-type worms to a heat-shock stress and found CCE to take place normally. However, following heat shock stress, mutants for the genes *sqst-1* and *uba-1* show significantly hampered CCE. The gene *sqst-1* encodes for the *C. elegans* ortholog of SQSTM1/p62, a scaffold protein that targets and anchors ubiquitinated proteins to the autophagosome membrane, promoting their degradation by selective autophagy. The gene *uba-1* encodes for the *C. elegans* ortholog of the E1 ubiquitin-ligase enzyme, UBA1. Together these data implicate selective autophagy in aiding CCE under stress.One degradation target of SQSTM1/p62 is KEAP1, a negative regulator of the stress response transcription factor NRF2. SQSTM1/p62 activates NRF2 by inactivating KEAP1. NRF2 is best known as a regulator of antioxidant and xenobiotic defense and is recently implicated in additional functions that include proteostasis and metabolic regulation. In worms, SKN-1 is the ortholog of NRF2. SKN-1 has been showed to promote DNA damage-induced germline apoptosis in worms and the NRF2-KEAP1 pathway has been linked to in neuronal remodeling in flies. We find that *skn-1* mutants also have a CCE defect under stress conditions and that SKN-1/NRF2 translocates to the phagocyte nucleus following heat stress. One transcriptional target of SKN-1/NRF-2 is the lysosomal trafficking gene *lyst-1.* We find that *lyst-1* mutants also have a similar CCE defect, as do mutants for *lmp-1*/Lamp-1, an important lysosomal gene. LMP-1/LAMP-1 also localizes discretely in the phagocyte following heat stress. Together, our data suggests autophagy and cellular stress resistance mechanisms bolster a developmental death program of complex cells under stress conditions by enhancing lysosomal trafficking and subsequent corpse digestion.

M89

**Unconventional binding of *Salmonella* effector SipA to F-actin mimics the structural effects of inorganic phosphate and phalloidin.**

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Non-typhoidal *Salmonella enterica* is a pathogen contracted by consuming contaminated food, which causes disease ranging from self-limiting diarrheal illness to life-threatening invasive infection. *Salmonella enterica* Typhimurium (ST) is the serovar most associated with disease and has been responsible for outbreaks in sub-Saharan Africa and Vietnam. After passing into the small intestine, ST injects factors into the host cell via its type III secretion system. This allows ST to stay attached to the

cell, reorganize the host's cytoskeleton, and become engulfed into a compartment called the Salmonella containing vacuole (SCV), where it resides, thrives, and multiplies. One of the key proteins in this process is Salmonella invasion protein A (SipA). The C-terminal half of SipA ($SipA_C$) binds to F-actin using its central globular domain and disordered arms, "stapling" actin subunits and protecting actin filaments from depolymerization. Existing structures of the $SipA_C$/F-actin complex are low resolution, and its biochemical characteristics are poorly defined. To further our understanding of $SipA_C$ and therefore ST pathogenicity in general, we solved a high-resolution Cryo-EM structure of $SipA_C$ decorated F-actin and characterized their interaction at the biochemical level. We show that $SipA_C$ binds F-actin in a 1:2 ratio via its globular core domain and its disordered Arm2 domain, which penetrates the inter-strand space. We show this binding mode anchors $SipA_C$ to F-actin, inhibits severing and depolymerization by ADF, inhibits thermal denaturation, and increases filament stiffness. Finally, we show that Arm2 can't bind F-actin alone and requires a prior binding of the core domain. These data, combined with previous observations that $SipA_C$ accumulates at the injection site and forms an actin cage around the SCV, indicate that the pathological mechanism of $SipA_C$ is to stabilize F-actin structures to assist in invasion and intracellular survival of the pathogen.

M90

**The Integrated Stress Response Unconventionally Activates the Unfolded Protein Response Sensor IRE1.**

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The integrated stress response (ISR) and the unfolded protein response (UPR) are conserved signaling networks governed by stress sensor kinases. In the ISR, four sensor kinases, GCN2, HRI, PERK, and PKR, phosphorylate a common node, the master translation initiation factor eIF2α, in response to multiple stresses. As a result, global protein synthesis shuts down while select mRNAs, including those encoding master ISR transcription factors, are translated. This bipartite mechanism leads to transcriptome and proteome reprogramming, and the reinstatement of homeostasis. In the UPR, endoplasmic reticulum (ER) stress activates three ER-resident sensors: PERK—a shared node between the ISR and the UPR—the kinase/endoRNAse IRE1, and the membrane-tethered transcription factor, ATF6. Active IRE1 induces the key UPR transcription factor XBP1 and degrades select transcripts, reducing ER load and safeguarding ER proteostasis. We and others have shown that the ISR-UPR sensors share common signaling principles, specifically, the assembly of sensor kinases into dynamic clusters that regulate signaling, suggesting profound similarities between the ISR and the UPR. Motivated by these findings, we sought to investigate the extent of interconnectivity of signaling mechanisms between the ISR and the UPR. Here, using a synthetic, stress-input agnostic approach, we show that activation of the ISR leads to the unconventional activation of IRE1—and not ATF6 or PERK—revealing crosstalk that is not contingent on canonical UPR signaling. In this context, IRE1 activation required neither IRE1's unfolded protein sensor domain nor its lipid bilayer stress sensing capability, suggesting that IRE1 conscription by the ISR is hard-wired. Moreover, we found that inhibiting protein synthesis in an ISR-independent manner also induced IRE1 activation in time scales that are inconsistent with unfolded protein accumulation in the ER lumen, suggesting that IRE1 is capable of monitoring translation rates and lending support to our previous findings on IRE1-ribosome interactions. Together, our data provide new mechanistic insights into fundamental aspects of stress sensor kinase signaling, linking the UPR and ISR outside their common node PERK, and shed light on moonlighting functions of IRE1.

## Minisymp: Cellular Genome Organization and Gene Regulation

M91

**Mechanisms of genome scaling during embryogenesis and evolution.**

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During the rapid and reductive cleavage divisions of early embryogenesis, the physical dimensions of chromosomes adjust to decreasing cell size to facilitate their accurate distribution to daughter cells. While previous research has revealed mechanisms by which the nucleus and spindle scale to cell size, the basis of mitotic chromosome scaling has remained elusive. We combined in vivo and in vitro approaches using eggs and embryos from the frog *Xenopus laevis* to show that mitotic chromosome scaling is mechanistically distinct from other forms of subcellular scaling. We found that mitotic chromosomes scale continuously with cell, spindle, and nuclear size in vivo. However, unlike for spindles and nuclei, mitotic chromosome size cannot be reset by cytoplasmic factors from earlier developmental stages. In vitro, increasing nuclear-cytoplasmic (N/C) ratio is sufficient to recapitulate mitotic chromosome scaling, but not nuclear or spindle scaling, through differential loading of maternal factors during interphase. An additional pathway involving importin α scales mitotic chromosomes to cell surface area/volume ratio during metaphase. Finally, single-chromosome immunofluorescence and Hi-C data suggest that mitotic chromosomes shrink during embryogenesis through decreased recruitment of condensin I, resulting in major rearrangements of DNA loop architecture to accommodate the same amount of DNA on a shorter chromosome axis. Together, our findings demonstrate how mitotic chromosome size is set by spatially and temporally distinct developmental cues in the early embryo. This work also sets the stage for investigating the mechanisms and functions of chromatin re-organization during embryogenesis and the effects of altering genome copy number (ploidy) that has occurred frequently in the evolution of different *Xenopus* species and is characteristic of many cancers.

M92

**Tackling mitotic chromosome (un-)folding.**

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Major advances in understanding the spatial genome organization have been made throughout the past decade due to the power of biochemical crosslinking and sequencing (HiC-type) methods. However, a detailed understanding of chromatin structure and its underlying modulators has been restricted to interphase, because sequence-specific architectural features are lost upon entry into mitosis and cannot be detected by population-based readouts. The folding of mitotic chromosomes into their characteristic stiff rod-shape required for faithful chromosome segregation and their dynamic transition back into the interphase state therefore has remained enigmatic. Using LoopTrace, a structure-preserving sequential DNA-FISH method for super-resolution tracing of chromatin, we can now describe the organization of compacted chromosomes in all mitotic stages with high precision. We will present native mitotic chromosome folding structures from the kilobase scale to the scale of entire chromosomes as well as the impact of perturbing key mitotic architecture regulators. Furthermore, using highly time-resolved quantitative imaging throughout mitosis and mitotic exit, we systematically characterize the dynamic chromatin association of the DNA-loop extrusion machinery that governs chromosome organization

during mitosis (Condensins) and interphase (Cohesins & CTCF). We identify a transition period during telophase, during which both major loop extrusion complexes are present on chromatin, as well as a differential re-binding pattern of the interphase-relevant loop extruders Cohesin-STAG1 and Cohesin-STAG2. Combined, our data allow us to formulate a quantitative mechanistic model of mitotic chromosome folding and its subsequent unfolding into the interphase state.

M93

**Dynamics and mechanics of R-loop formation during DNA interrogation by Cas12a from different species.**

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Cas12a is a CRISPR endonuclease that interrogates DNA sequences by forming an R-loop structure containing an RNA:DNA heteroduplex before cleaving a target site that matches a guide RNA. The sequence specificity of R-loop formation and cleavage has enabled wide applications in genetic engineering. R-loop substeps are affected by mismatches with the guide RNA and by target DNA topology, which must be understood to arrive at mechanochemical descriptions of the mechanisms underlying specificity in different CRISPR endonucleases such as Cas12a and Cas9. We have used gold rotor bead tracking (AuRBT), a high-resolution single-molecule torque spectroscopy method, to observe the dynamics of R-loop formation on supercoiled DNA. By observing torque changes corresponding to DNA duplex unwinding, we can measure the dynamics and mechanics of DNA interrogation. We quantify torque-dependent transition rates between identified R-loop states to construct models of energy landscapes whose properties are modulated by DNA supercoiling and mismatches. Previous work with Cas9 found that R-loop formation proceeds through a discrete intermediate corresponding to unwinding of the seed region and showed that DNA supercoiling strongly modulates Cas9 activity and specificity. Here we measure R-loop dynamics for Cas12a from two different species of bacteria, revealing differences between species and from Cas9. We find that R-loop formation proceeds through a complex series of intermediates depending on species, target sequence, and mismatch locations. We directly observe and characterize an intermediate corresponding to a previously described ~5 bp seed region, and also investigate dynamic unwinding beyond the 20 bp seed region. As seen for Cas9, supercoiling promotes promiscuous activity in the presence of mismatches. Detailed mechanochemical comparisons between Cas9 and Cas12a, as well as between Cas12a enzymes from different species, should provide important insights for optimized usage and engineering of CRISPR endonucleases.

M94

**Lamins A and B1 are essential for mouse development by conferring proper 3D genome organization and cell fate in the extraembryonic endoderm.**

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Lamin proteins are intermediate filaments which form the nuclear lamina (NL), a proteinaceous network found beneath the nuclear envelope. The NL has many cellular roles, including organizing DNA into lamina-associated DNA domains (LADs) and contributing to proper 3D genome organization. Mammals have three lamin genes, Lmna which expresses isoforms lamin-A and lamin-C (collectively: LA), and

Lmnb1 and Lmnb2 which express lamin-B1 (LB1) and lamin-B2 (LB2). Mutations in individual lamin genes cause tissue-specific diseases called laminopathies, but the diverse functions of different lamins complicates our understanding of how lamin mutations cause disease. Our lab utilizes mouse development as a model to study the essential functions of lamins. We have found that LA/LB1 double knockout (DKO) embryos have retarded growth and exhibit lethality by embryonic day (E) 10.5, which is much earlier than lamin single KO and LB1/LB2 DKO embryos. By performing single cell RNA-seq on LA/LB1 DKO embryos, we found lipid processing transcriptional defects and upregulation of alternate cell fate genes in extraembryonic endoderm (ExE endoderm) cells. ExE endoderm expresses both LB1 and LA protein at E9.5, suggesting LA and LB1 have essential redundant roles in this tissue. The transcriptional defect in LA/LB1 ExE Endoderm could explain the embryonic growth defect due to reduced lipid transport to the embryo, which is supported by decreased Oil Red O staining in LA/LB1 DKO embryos. To study how altered 3D genome organization in LA/LB1 DKO ExE Endoderm cells contributes to the observed transcriptional defect, we performed LAD mapping and high-throughput chromosome conformation capture (HiC) of LA/LB1 DKO and control ExE Endoderm cells. Surprisingly, LADs in control ExE Endoderm cells are inverted compared to other cell types, suggesting that the unique metabolic functions of these cell types necessitates a unique LAD organization. We found that LAD organization is disrupted in LA/LB1 DKO ExE Endoderm cells, which helps explain the observed transcriptional defects. Our ongoing work is focused on deciphering the unique genome organization of ExE Endoderm cells, and how lamin loss changes chromatin interactions that influence cell fate and function.

M95

**Identifying the mechanism of olfactory receptor gene regulation in primary olfactory neurons via live-cell imaging of genomic loci and transcription factors.**

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Detection and identification of volatile chemicals in the mammalian olfactory system is built upon the “one receptor per neuron” rule, whereby each mature olfactory sensory neuron expresses a single olfactory receptor (OR) gene from over 2,000 possible alleles. Singular expression is critical for olfactory perception, defining both the receptive field of the OSN and the circuitry of its axon. This singular expression is contingent on orchestrated changes in nuclear architecture.

OR gene activation is accomplished by the formation of a hub that brings together loci from various chromosomes and associates with and activates a single OR gene. This hub forms under the control of the transcription factors, Lhx2 and Ebf, and the adaptor protein Ldb1. By fluorescently labeling the genomic loci of the expressed OR and these regulatory proteins, we can monitor their dynamics in living primary mouse neurons.

The formation of this hub allows the chosen OR to overcome repression, and this hub represents a greater enrichment of transcription factors than can be explained simply by the stoichiometry of enhancer binding sites within the hub. with single-particle tracking and super-resolution imaging, we have characterized the biomolecular interactions that allow the hub to recruit and concentrate Lhx2/Ebf/Ldb1 specifically at the genomic loci of the expressed OR.

M96

**Towards a Comprehensive Characterization of Human DNA Replication Kinetics.**

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The organization of DNA replication, both spatially along chromosomes and temporally throughout S phase, is a fundamental function of nuclear biology that affects genome structure, expression, stability and evolution. Genome-wide replication kinetics have been characterized at the population level, but such bulk analysis only reveals the average timing and location of replication, masking the significant heterogeneity of the replication program. Single-molecule characterization of replication kinetics has elucidated this heterogeneity and been used to characterize specific loci, but has not been sufficiently high throughput to characterize replication kinetics genome wide. Therefore, it has been difficult to test models of origin regulation. In particular, it has been difficult to distinguish between deterministic models, in which specific origins fire at specific times, and stochastic models, in which an origin's probability of firing determines its average firing time, but there is no pre-determined timing program. We have developed optical replication mapping (ORM), a technique that allows for the first time genome-wide, single-molecule characterization of metazoan replication kinetics <https://pubmed.ncbi.nlm.nih.gov/34157308>. Our analysis of replication in human cell lines, and our modeling of replication kinetics based on that data, shows that replication initiation is distributed stochastically across the genome. The probability of firing varies widely between origins, with higher-probability origins firing, on average, earlier that lower-probability origins. Importantly, there is no correlation in firing between adjacent origin.

Our results demonstrate how cells maintain a robust timing program without having a mechanism that fires specific origins at specific times. Instead, timing emerges as a consequence of firing probability, with higher-probability origins having a higher probability of firing early, and therefore having an earlier average firing time. This insight will focus future work on understanding the mechanisms that regulate the probability of origin firing across the human genome.

## Minisymp: Emerging Frontiers in Tumor Microenvironment Research and Therapeutic Innovations

M97

**Microphysiological systems to study the tumor microenvironment: from memory-like NK cells to immune exhaustion.**

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Solid tumors generate a microenvironment characterized by hypoxia, nutrient starvation, waste product accumulation. These microenvironmental gradients compromise the immune response, reducing T and NK cell cytotoxicity. However, capturing the tumor microenvironment with traditional in vitro model based on Petri dishes is challenging. Thus, we have developed microphysiological systems (MPS) that mimic the tumor structure, including the presence of patient-derived tumor samples, 3D extracellular matrix, oxygen and nutrient gradients, stromal cells, vasculature, and multiple immune cells (e.g., macrophages, NK, CD4, and CD8 T cells). Furthermore, we have applied these MPS to study a variety of solid tumors such as head and neck cancer and breast cancer. Using this approach, we have evaluated multiple aspects of the anti-tumor immune response such as immune infiltration, antibody tumor penetration, and antibody cell-dependent cytotoxicity, and immune exhaustion induced by metabolic starvation. We demonstrated that memory-like NK cells have increased cytotoxicity capacity compared

with naïve NK cells. Additionally, memory-like NK cells did not require the presence of CD4 T cells to extravasate from the tumor-surrounding vasculature and penetrate into the tumor spheroid. Using fluorescent antibodies targeting the tumor, we demonstrated solid tumors exert a barrier effect that excludes antibody penetration, limiting antibody-dependent cell cytotoxicity mediated by NK cells. Finally, we also observed that nutrient starvation and acidic pH at the tumor core led to irreversible NK cell exhaustion that was partially prevented by leveraging several treatments.

M98

**Nutrient stress enhances DNA repair pathways in pancreatic cancer.**

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Many cancers, including pancreatic ductal adenocarcinomas (PDAC), are defined by poor vascularization and substantial fibrosis which contribute to impaired nutrient and waste exchange, leading to altered nutrient availability within the tumor. As a result, PDAC cells must adapt to grow under conditions of significant nutrient stress. To better understand how PDAC tumors survive under nutrient stress, we developed Tumor Interstitial Fluid Medium (TIFM), a cell culture medium which models 115 metabolites at concentrations found in murine PDAC tumors. Surprisingly, we found that TIFM-cultured PDAC cell lines are robustly resistant to a diverse array of non-nutrient stressors, including DNA damaging chemotherapies. Notably, culture in TIFM does not prevent chemotherapy-induced DNA damage, but rather enables PDAC cells to evade cell death secondary to DNA damage. This suggests that nutrient stress in PDAC tumors endows cancer cells with the ability to survive otherwise lethal stresses, including chemotherapy-induced DNA damage.
To understand the underlying mechanisms of nutrient stress-induced drug resistance, we conducted a whole genome CRISPRi screen with the commonly used chemotherapy drug gemcitabine to identify genes responsible for nutrient-mediated chemoresistance. The screen revealed that genes encoding members of the ATR pathway, which regulates DNA damage repair, and downstream ubiquitin-proteasome effectors were critical for chemoresistance. This suggests nutrient stress may enhance the activity of DNA damage repair in cancer cells, enabling PDAC cells to survive non-nutrient stressors like chemotherapy. In future studies, we will determine if nutrient stress improves DNA damage repair and identify the nutrient(s) causing this change in DNA repair. Overall, these studies identify nutrient stress in tumors as triggering collateral resistance to other stresses, particularly chemotherapy challenge, and provide insight into the molecular basis underpinning this phenomenon.

M99

**Emerin modulates mechanotransduction to facilitate breast cancer metastasis.**

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Breast cancer metastasis accounts for most of the breast cancer-related deaths. During metastasis, cancerous tumor cells spread through the body by moving into and out of nearby blood vessels and squeezing through very small gaps in the endothelium. Nuclei in cancer cells are often smaller and more malleable, allowing for easier metastasis. Our lab has shown invasive breast cancer cell lines and patient samples have less emerin expression, misshapen nuclei, and higher metastasis rates than non-cancerous controls. We hypothesize that reduced emerin expression compromises the structural integrity of the

nucleus to increase its compliance, which serves as a key driver of breast cancer metastasis. However, the mechanism by which emerin modulates nuclear stiffness is not well understood. Increasing stiffness of the tumor microenvironment (TME) was shown to increase nuclear softening. Therefore, we predict emerin plays an important role in responding to changes in the TME by altering nuclear structure. Interestingly, this increased stiffness in the TME and subsequent nuclear softening also correlates with increased metastasis. Here, we present data showing that emerin protein expression decreases upon extracellular matrix (ECM) stiffening. Importantly, ECM stiffening in control cells (MCF10A and MCF7) reduced emerin expression to TNBC levels concomitant with nuclear structure abnormalities. We posit emerin is acting as a mechanosensor that responds to extracellular stimuli through the linker of nucleoskeleton and cytoskeleton (LINC) complex by modulating structural and/or transcriptional outcomes, resulting in nuclear softening to enable metastasis in triple-negative breast cancer (TNBC). We postulate loss of the interaction between emerin and LINC is critical for enabling metastatic transformation. We used cell culture plates of differing substrates and emerin and LINC mutants to perturb LINC interaction(s) with emerin and evaluated changes in TNBC cell nuclear size and shape, volume, invasion, and migration upon substrate stiffening. Our analysis provides key insight into *how* emerin responds to substrate stiffening in metastatic progression and the mechanism of how emerin and the LINC complex respond to changes in the tumor microenvironment.

M100

**Multidimensional single-object screening of pancreatic cancer spheroids/organoids reveals cooperative vulnerabilities in mitotic and cell adhesion pathways that associate with KRAS mutations.**

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy, with a median survival of less than 12 months and a 5-year survival of less than 10%. These poor outcomes are due, in part, to few targeted therapy options. Due to their tractability, ease of manipulation and pathophysiological relevance, three-dimensional (3D) spheroids and organoids can serve as powerful models for uncovering mechanisms of solid tumor progression and therapy response. Here, we have established a multidimensional single-object screening pipeline and used it to interrogate the effects of pathway antagonists/agonists and/or mesenchymal stromal cells (MSC) on PDAC spheroid/organoid compaction, organization, morphology and growth. Interestingly, mutant KRAS PDAC cells with mixed epithelial/mesenchymal character formed loosely aggregated spheroids and more eccentric/invasive organoids. While mutant KRAS PDAC cells varied in their response to our panel of pathway antagonists/agonists, spheroid and organoid growth was consistently reduced by 20% following inhibition of polo-like kinase 1 (PLK1). Independent of genetic background, PDAC/MSC multicellular spheroids/organoids self-organized into structures with an MSC-dominant core; however, MSCs selectively induced compacting and growth effects in mutant KRAS PDAC spheroids. Interestingly, we discovered that MSC-induced mutant KRAS PDAC spheroid growth was completely blocked by pharmacological inhibition of integrin beta 1 (ITGB1) or calcium chelation (but not integrin alpha 1, ITGA1 knockdown in PDAC cells) suggesting that non-ITGA1 ITGB1-containing integrins mediate growth of these multicellular spheroids. In contrast, our observations that exogenous collagen 1 increased mutant KRAS PDAC spheroid compaction by only 25% while ITGA1 knockdown in mutant KRAS PDAC cells (but not pharmacological inhibition of ITGB1) completely blocked MSC-induced spheroid compaction suggest that inside-out ITGA1 signaling may cooperate with non-integrin cell-cell adhesion mechanisms to mediate compaction of these multicellular spheroids. In agreement with this model,

calcium chelation also blocked MSC-induced mutant KRAS PDAC spheroid compaction. Finally, we report that PLK1 and ITGA1 cooperatively regulate paxillin-positive adhesions in KRAS-mutant PDAC cells and that their concurrent upregulation in PDAC is associated with poor patient outcomes. Taken together, this work employs a quantitative method for single-spheroid/organoid analysis to uncover new mechanisms of therapeutic vulnerability in PDAC that are relevant to the pathogenesis and progression of this stromal cell-rich malignancy, and which may reveal strategies for improving patient outcomes.

M101

**Neoadjuvant Niraparib Therapy and Chemotherapy Reverse HRD-driven Immunosuppression of Tumor Microenvironment in Ovarian Cancer.**

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Homologous recombination deficiency (HRD) is a widespread cancer hallmark and renders tumors vulnerable to PARP inhibition. However, the effects of HRD and related therapies on the tumor microenvironment (TME) remain poorly understood. Here we characterize 93 tumor samples from 52 patients with high grade serous ovarian cancer (HGSOC) using single-cell RNA and T cell receptor (TCR) profiling to establish the most comprehensive HGSOC TME atlas to this day with >850,000 cells. Combined with multiplex immunofluorescence imaging, flow cytometric analysis, and bulk TCR-seq of tumors and matched peripheral blood, we systematically assess HGSOC TME compositions before and after neoadjuvant niraparib therapy or chemotherapy. Treatment-naïve HRD tumors are typified by weaker adaptive immune infiltration but show a more dramatic post-treatment relief from immunosuppression, characterized by a global shift of T lymphocytes away from hyper-expanded, immunosuppressive regulatory $CD4^+$ T and exhausted $CD8^+$ T cells as well as local deviations from their terminal effector subsets. The synergestic effect of Treg depletion and PARP inhibition on TME rejuvenation is then validated in a humanized syngeneic HGSOC mouse model. Mechanistically, the widespread, chronic activation of interferon signaling across TME components orchestrates T cell anergy induction, including the secretion of CXCL9/10 by M1-like macrophages and MHC-II-dependent direct engagement by cancer cells. Our study thus reveals an underappreciated effect of neoadjuvant chemotherapy and PARP inhibitor in reversing HRD-driven immunosuppression of TME, suggesting the utility of genotoxic anticancer agents to revive TME immunoactivity in genomically unstable tumors.

M102

**HER2-dependent prostate cancer cells downregulate androgen receptor activity to drive resistance to therapy.**

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Prostate cancer is the second-leading cause of cancer-associated death among men in the United States, due to the prevalence of treatment-resistant micrometastatic spread, driving relapse. A majority of prostate tumors are driven by androgen receptor (AR) signaling, which provides an opportunity to treat prostate cancer with androgen deprivation therapy (ADT). In patients with advanced prostate cancer, hormone-based therapies targeting androgen-dependent malignant cells, such as neoadjuvant ADT, often represent the last chance for a cure, although a substantial subset of these tumors will ultimately develop resistance to ADT. To determine novel mechanisms of treatment resistance, we tested the

hypothesis that mechanisms of treatment resistance are present at diagnosis, which would predict poor response to ADT before therapy begins. Pre-treatment tumors from 37 patients with locally advanced prostate cancer were subjected to IHC and laser capture microdissection to obtain 143 distinct foci. RNA was isolated from each focus and used to generate RNAseq libraries. Histologic features were obtained from IHC of pre- and post-treatment tumor foci. Tumors that exhibited worse response to ADT had lower AR activity prior to treatment, due to a decreased dependency on AR-mediated proliferation. These poorly responding tumors also had increased expression of EGFR family signaling, primarily through HER2-mediated activity. Additionally, HER2 was the most abundantly expressed EGFR family protein in post-treatment tumor samples. Given the multitude of receptor tyrosine kinases (RTKs) regulated by EGFR family signaling, we further interrogated the mechanism of treatment resistance using a panel of prostate cancer cell lines treated with RTK inhibitors (RTKi), ADT, and a combination of RTKi and ADT. We found that afatinib (HER2 inhibitor) and neratinib (HER2 and EGFR inhibitor) showed the greatest anti-tumor efficacy *in vitro*. Treatment with these agents rapidly and dynamically increased AR expression and activity. Cells treated with ADT exhibited increased activation of HER2 and downstream PI3K signaling pathways. When combining ADT and RTKi treatments, we observed synergistic effects on cell viability and proliferation. These data highlight the role of HER2 signaling in bypassing androgen inhibition to promote AR-independent PI3K-mediated proliferation pathways, and represent new opportunities for combination therapy in newly diagnosed high-risk prostate cancer. As these effects were observed in a treatment-naive patient population, they represent an early divergent path in tumorigenesis, where prostate tumors initially develop with intrinsically lower AR activity, contributing to treatment resistance and metastatic spread.

## Minisymp: Emerging Technologies to Study Cellular Dynamics

M103

**Visual Proteomics of *C. reinhardtii* using High-throughput Collaborative in situ Cryo-ET.**

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Cryo-electron tomography (cryo-ET) and subtomogram averaging are structural biology tools that can address biomolecules in complex environments. In combination with cryo-FIB, which allows thinning of thick specimens, this technique enables visualization of macromolecules embedded in their native environment within cells. Subtomogram averaging has been used to determine structures of purified proteins and complexes at a resolution needed for model building (<4 Å). On the other hand, vitrified and thinned cellular samples have been largely limited to resolutions of 10 Å and worse. The common reasons were poor lamellae quality, low throughput, and beam-induced motion. The new generation of cryo-FIB from Thermo Fisher Scientific uses plasma instead of Gallium ions, which reduces redeposition and ion beam damage, and substantially improves throughput. To explore the potential and benefits of plasma cryo-FIB for high-throughput cryo-ET, we initiated a study of a model organism, *C. reinhardtii*. So far, we have prepared such amount of lamellae that was sufficient for acquisition of more than 1500 tomograms of different cellular compartments. Our preliminary results indicate that subtomogram averaging can reach sub-nanometer resolution for selected complexes using a fraction of the dataset. Using 6 tomograms acquired on a single lamella, we determined the structure of 80S ribosome at 6 Å. In addition, we present a 7 Å structure of in situ ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) within the pyrenoid compartment. Furthermore, we would like to use the data to generate a

molecular atlas of the abundant cellular machineries and complexes. However, the vast number of biomolecular complexes within living cells makes it challenging to identify these complexes, confirm their identity, and determine their structure, necessitating for collaborative efforts. We would therefore like to create an open access database for *C. reinhardtii* tomograms to accelerate annotation and curation, enable further cell biology research, and develop new computational tools for in situ cryo-ET. We believe that this project will provide invaluable insights into fundamental cellular processes and lay the foundation for similar large-scale studies of other species.

M104

**De novo protein identification in mammalian sperm using in situ cryo-electron tomography and AlphaFold2 docking.**

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To understand molecular mechanisms of cellular pathways, contemporary workflows typically require multiple techniques to identify proteins, track their localization, and determine their structures *in vitro*. Here, we combined cellular cryo-electron tomography (cryoET) and AlphaFold2 modeling to address these questions and understand how mammalian sperm are built *in situ*. Our cellular cryoET and subtomogram averaging provided 6.0 Å reconstructions of axonemal microtubule structures. The well-resolved tertiary structures allowed us to unbiasedly match novel densities with 21,615 AlphaFold2-predicted protein models of the mouse proteome. We identified Tektin 5, CCDC105 and SPACA9 as novel microtubule-associated proteins. These proteins form an extensive interaction network crosslinking the lumen of axonemal doublet microtubules, indicating their roles in modulating the mechanical properties of the filaments. Indeed, *Tekt5 -/-* sperm possess more deformed flagella with 180° bends. Together, our studies presented a cellular visual proteomics workflow and shed light on the *in vivo* functions of Tektin 5.

M105

**Live cell resolution of mRNA decapping subunit interaction dynamics within condensates.**

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Ribonucleoprotein (RNP) granules are membrane-less organelles that concentrate RNA and protein throughout the cell by liquid-liquid phase separation. A major challenge with understanding the true biological impact of RNP granules on cellular function lies in separating functions between the two phases: condensate versus dilute. We have developed an approach to visualize condensates which allows for the separation between the two phases in live cells. The lifetime of a fluorophore is sensitive to its environmental conditions. Using fluorescence lifetime imagine microscopy (FLIM), we can harness this sensitivity to distinguish the condensed and dilute phases within the cell, allowing us to monitor protein-protein interactions which take place in RNP granules. mRNA processing bodies (P-bodies) are RNP granules conserved from yeast to humans, which hold high catalytic potential due to their enrichment of enzyme complexes involved in mRNA decay, mRNA silencing, and mRNA editing. Although mRNA decapping complex subunits are clearly enriched in P-bodies, it is unclear whether subunits can form functional complexes within P-bodies. Here, we combined our ability to separate P-bodies from dilute phase by fluorescence lifetime with FRET to evaluate whether the decapping complex

can form within P-bodies in live cells under different cellular contexts. We used fluorescently-tagged decapping enzyme, Dcp2, as our FLIM-FRET donor-fused protein, and Dcp2 coactivators, such as Dcp1A, as our FLIM-FRET acceptors to show that decapping complex formation within P-bodies is actively regulated during the cell cycle and when cells are challenged with stress, thus providing evidence to support the model that these organelles can serve as sites of mRNA storage and degradation.

M106

**Novel microscopy tools reveal dynamic sub-cellular distributions of core clock components in *Neurospora crassa*.**

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Circadian rhythms are endogenous daily oscillations driven by a molecular clock that helps organisms better coordinate with the environment. Organisms from fungi to animals share a similar phosphorylation-driven transcription/translation negative feedback loop as the core clock mechanism, an oscillator composed of positive and negative elements. Research on the filamentous fungus model organism, *Neurospora crassa*, has provided answers to many fundamental clock-related questions. In *Neurospora crassa*, the transcription factor White Collar Complex (WCC) serves as the positive element driving the transcription of *frequency* (*frq*). The intrinsically disordered protein Frequency (FRQ), with other regulators, forms the negative element complex that inhibits the function of WCC and stops *frq* transcription. Molecular components of the circadian clock have been described over decades of genetic and molecular biological studies. However, little is known about their dynamics and regulation at the subcellular level. *Neurospora crassa* grows as a syncytium analogous to muscle cells, thus the subcellular distribution of molecules must facilitate precise temporal control throughout the syncytium. Live-cell imaging has emerged as a valuable tool in circadian research. We implemented novel strategies and microscopy tools for *Neurospora*, including 4-color imaging and microfluidics compatible with multi-day growth, to facilitate live-cell imaging of low-abundance circadian proteins. Through multi-color live-cell imaging in single cells, we tracked the circadian dynamics of the subcellular localization of WCC and FRQ in high spatiotemporal resolution. We also observed *in vivo* highly dynamic liquid-liquid phase separation (LLPS)-like behaviors of FRQ using the super-resolution SoRa microscope. Furthermore, by employing FRAP, we have unraveled the circadian-regulated nuclear import of FRQ and its underlying mechanism. We also optimized photoconvertible fluorescent proteins to facilitate further exploration of the nucleocytoplasmic transport of clock proteins. Our work showcases the successful application of advanced microscopy techniques in a conventional fungal model organism to gain insights into the intricate subcellular dynamics of circadian proteins, paving the way for a deeper understanding of circadian rhythms.

M107

**Investigating the impact of G2019S LRRK2 on ultrafast endocytosis at hippocampal presynapses.**

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Neurons maintain seamless signaling by recycling presynaptic components through ultrafast endocytosis. Leucine rich repeat kinase 2 (LRRK2), a protein highly associated with Parkinson's disease, phosphorylates proteins involved in the mechanism of ultrafast endocytosis. A hyper-kinase mutant form of LRRK2, called 'G2019S-LRRK2', can reportedly change the localization and function of ultrafast

endocytic proteins in fly presynapses. It is unclear if this mislocalization of key proteins also occurs in mammalian presynapses, and if sequestering such proteins away from their typical roles negatively impacts the ultrafast endocytosis pathway. Additionally, it remains unknown if disrupting ultrafast endocytosis membrane trafficking can lead to vulnerability and early-stage synaptic dysfunction in diseases like Parkinson's. To explore these questions we use an advanced electron microscopy (EM) technique, 'zap-and-freeze' EM, to compare vesicular trafficking dynamics in cultured hippocampal neurons from Wild-type and G2019S-LRRK2 mice. Zap-and-freeze EM utilizes electric field stimulation and high-pressure freezing to visualize synaptic membrane trafficking dynamics with high temporal and spatial resolutions (milliseconds and nanometers). Using our technique, we have discovered that the G2019S-LRRK2 mutation stalls ultrafast endocytic pits formed at the presynaptic plasma membrane in hippocampal neurons—indicating a negative impact on the overall progression of the pathway. Further testing using super-resolution microscopy is being done to determine if this mechanism is being driven through mislocalization of key proteins (i.e. Dynamin1xA, Endophilin, and Synaptojanin). Furthermore, we are testing the necessity of LRRK2 in ultrafast endocytosis by comparing vesicular dynamics in Wild-type and LRRK2 constitutive knockout neurons. We hope our findings will add unique knowledge to the growing body work on LRRK2's role in synaptic membrane trafficking, and potentially offer insights into pre-disease pathways that could be impacted to leave synapses susceptible to pathogenesis later on.

M108

**Defining ciliary extracellular vesicle subtypes by single-EV molecular analysis.**

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Intercellular signaling via extracellular vesicles (EVs) requires mechanisms for selecting and targeting molecular cargo. Understanding the composition of individual EV subtypes is crucial for unraveling their bioactivity yet remains challenging. Here, we use proximity labeling, super-resolution microscopy, and genetic perturbations in *C. elegans* to define distinct EV subtypes. By focusing on ciliary EVs carrying conserved polycystin channels, we shed light on their signaling mechanisms. Polycystins, when disrupted in vertebrates, lead to cyst formation in internal organs (ADPKD - autosomal dominant polycystic kidney disease) and randomization of left-right body plan during embryogenesis, in the latter case with polycystins acting through EVs. In *C. elegans*, polycystin-carrying EVs function in animal-to-animal communication. However, the signaling mechanism and cargo of polycystin EVs are unknown. We identified and validated eight polycystin interactors, including TRAFs (tumor necrosis factor TNF receptor-associated factors), transmembrane lectins, and putative cation channels. Our findings demonstrate that polycystins are crucial for selecting and targeting cargo to ciliary EVs. We discovered that TRAF signaling adaptors rely on polycystins for loading to ciliary EVs, but not for entering the cilium. Disrupting polycystin-1 leads to the ciliary release of polycystin-2 EVs lacking TRAFs. These findings suggest that the polycystin pathway employs TRAFs for EV-based signaling, in line with clinical observations of aberrant TNFα signaling and altered urinary EV proteome in ADPKD. Furthermore, transmembrane lectins require polycystin-1 for their localization to cilia and EVs, potentially serving as ligands for receptors on targeted cells, of which are vulvae of mating partners in *C. elegans*. These data prompt investigation into the potential role of EV surface lectins in mate recognition. The regulated loading of cargo into EVs supports their role as signaling devices and warrants studies addressing mechanisms for cargo selection at the ciliary tip. Our proximity labeling approach holds promise for systematically defining numerous EV subtypes. This study is the first to demonstrate the profound

impact of a single genetic perturbation on the composition of a single EV subtype, highlighting the tremendous diagnostic potential of a single-EV analysis.

## Minisymp: Navigating Stem Cell Fate and Tissue-specific Function

M109

**Early Embryonic Cell Fate Decisions directed by Differential RNA Localisation and Translation.**

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How equipotent cells develop into complex tissues containing many diverse cell types is one of the most fundamental questions in biology. The organisation of a cell's interior, the cytoskeleton and organelles, is pivotal for every cell's functionality. However, unlike most differentiated cells, our knowledge about the contribution of the sub-cellular architecture to pluripotency remains scarce. Using cutting-edge live imaging technologies, we uncovered polarised Calmodulin regulated spectrin-associated protein 3 (CAMSAP3)-dependent non-centrosomal microtubules as central player for spatiotemporal RNA heterogeneities during early mammalian embryogenesis, determining differential translation capacities unevenly inherited by outer and inner daughter cells as they adopt different cell fates. Outer cells, marked by transcription factor Cdx2, undergoing differentiation into extraembryonic tissue, require an elevated translation efficiency shown by a higher mRNA, tRNA, endoplasmic reticulum and ribosome content, fortified by the live visualisation of translation events. Contrary, Sox2-positive inner cells, giving rise to the pluripotent inner cell mass, are characterised by a higher rRNA content compared to outer cells, and thus being less translationally active. To enable the spatio-temporal manipulation of cytoplasmic organelle and RNA localisation, we have successfully engineered an optogenetical-controllable CAMSAP3-Halo (= Opto-CAMSAP3-Halo) cassette. Our innovative light-inducible tool can overcome the current limitations of typical microtubule drugs to non-invasively and with spatial-temporal precision manipulate the organisation of the non-centrosomal microtubule network in real-time. By translocating the origin of microtubule growth in pluripotent stem cells, it can provide a direct link between the cell biology and the potency of stem cells *in vivo* and *in vitro*. Dissecting how intrinsic cellular regulation contributes to pluripotency, complementing the genetic and epigenetic regulation, may lead a revolutionary era of regenerative and reproductive medicine.

M110

**Non-cell autonomous competition eliminates karyotypic mosaicism in human embryonic stem cells.**

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Aneuploidy, an abnormal number of chromosomes, is the leading cause of pregnancy loss during human development. Yet, ~30% of mosaic human pre-implantation embryos that consist of a mixture of diploid and aneuploid cells develop normally and result in a healthy baby with a diploid karyotype. Thus,

aneuploid cells can be outcompeted by diploid cells during development, but how this occurs remains unknown. Here, using a combination of immunofluorescence microscopy and fluorescent in-situ hybridization, we investigate cell competition in a mosaic population of human embryonic stem cells (hESCs) possessing either 2 copies (disomic and diploid) or 3 copies (trisomic and aneuploid) of chromosome 17 (Chr 17). We initially find that trisomic Chr 17 hESCs are at a selective disadvantage and are outcompeted by disomic Chr 17 hESCs. However, upon prolonged propagation, a switch occurs and trisomic Chr 17 hESCs gain a selective advantage and outcompete disomic Chr 17 hESCs. Moreover, we demonstrate that cell fitness correlates with the relative protein levels of the transcription factors Myc and p53. This fitness correlation is non-cell autonomous, as it is dependent on cell-cell interactions between neighboring cells. Disomic Chr 17 hESCs with increased Myc or decreased p53 protein abundance, relative to their trisomic neighbors, possess a fitness advantage. Conversely, disomic Chr 17 hESCs with decreased Myc or increased p53 protein abundance, relative to their trisomic neighbors, have a fitness disadvantage. Thus, non-cell autonomous competition eliminates karyotypic mosaicism in hESCs. Importantly, our work provides a mechanism of how mosaic embryos can lead to a normal birth.

M111

**The impact of dysfunctional mitochondria from astrocytes in Rett Syndrome on the human brain.**

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Rett Syndrome (RTT) is a devastating neurodevelopmental disorder. Classical RTT diagnosis, most commonly due to mutations in Methyl CpG binding protein 2 (MECP2), is associated with severe mental disability and autism-like syndromes that manifests during early childhood. Studies on the function of MECP2 and the consequence of MECP2 deficiency have largely focused on neurons. The function of MECP2 in human glia, along with the comprehensive understanding of glial function in neurodevelopmental disorders, is much less understood. Using female and male human embryonic stem cell (hESC) lines to model MECP2 loss-of-function in RTT in the developing brain, we investigated the molecular underpinnings of astrocyte (AST) development and dysfunction, and the mechanisms by which AST contribute to neuronal activity. Here we show that hESC-derived RTT ASTs have altered reactivity and fewer mitochondria yet similar levels of reactive oxygen species compared to isogenic controls (CTR). We identified significantly diminished mitochondrial respiration, and that the molecular mechanism behind mitochondrial dysfunction were reduced key proteins around the tricarboxylic acid cycle and electron transport chain. We found an increased abundance of cytosolic amino acids in RTT ASTs under basal conditions that were readily depleted when energy demands were increased. We determined that isolated mitochondria from RTT ASTs taken up by cortical neurons increases abundance of reactive oxygen species and are sufficient to cause significant changes to neuronal activity, increasing local field potentials to a hyperexcitable state. To examine mitochondrial health in the developing brain, we derived cerebral organoids. Ultrastructural analysis indicated that mitochondria from RTT hESC-derived organoids were significantly smaller compared to mitochondria from CTR organoids, indicating decreased connectivity and function, and this phenotype was stronger in glia compared to neurons. Using a multiomics epigenetics approach, we found hallmarks of RTT developmental delay and glial specific gene expression changes that corroborate altered energy metabolism and mitochondrial dysfunction. Based on these results, we propose that release of dysfunctional mitochondria from RTT ASTs to neurons furthers pathophysiology of the syndrome.

M112

**Intracellular Cell Tension Regulates Stem Cell Conversion to the Mesoderm Lineage Downstream of the Apoptotic Pathway.**

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The heart is the first functional organ to form during morphogenesis and failure to specify cardiac progenitors can result in severe congenital heart defects, affecting ~1% of all US newborns. Activation of the WNT pathway in human induced pluripotent stem cells (hiPSCs) recapitulates the initial stages of cardiac lineage commitment, driving sequential expression of primitive streak, mesoderm, and cardiac mesoderm markers. In parallel, cells undergo an epithelial-to-mesenchymal transition (EMT), similar to gastrulation in vivo, during which drastic morphological changes occur, including tension-dependent apical constriction. While the relations between EMT and lineage specification have been widely studied, our understanding of the connection between cell shape and lineage identity remains poor. During development, changes in cell morphology can be the result cell division orientation, increase of cell number in a constrained space and active contractile forces, together driving morphogenesis. The acto-myosin cytoskeleton, a highly dynamic network of proteins that maintain cell morphology, is a crucial interface to translate physical signals into internal biochemical responses. While geometrical organization of “gastrulation-like” morphogenesis has been shown to affect WNT signaling and cell fate specification, it remains unclear how, mechanistically, cells communicate and integrate the state of their environment to coordinate developmental responses at the tissue level.

We hypothesize that mechanical forces, such as cell tension, regulate mesodermal identity in hiPSCs. Pharmaceutical inhibition of ROCK to reduce tension during differentiation, increases mesoderm gene expression. On the other hand, overexpression of a constitutively active ROCK2 to increase tension totally blocks cardiac mesoderm identity. These cells also maintain their epithelial features, suggesting a blockage of the EMT, a crucial step during lineage specification. Probing for a mechanism, we investigated the effect of the apoptosis pathway. We previously published a crucial role for apoptosis during stem cell commitment to the mesoderm lineage. Surprisingly, while cells treated with apoptosis inhibitor fail to differentiate, adding ROCK inhibitor overcomes this blockage. This epistasis experiment suggests that contractility acts dominantly, downstream of the apoptotic pathway.

Together, our data suggest that apoptosis and contractility work together in balancing mesodermal identity. We are currently investigating the molecular mechanisms linking these two pathways to the genetic reprogramming occurring during mesoderm differentiation.

M113

**Myosin 2A-driven planar cell division ensures lumen integrity in intestinal organoids.**

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Mouse intestinal organoids retain the crypt-villus topology of the intestine in the form of a three-dimensional, stem cell-driven tissue model that is amenable to structure: function analysis. Here we investigated the role that myosin 2A (M2A) plays in maintaining a single lumen in growing organoids. Airyscan images of organoids made from a GFP-M2A knockin mouse show that the myosin localizes along the lateral surface of organoids, consistent with its known role in supporting cadherin-based cell: cell adhesion, and within stress fibers at the base, consistent with its known role in supporting integrin-based cell: ECM adhesion. In dividing cells, M2A is seen not only in the cleavage furrow, but also in

evenly-spaced strings of cortical mini-filaments that span the long axis of the cell prior to contractile ring formation. shRNA-mediated knockdown (KD) of M2A in GFP-M2A KI organoids leads to the appearance of a multilobed phenotype that scales with the degree of KD (the dimmer the GFP-M2A signal, the more multilobed the organoid appears). Imaging with a membrane marker shows that each lobe possesses its own, separate lumen. Lattice Light Sheet imaging of stem cell-marked organoids treated with Blebbistatin (BB), which phenocopies M2A KD, shows that individual lobes arise from stem cells undergoing cell division. In the absence of M2A-dependent force, interkinetic nuclear migration in these cells is inhibited and their axis of cell division changes from planer to orthogonal. As a result, the daughter cells are no longer side-by-side in the plane of the epithelium, but rather stacked one on top of the other. This leads to epithelial stratification (the formation of multiple cell layers). Staining of the daughter cells arising from orthogonal divisions with ZO-1 to mark their apical domains shows that the nascent lumen of the forming lobe is created at the interface between the two stacked cells where their apical domains abut. Interestingly, restoration of M2A contractility by BB washout, if done before the multilobed phenotype progress too far, results in lobe merger to restore the single lumen. We conclude that M2A-dependent contractility serves to maintain a single lumen in growing organoids by maintaining a planar axis of cell division.

M114

**Planarians employ diverse and dynamic stem cell microenvironments to support whole-body regeneration.**

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Stem cells enable regeneration by self-renewing and differentiating as instructed by a local microenvironment called a niche. In most cases, the repair or replacement of tissues is fueled by tissue-specific or lineage-restricted stem cells that proliferate in response to local injury and apoptosis. However, in organisms that regenerate using abundant adult pluripotent stem cells, the stem cell niches that support tissue repair have not been identified or characterized. Since these adult pluripotent stem cells are often more widely distributed and plentiful than lineage-restricted stem cells of other organisms, defining their microenvironments may uncover alternative forms of stem cell regulation. Here we used unbiased spatial transcriptomics to define the cellular and molecular environments that support pluripotency in the highly regenerative freshwater planarian *Schmidtea mediterranea*. We determined that stem cells associate with a diverse collection of differentiated cell types, and these associations are dynamic during regeneration, indicating that regenerative planarians do not possess a single regenerative niche. We explored associations with two distinct cell types: secretory cells we term 'hecatonoblasts,' and intestinal cells, which have previously been identified as a potential niche tissue for stem cell function. We found that the two cell types had distinct spatial relationships to stem cells and used single cell sequencing and a new method for HCRv3-based enrichment and RNAseq to identify genes expressed in the two cell types required for stem cell maintenance or normal proliferation during regeneration. Together, our results indicate that the planarian stem cell pool is maintained by a dynamic collection of distinct microenvironments that cooperatively power whole-body regeneration.

## Minisymp: Physical and Engineering Approaches to Cell Biology

M115

**Synthetic control of dynamic pattern formation at the cortex.**

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The cell cortex undergoes dramatic remodeling to drive cell shape changes necessary for cell division, motility, and morphogenesis. Cortical dynamics are often regulated by Rho family GTPases, which cycle between an active, GTP-bound state, and an inactive, GDP-bound state. Cycling between Rho's active and inactive state is primarily regulated through Guanine nucleotide exchange factors, or GEFs, which promote activation of Rho via nucleotide exchange, and GTPase activating proteins, or GAPs, which promote Rho inactivation via GTP hydrolysis. This cycling of Rho generates patterns at the cortex such as patches, rings, or stripes, in addition to propagating, complementary waves of Rho GTPase activity and F-actin disassembly – referred to as cortical excitability. Cortical excitability is subject to both internal and external regulation, including internal signaling which prompts cell cycle progression and development. We have previously shown that cortical excitability can be induced in *Xenopus* oocytes (which are not normally excitable) via co-expression of two Rho regulators essential for cytokinesis: Ect2 (a GEF) and RGA-3/4 (a GAP). To further investigate this excitable circuit, and cortical excitability overall, I have used a synthetic biology approach. Specifically, I employed a synthetic GEF construct to provide Rho-based positive feedback by utilizing the Rho GEF domain of LARG fused with rGBD, a domain that binds specifically to active Rho. When expressed with RGA-3/4, a natural participant in Rho-based negative feedback, the synthetic positive feedback construct, *Rho Positive Feedback 1 (RhoP1)*, produces "semi-synthetic waves" of Rho activity and F-actin. The waves are elongated and propagate slowly, with a relative amplitude of 1.18 and period of 436 seconds, compared with non-synthetic waves produced via co-expression of the two natural excitability regulators, Ect2 and RGA-3/4 (relative amplitude: 0.82; period: 152 sec). Relative to Ect2, expression of *RhoP1* results in heightened cortical contractility. Similarly, a synthetic inhibitor, dubbed *Rho Negative Feedback 1 (RhoN1),* containing a Rho GAP domain and tropomyosin (to bind F-actin), supports semi-synthetic, pulsatile waves when co-expressed with Ect2. These waves show a relative amplitude of 0.17 and period of 320 seconds. Interestingly, co-expression *RhoP1* and *RhoN1* produce fully synthetic, semi-stable patterns of extremely high amplitude. Use of other synthetic inhibitors alongside Ect2 alters resulting wave period and morphology. Further study of this system and development of additional synthetic constructs designed to interact with Rho, F-actin, or their regulators will provide further information about excitable dynamics of this circuit.

M116

**Light-directed evolution of dynamically changing and computing proteins.**

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Directed evolution has been a powerful method for refining and creating new steady-state functionalities of proteins. However, existing methods are ill-suited for the evolution of dynamic, multi-state, or computational properties, which require ways to simultaneously screen all states of the proteins and their transitions. I will present a novel directed evolution paradigm, called `optovolution', which is inherently germane for this task. It is continuous, in vivo, based on self-selection, and was robust and efficient in our tests. We realized that the desired selection pressures can be applied to

proteins by inserting them between optogenetic control and a substantially modified cell cycle control system in budding yeast. The signaling cascade is engineered to have to oscillate on and off for cells to proliferate, which is additionally tunable by modulating the pacemaker, light. This allows directed evolution to act on different input-output relations, transient states, and dynamics of proteins at every cellular replication cycle. We applied optovolution to two switchable relay proteins: LOV domains constitute the most widely used core of optogenetic systems apart from ion channels. We evolved new variants, needed for diverse applications, which are stronger, less leaky, or have green- or UV-shifted light excitation spectra, which was thought to be impossible. Further, we created stronger doxycycline-senstive rtTA transcription factors. Optovolution is particularly well-suited for the emerging era of AI-designed proteins with sophisticated new functionalities.

M117

**Diffusion in the cytoplasm increases with cell size.**

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Cells overall exist within a wide range of size, yet cells within a cell type have a narrow size range, suggesting that size may be important for cell function. Previous studies have suggested that in abnormally large budding yeast and mammalian cells, the cytoplasm becomes more dilute and the proteome is remodeled (Neurohr et al. 2019, Lanz et al. 2021). Here, we used genetically-encoded 40-nm nanoparticles (GEMs) (Delarue et al. 2018) to measure cytoplasmic diffusion in fission yeast *S. pombe* cells of cell sizes larger and smaller than wildtype. Using mutants to alter cell size (e.g. *cdc25-22*, *cdc2-asM17*, and *wee1-50*), we found that GEMs diffusion increased in larger cells and decreased in smaller cells. To test if this change in diffusion was due to DNA-to-cytoplasm ratio, we used cytokinesis mutants (e.g. *sid2-as* and *cdc11-119*) to maintain wildtype DNA-to-cytoplasm ratio in large multinucleate cells. Indeed, larger cells with scaled DNA content have comparable GEMs diffusion rates as their normal-sized counterpart. To investigate the cause of increased GEMs diffusion, we assessed the concentration of ribosomes, a major crowding agent in the cytoplasm (Delarue et al. 2018). Our preliminary results suggest that large cells have a lower cytoplasmic ribosomal concentration than wildtype, consistent with their increased GEMs diffusion. Finally, we are currently investigating how cell size corresponds to changes in the proteome of *S. pombe*. In conclusion, we have shown that diffusion in the cytoplasm increases with cell size due to decreasing DNA-to-cytoplasm ratio, suggesting that size plays an important role in determining the biophysical properties of the cytoplasm.

1. Neurohr et al. Excessive Cell Growth Causes Cytoplasm Dilution and Contributes to Senescence. *Cell* (2019).
2. Lanz et al. Increasing cell size remodels the proteome and promotes senescence. *Molecular Cell* (2022).3. Delarue et al. mTORC1 Controls Phase Separation and the Biophysical Properties of the Cytoplasm by Tuning Crowding. Cell (2018).

M118

**Confinement as a critical factor in guiding rheotactic cell behavior.**

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This study examines how confinement influences cell migration direction under various flow conditions and uncovers the molecular mechanisms governing the distinct migratory responses observed in confined and unconfined microenvironments. Variations in pressure across different regions within the human body are responsible for fluid flow, including interstitial and blood flow. Previous studies have

reported divergent migratory responses of cells to flow, with some showing migration towards low-pressure regions and others showing the opposite. These discrepancies may be attributed to the use of discrete 3D hydrogels, which may evoke different migratory behavior due to variations in pore sizes/degree of confinement. Thus far, the impact of confinement on flow-induced cell migration remains elusive because most flow experiments are conducted on planar surfaces or in 3D hydrogels that do not permit tuning of pore size independent of other physical properties. To address this limitation, we studied cell migration in microchannel devices that enable precise control of fluid flow and confinement levels. Using this assay, we were able to assess how (patho)physiologically relevant fluid velocities ranging from ~40 to ~1000 μm/s influenced confined vs unconfined migration. In confined microchannels, where cells occupied almost the entire channel cross-sectional area, the fraction of cells moving against the flow increased monotonically with fluid velocity. In contrast, in unconfined microchannels, fluid flow triggered predominantly downstream migration. These observations held true for HT-1080 fibrosarcoma cells, MDA-MB-231 breast cancer cells, bone marrow-derived, and umbilical cord-derived mesenchymal stem cells and were independent of seeding density. Furthermore, by integrating chemical and genetic interventions with live-cell probes and optogenetic tools, we demonstrated that in confinement, flow-induced activation of mechanosensitive ion channels (MICs) triggered extracellular calcium influx, which in turn activated actomyosin contractility at the cell trailing edge, propelling migration against the flow. On the other hand, in unconfined microenvironments, the absence of this robust calcium influx and the failure to polarize myosin II prevented cells from moving upstream. Importantly, we identified the nuclear envelope protein LMNA as a key determinant of upstream migration. LMNA mediated its effects by inducing the expression of different MICs and thereby increasing the sensitivity of confined cells to flow. Collectively, our data reveal the key role of confinement in regulating cell responses to pressure-generated flow fields and demonstrate how LMNA and MICs facilitate upstream cell migration.

M119

**Optogenetic programming of large-scale collective cell migration.**

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Epithelial tissues are critical for maintaining organ compartmentalization and protective barrier functions, and were essential for the evolution of multicellular life. A key feature of these tissues is from their self-healing ability through collective cell migration. The coordinated mobilization of cells is critical to close gaps caused by trauma or diseases such as diabetes or vascular insufficiency, as well as enable complex developmental processes. Controlling this self-healing can help us to better treat injuries and engineer tissues. Among all the tools to control cellular behavior, optogenetics is one of the most powerful tools since it enables precise spatiotemporal control of specific signaling pathways. However, while optogenetic tools have been applied to subcellular contractility, they have not yet been developed to effectively program large, multicellular tissue dynamics. Here, we precisely programmed mm-cm scale collective cell migration in engineered retinal pigment epithelial (RPE) cells by optogenetically activating EGFR signaling using custom photo-masks. Both the center and edge of epithelial monolayers reacted to illumination patterns, but their responses were strikingly different. Stimulating a small zone (500 μm diameter) in the center of a large a tissue (5000 μm diameter) triggered rapid, powerful, convergent migration from outside the illuminated zone towards the interior, resulting in 50 μm high, 3D cell mounds. However, at the boundary of the tissue, local illumination caused the illuminated

portions of the tissue boundary to grow outwards significantly faster and more persistently than in the unilluminated regions. Using these two modes of light-induced, large scale migration, we could sculpt tissues into specific shapes. We are now investigating the signaling and cytoskeletal mechanisms underpinning this using inhibitors and mosaic co-cultures, and our preliminary data suggest that this process is independent of ERK signaling itself, and may relate to focal adhesion dynamics. In parallel, we are attempting to demonstrate accelerated scratch wound closure using dynamic patterns of light at the wound margin. Our study demonstrates how optogenetics can be harnessed for healing and tissue growth control and could offer an exciting new avenue for programming large-scale tissue mechanics.

M120
**Behavioral cartography: Mapping cellular behavior in the wild.**
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It is difficult to understand living systems without ecological context in which they live. Ecology shapes every aspect of a cell including cellular behavior, metabolism, growth and development. Here we present a new framework for mapping single cell behavior in an unbounded context of an aquatic environment (marine and fresh water). We share instrumentation and software tools that enable a unique class of behavioral assay capable of "free swimming" motility of a living cell in an unbounded landscape. Creating a virtual reality arena for a cell by modulating light, pressure, gravitational orientation, salinity and other chemical cues - we can collect unbiased behavioral datasets in the field. Here we share our large scale efforts over the last few years to build a global cell motility atlas - from the Arctic to Antarctica, in Pacific, and Atlantic Ocean and marine and fresh water species. The database represents largest collection of behavioral data collected over more than 10 expeditions with partners in US and Europe. We will further describe our framework to scale these technologies and platforms to broaden our understanding of cell behavior in the wild.

## Minisymp: Protein Compartmentalization and Sorting

M121
**Mechanisms Regulating Organellar Protein Localization.**
**S. Shao**, M. J. McKenna, M. Vasandani; Harvard Medical School, Boston, MA.

Accurately localizing proteins to different organelles is critical for maintaining cellular homeostasis and organism health. Achieving this requires high-fidelity protein targeting pathways as well as efficient quality control mechanisms. Our lab identified the ATP-dependent transporter ATP13A1 as a protein dislocase that removes mistargeted mitochondrial membrane proteins from the endoplasmic reticulum (ER). This discovery highlighted how mistakes in protein targeting occur more often than previously appreciated and how active correction mechanisms play essential roles in maintaining steady state subcellular protein distributions. with these concepts in mind, we pursued an unbiased crosslinking and mass spectrometry approach to identify additional factors that mediate organellar protein localization, with a particular focus on proteins that localize to mitochondria through uncharacterized molecular signals. These experiments revealed a putative shuttling factor required for the localization and stability of a class of proteins that regulate mitochondrial trafficking and morphology. Evidence from biochemical fractionations, cell-free reconstitutions, and interaction analyses guided by Alphafold-multimer models suggest that this shuttling factor associates with distinct mitochondrial protein complexes to control the activities of its substrates. Based on our findings, we discuss a working model for a molecular

mechanism that dynamically regulates mitochondrial distribution and morphology, potentially explaining why mutations in these factors disrupt mitochondrial homeostasis and are implicated in numerous cancers.

M122

**Using Photoswitching FRET to define the interaction boundaries between Rab1 and secretory cargo.**

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FRET is a powerful tool to simultaneously establish and localize interactions between fluorescently tagged proteins with high spatial resolution. Rainey K.H. and Patterson G. H. (Rainey, K.H., and Patterson, G.H. 2019. PNAS USA *116*, 864-873) introduced Photoswitching FRET (psFRET) using the Dronpa photoswitchable fluorescent protein. We present a straightforward method, including a detailed experimental protocol and a software tool allowing direct assimilation of psFRET to diverse experimental setups, yielding a full pixel-level FRET interaction channel. Image stacks, recording the decay of the Dronpa donor, serve as input to the software utility that includes several fundamental preprocessing options preceding the calculation of exponential decay coefficients for an entire field of view at the single pixel level. We applied psFRET to generate interaction maps analyzing and localizing diverse interactions among a cargo protein, the GTPase Rab1, GRASP65, and COPII. Cargo-Rab1 interactions were found to be restricted to the transit period from ER to Golgi. This interaction was severed upon the arrival of both interactors to the Golgi apparatus. The psFRET analysis of cargo-COPII showed that the interaction was exclusively restricted to the COPII collar at the ER-ER exit site boundary. Our psFRET method is applicable to living intact cells as the photoswitchable Dronpa molecule can be reused multiple times on the same sample. Thus, for the first time to our knowledge, we can directly obtain time-resolved psFRET dynamic information during an intracellular process such as ER to Golgi transport.

M123

**Advanced microscopy time-resolves the higher-order structure of the exocyst and it reveals an unexpected function for Sec18.**

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This study aims to resolve the higher-order mechanism that regulates the concerted action of multiple exocyst complexes in constitutive exocytosis (hereafter exocytosis). Exocytosis is a vesicle trafficking pathway universal to all eukaryotes that delivers molecules to the cell surface and the extracellular space. In just a few seconds, exocytosis progresses uninterruptedly through a cycle of vesicle transport, tethering and fusion. Each exocytic event is controlled by 10 to 15 copies of the exocyst complex, which cooperate to tether the secretory vesicle to the plasma membrane. Thereafter, the exocytic SNARE complex drives vesicle fusion. Ultimately, Sec18/Sec17 dismantle the SNARE complex to enter in a new cycle of exocytosis. Although the study of the structure and mechanism of individual proteins has progressed notably, the investigation of the higher-order mechanism that coordinates multiple exocysts has been hampered by its intrinsic complexity and dynamism. To bridge this gap, we integrated complementary microscopy techniques to image exocytosis at the plasma membrane of *Saccharomyces*

*cerevisiae*. We used particle-tracking to determine the temporal map for the main components of the exocytic machinery with ms temporal resolution. Correlative cryo-light microscopy and cryo-electron tomography allowed us to image the ultrastructure underlying this pathway. We resolved how multiple exocyst organize in time and space and the dynamics of the higher-order structures they form during the tethering and the fusion steps. Correlation of diffraction-limited fluorescent temporal markers and single-molecule localization microscopy demonstrated that, during the tethering, multiple exocysts cluster together in patches that grow in diameter for 4s. Concurrent to the departure of Sec2, the exocyst higher-order structure switches to a stable ring-shaped architecture that requires the presence of SNAREs to be formed. We interrogated molecular activities using genetics and discovered an unexpected role for Sec18 (NSF homolog) in the disassembly of ring-shaped exocyst-SNAREs supra-structures ~2s before it culminates the SNARE complex dismantling. We propose a quantitative mechanistic model of how multiple exocysts coordinate their action during vesicle tethering and fusion, including their interplay with SNAREs and Sec18/Sec17.

M124

**Filamentation of Lipoprotein Lipase in Secretory Vesicles.**

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Lipoprotein lipase (LPL) is the enzyme that hydrolyzes triglycerides from triglyceride-rich lipoproteins (TRLs) in the capillaries. LPL is synthesized in the adipose, heart, and skeletal muscle tissue and then secreted into the interstitial space where it binds to endothelial protein glycosylphophatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1) for subsequent transport into the capillary. Recent structural work has uncovered an inactive and concentration dependent oligomer of LPL which forms a helical filament. Super-resolution microscopy shows that LPL forms filament-like structures inside of adipocyte storage vesicles, indicating that the quaternary structure of LPL in these vesicles could be the filamentous oligomer. Syndecan-1 (SDC1) a heparan sulfate proteoglycan (HSPG) decorates the LPL secretory vesicles and has been shown to facilitate LPL secretion. Overexpression of LPL and SDC1 in HEK293 cells recapitulates the filament-like structures of LPL observed in adipocytes. Utilizing this system, we isolated LPL containing vesicles from cells using ultracentrifugation and sorbitol gradients. We used a cytoplasmic facing tag on SDC1 to label putative LPL containing vesicles with immunogold for data collection. We then characterized the gold labeled vesicles using cryogenic electron tomography (cryoET). Reconstructed tomograms reveal the presence of filaments adjacent to the membrane inside of SDC1 vesicles. The filament is ~12 nm in diameter and corresponds to a newly identified LPL filament that we have imaged with cryogenic electron microscopy (cryoEM). In conclusion, these studies reveal that LPL adopts the inactive oligomer conformation inside of secretory vesicles. We propose that the local concentration of LPL in vesicles triggers filamentation and provides a regulatory mechanism, which renders LPL inactive in the cell where LPL activity may be detrimental but restores LPL activity following the rapid dilution of the LPL filament during secretion.

M125

**A unifying mechanism underlying the trafficking of proteins to the primary cilium membrane.**

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The primary cilium membrane has a distinct protein and lipid composition despite being contiguous with the plasma membrane. To understand the role of cilium-generated signaling in health and disease, it is necessary to decipher the mechanisms that drive such unique compartmentalization. We discovered that the tubby family protein, TULP3, transports integral membrane and membrane-associated proteins into cilia through binding with the intraflagellar transport complex-A (IFT-A) and phosphoinositides. We also showed that these cargoes have short motifs that are necessary and sufficient for TULP3-mediated trafficking. However, the mechanism by which such diverse cargos are trafficked to cilia by TULP3 is not well understood. Here we used structural, cell biological and genetic methods to solve this puzzle. First, we solved the cryogenic electron microscopic structure of the TULP3-IFT-A complex at atomic resolution, and identified multiple residues in the N-terminus of TULP3 that interacted with IFT-A. We used mutational studies to demonstrate that many of these TULP3 residues were critical in regulating the ciliary trafficking of both transmembrane cargo, GPR161, and membrane associated cargo, ARL13B. Next, we sought to understand the molecular details of cargo recognition characteristics of TULP3. Using proximity biotinylation-mass spectrometry screens and mutational analysis, we identified critical residues in the β-barrel of TULP3's tubby domain that were crucial in promoting ciliary trafficking of both lipidated and transmembrane cargoes. While these residues were not involved in binding between TULP3 and IFT-A or phoshoinositides, they were critical in mediating interactions with cargos. All of these residues were located on one side of the β-barrel and away from the phosphoinositide binding site, thereby establishing the orientation of the cargo binding surface of TULP3 with respect to membrane and IFT-A. Further, we found that some of these residues were also mutated in human subjects with renal and liver cystogenesis. Overall, these findings indicated an expanded role of a shared tubby domain surface in TULP3 that captured short sequences of diverse cargoes to directly mediate their transport into cilia in concert with IFT-A. Finally, targeting the TULP3-cargo interactions could provide therapeutics in ciliary trafficking regulated diseases such as polycystic kidney disease and peripheral obesity.

M126

**Transporting the Gli transcription factors to the cilium tip compartment for Hedgehog signaling: A tale of two motor systems.**

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The Hedgehog (Hh) pathway in vertebrates requires the primary cilium. A key step in pathway activation is the accumulation of the major pathway effectors, the Gli transcription factors, at the distal cilium tip. However, the movement of Gli within the cilium has not been directly observed, and the underlying trafficking mechanisms remain poorly understood. We developed a TIRF-microscopy-based live cell imaging assay to visualize Gli2 molecules in the cilium. Multi-hour imaging revealed that Gli2 tip accumulation occurs early in the pathway, with the maximum level of Gli2 at the tip achieved within ~90 minutes of pathway activation. By imaging at higher temporal resolution, we observed the directional movement of Gli2 molecules towards the distal cilium tip specifically during this initial time window. Through co-imaging Gli2 and the IFT88 subunit of the Intra Flagellar Transport (IFT) machinery, we conclusively showed that Gli2 is a bonafide IFT cargo. Next, we examined the contribution of Kif7, a non-

motile kinesin that is proposed to organize a ciliary tip compartment for Hh signaling. We found that in the absence of Kif7, Gli2 is not efficiently loaded on IFT and is not exclusively concentrated at the cilium tip. This finding underscores the synergy between two cilium-specific cytoskeleton systems in enabling Hh-responsive trafficking of Gli2 to the cilium tip. Significantly, our findings address a long-standing conundrum and suggest that the function of IFT in vertebrate Hh signaling extends beyond maintaining cilium architecture. Instead, IFT is an integral component of the Hh pathway, with a direct role in Gli transport.

## Minisymp: Signaling Networks in Cell Migration

M127

**Secretion of exosome-associated DNA (SEAD) regulates neutrophil chemotaxis.**

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Neutrophils are the first line of defense in response to injury or infection. Once neutrophils reach affected sites, they contain the area by phagocytosing pathogens and releasing powerful proteases from cytoplasmic granules. In addition, neutrophils release their genomic DNA, studded with toxic enzymes, that go on to ensnare and kill microbes in neutrophil extracellular traps (NETs). Excessive NET release aggravates inflammation, delays resolution, and subsequently leads to host tissue injury during various autoimmune diseases, and infections. Neutrophils migrating towards shallow chemoattractant gradients emanating from injured/infected tissues amplify their recruitment range by releasing the secondary chemoattractant leukotriene $B_4$ ($LTB_4$). $LTB_4$ is synthesized from arachidonic acid through the action of the cytosolic enzyme 5-lipoxygenase (5-LO), the endoplasmic reticulum/nuclear envelope-resident protein 5-lipoxygenase activating protein (FLAP), and leukotriene $A_4$ hydrolase. All these enzymes and $LTB_4$ are packaged in and released from extracellular vesicles called exosomes. We recently showed that the biogenesis of $LTB_4$-containing exosomes originates at the nuclear envelope (NE) of activated neutrophils. We found that the neutral Sphingomyelinase 1 (nSMase1)-dependent generation of ceramide-rich lipid-ordered membrane microdomains is required for FLAP and 5-LO clustering at the NE and subsequent NE-budding. with nano-scale microscopic resolution achieved by 4x isotopic expansion of samples, we found 5-LO-positive intraluminal vesicles within NE-resident Lamin B Receptor (LBR)-positive limiting membranes, generating NE-derived multivesicular bodies (NE-MVBs). Interestingly, we discovered that both NE-derived MVBs and exosomes are distinct from conventional plasma membrane-derived CD63-positive MVB/exosomes. We also observed the presence of DNA in the lumen of the NE-MVBs. Using SYTOXgreen, a membrane-impermeable DNA-binding dye, we visualized in real time the secretion of DNA from the back of chemotaxing neutrophils - a process that was dependent on nSMase1 activity and on the presence of LBR, which is known to bind DNA. We further determined that decondensed chromatin is present in the lumen of NE-MVBs and found that increasing chromatin decondensation, by inhibiting histone deacetylases (HDAC), increases NET secretion. Finally, we determined that DNase-induced dissolution of secreted exosome-associated DNA (SEAD) leads to erratic neutrophil migration with a loss of directionality. Taken together, our study provides novel mechanistic insights into an inside-out pathway of DNA and exosome secretion originating from the NE of activated neutrophils, involved in the physiological regulation of neutrophil chemotaxis.

M128

**Mechanocontrol of RhoA dynamics by Caveolae is required for directional migration.**

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Caveolae are PM invaginations made of caveolin-1 (Cav1), cavins, cholesterol, and sphingolipids. Caveolae are mechanosensors that serve as a membrane reservoir by flattening to accommodate abrupt increases of membrane tension. Caveolae flattening also triggers signaling events establishing these organelles as mechanotransducers. While membrane tension appears as a master regulator of cell motility, it is not known whether membrane tension buffering by caveolae and the consecutive impact on signaling are instrumental during cell migration. We observe that cells migrating in 2D & 3D micro-environment display a heterogeneous distribution of caveolae, as they accumulate in the rear creating a 'front-rear" polarity. We observed that increased membrane tension resulted in dissipation of the rear Cav-1 pool and instantaneously stopped migration. Cav1 front-rear polarity and migration were re-established when tension homeostasis was restored. Furthermore, we found that optimal cortical tension is crucial to maintain Cav1 front-rear polarity and directional migration. Re-distribution of the Cav1 rear pool to a homogenous localization was coupled with a halt in migration. Restoration of Cav1 front-rear polarity occurred before the cell re-started to migrate with new cell front and rear, implying that Cav1 polarity is a pre-requisite for effective directional migration. In randomly migrating cells, at the site of cell contraction, caveolae accumulated first, followed by activation of RhoA. Further, local activation of RhoA GTPase in front of cell using optogenetics tool exhibits recruitment of caveolae at the site of RhoA activation, which precedes cell contraction. Further, cells lacking caveolar components resulted in delayed contraction at the local RhoA activation site. In summary, we establish that caveolae mechanosensing property and its interaction with active RhoA is crucial in regulating the directional migration of cells.

M129

**The activation of INF2 by Piezo1/$Ca^{2+}$induces de-adhesion and amoeboid migration in confined environments.**

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Metastatic cells showcase phenotypic plasticity to adapt to different physiological environments. Mesenchymal to amoeboid transition ensues when cells encounter confined microenvironments. Confinement sensing by metastatic cells has been attributed to calcium signaling pathways. However, the understanding of mechanistic essentials of amoeboid migration in confinement is still limited. In this study, we demonstrate the interplay between Piezo1, a mechanosensitive calcium ion channel, and inverted formin 2 (INF2) in regulating the phenotypic transition of migrating melanoma cells under confinement. A confined environment was simulated *in vitro* using 2-D vertical confinement and 3-D microfluidic channels. Our study indicates a surge in the level of intracellular [$Ca^{2+}$] upon 2D confinement, which is in correlation to confinement height. However, cells lose this correlation upon depletion of Piezo1 activity. Moreover, chelation of calcium or depletion of Piezo1 restricts transition from mesenchymal to fast amoeboid migration. In straight VCAM-1 coated microchannels, cells predominantly display hybrid phenotype, featuring attributes of both mesenchymal and amoeboid migration. Whereas in undulated VCAM-1 coated microchannels, cells are amoeboid phenotypically. In both types of channels however, cells predominantly adopt mesenchymal phenotype upon calcium

chelation or Piezo1 depletion. Downstream of Piezo1/ $Ca^{2+}$, we found a significant role of INF2 in promoting amoeboid migration in adhesive confined environment, as in its absence cells tend to show mesenchymal phenotype. In line with this finding, we demonstrate that Piezo1 activator could initiate actin remodeling and focal adhesion disintegration only in presence of INF2, thereby promoting the aforementioned phenotypic transition. Apart from actin reorganization, myosin contractility also plays a major role in amoeboid migration. Consistent with existing literature, we found that ROCK2 contributes to the same in a different pathway. Overall, our study demonstrates that melanoma cells under confinement sense plasma membrane tension through Piezo1, which causes calcium influx in the cells. Calcium then mediates actin reorganization and cell de-adhesion with the help of INF2, consequently favoring amoeboid migration.

M130

**Investigating how FGF signaling guides cell migration *in vivo*.**

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Understanding how extracellular signals are translated by intracellular effectors to accomplish diverse functions is a fundamental goal in biology. We are using *C. elegans* to investigate signal transduction mechanisms that regulate cell migration *in vivo*. During larval development, muscle progenitors called sex myoblasts (SMs) migrate from near the tail of the worm to the center. This process requires fibroblast growth factor (FGF) signaling in the migrating SMs, and the FGF8 homolog EGL-17 (hereafter FGF) has been proposed to act as a diffusible chemoattractant that guides migrating SMs. However, FGF diffusion has never been demonstrated at native protein levels in any animal. To investigate FGF dispersal, we used cell type-specific rescue and misexpression to demonstrate that FGF is capable of diffusing over long distances and acts as an instructive cue to attract migrating SMs. Live imaging of endogenously tagged FGF combined with membrane tethering and extracellular trapping approaches revealed FGF is diffusible *in vivo* and that extracellular FGF dispersal is required for SM migration. Next, we sought to characterize the intracellular signal transduction mechanisms by which FGF guides migrating SMs. We used cell type-specific, auxin-inducible degradation to deplete individual proteins in the Ras-MEK-ERK signaling cascade, finding that homologs of GRB2, SOS, and Ras were required whereas homologs of RAF and ERK were not. Similar experiments also demonstrated that homologs of the PI3K pathway components PTEN, PI3K, and AKT are not required for SM migration individually, or in combination with ERK. We then sought to determine how SOS and Ras homologs regulate SM migration. We found that mNG::SOS-1 localized to the leading and ventral edges of migrating SMs, and redistributing a SOS catalytic domain uniformly around the cell arrested SM migration. Although activated Ras/LET-60(G13E) was capable of cell-autonomously rescuing SM migration after FGF loss of function, Ras overactivation in SMs did not recapitulate FGF overexpression or $SOS^{cat}$ redistribution. Combined, these results suggest that SOS-1 plays a key instructive function in SM migration while Ras is required as a permissive signal independent from common downstream effectors. This work provides new insights into how FGF ligands disperse and how FGF signals are interpreted to direct cell migration.

M131

**Change in RhoGAP and RhoGEF availability drives transitions in cortical patterning and excitability in *Drosophila*.**

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Precise control of force generation within cells by cortical actomyosin is critical to animal development. Actomyosin contractility is therefore subject to multiple layers of regulation, notably through regulation of the RhoA GTPase pathway. Feedback loops give rise to various spatiotemporal patterns in different cell types, including oscillations and traveling waves. We discovered a transition between isotropic contractility and traveling waves of actomyosin in nurse cells in late-stage *Drosophila* egg chambers. Here, we show that these actomyosin waves are regulated by the guanine nucleotide exchange factor (GEF) Pbl/Ect2, in addition to the GTPase-activating protein (GAP) RhoGAP15B previously shown to play a role. Both proteins exit the nucleus and become cortically enriched in individual nurse cells in an order mirroring but slightly preceding that of wave onset, suggesting increasing cortical availability of GAP and GEF induces actomyosin waves. To test whether increased GAP and GEF availability can induce waves in cells in a different context than the nurse cells, we overexpressed RhoGEF2 and RhoGAP71E/C-GAP in the *Drosophila* early embryo, in which cells are much smaller and apicobasally polarized. In this system, co-overexpression converts apical, normally pulsatile actomyosin into traveling waves during gastrulation. Additionally, embryos co-overexpressing both regulators in which cellularization fails display a large, continuous cortex in which actomyosin organizes into trains of wavefronts, which allowed us to determine that RhoA leads myosin by 3-4 seconds while myosin leads F-actin by ~1 second. Finally, we show that myosin pulse size, but not temporal behavior, during gastrulation scales with the net RhoA activity level (i.e. The GEF:GAP ratio). We are continuing to investigate whether F-actin plays a role in negative feedback onto RhoA in either system, as well as possible mechanisms that spatially confine pulses and prevent wave behavior during normal gastrulation. Overall, our results in both systems suggest transitions between cortical actomyosin patterns are controlled by the availability of GEF and GAP rather than by overall RhoA activity levels.

M132

**A dynamic partitioning mechanism for polarizing membrane protein distribution.**

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The plasma membrane is one of the most prominent hubs of signal transduction activities that directs a plethora of spatiotemporally dynamic cell physiological processes such as cell migration, polarity, and cortical pattern formation. Yet, how different classes of membrane proteins dynamically compartmentalizes into distinct domains of the membrane, remained unclear. Using multimodal live-cell imaging to study *Dictyostelium* as well as RAW 264.7 macrophages, here we first demonstrated that multiple endogenous and synthetic lipid-anchored proteins that were previously reported to be uniform

over the membrane, were surprisingly depleted from the membrane domains where Ras/PI3K/Akt/F-actin signaling network was activated, either spontaneously or under chemotactic stimulation. Our experiments ruled out the contribution of cytoskeletal supramolecular structures and targeted vesicular trafficking in organizing these dynamic compartmentalization events. Even though the dynamic asymmetric patterns of these proteins, during protrusion formation as well as ventral waves propagation, closely resembled to that of conventional peripheral back proteins such as PTEN, these lipid-anchored proteins did not translocate from membrane to cytosol upon global receptor activation. Our selective photoconversion-based protein tracking during wave propagation suggested that these asymmetric patterns may actually originate from the intrinsic ability of these membrane proteins to selectively partition into specific regions of the plasma membrane. The *in situ* single-molecule measurements further showed that these lipidated protein molecules have significantly different diffusion profiles in dissimilar domains of the membrane. When we utilized the single-molecule imaging data to parameterize an excitable network-based stochastic reaction-diffusion system that attributed the altered diffusion rates to altered binding affinity towards different domains of the membrane, computational simulations demonstrated that the spatially heterogeneous mobility-based "dynamic partitioning" is sufficient to generate the asymmetric propagating wave patterns on the membrane with familiar length and time scale. Furthermore, using novel optogenetic actuators that alters membrane domain specific binding affinity, we were able to partition normally uniform integral and lipid-anchored membrane proteins into specific compartments of the plasma membrane, in *Dictyostelium* as well as HL-60 neutrophils. Together, our results establish "dynamic partitioning" as a novel mechanism that can drive spatially large-scale and temporally dynamic polarization of a wide array of lipid-anchored and integral membrane proteins.

## Minisymp: Cell Division

M133

**Spatially distinct regulatory inputs modulate mitotic-phase GAP activity to locally limit RhoA activity for successful cytokinesis.**

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During cytokinesis active RhoA induces the formation of a contractile actin-myosin ring at the cell equator. Ring constriction forms the cleavage furrow and equally partitions the content of the mother cell between the two daughter cells. Opposing stimulatory and inhibitory signaling cascades spatially restrict RhoA activity to a narrow equatorial zone to promote F-actin polymerization and ring formation in the right location. While the mechanisms of the stimulatory signal are well characterized, how the inhibitory signals restrict RhoA activation to a spatially defined equatorial zone and prevent RhoA activity at the cell poles is unclear. We discover two regulatory inputs that modulate the activation and cortical targeting of the Mitotic Phase GTPase activating protein (MP-GAP, ARHGAP11a) for RhoA to restrict RhoA activity not only at the cell poles but also at the cell equator. At the cell poles, MP-GAP is a phosphorylation target of spindle-pole enriched Aurora A kinase. Interaction studies suggest that MP-GAP adopts an auto-inhibitory conformation. The three identified Aurora A target residues are located in a region of MP-GAP that interacts with its catalytic GAP domain suggesting the Aurora A phosphorylation opens MP-GAP and relieves its auto-inhibition. At the cell equator, active RhoA induced F-actin polymerization promotes MP-GAP targeting to the cell periphery. Equatorial enriched MP-GAP

opposes RhoA GEF activity and thereby accelerates RhoA flux through the GTPase cycle for the formation of a narrow active RhoA zone. Thus, MP-GAP activity and cortical targeting are modulated by two spatially distinct regulatory inputs: Aurora A phosphorylation relieves MP-GAP auto-inhibition to prevent RhoA activity at the poles, while F-actin promotes MP-GAP targeting to prevent the spreading of active RhoA away from the site of activation at the cell equator. By determining the mechanism of spatially confining RhoA activity at the equator and the cell poles during cytokinesis, our work has broad implications to how defects in cytokinesis impact animal development and disease.

M134

**Linker of Nucleoskeleton and Cytoskeleton (LINC) complex mediated signal is required for correct centrosome positioning in prophase, and maintenance of mitotic fidelity.**

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Mitosis is a highly dynamic process that requires a combination of intrinsic and extrinsic factors. Errors during its course can lead to chromosomal instability (CIN), an important mediator of cancer progression. Consequently, characterizing how the internal and external factors crosstalk to ensure faithful chromosome segregation can be a decisive step in better understanding carcinogenesis. Centrosomes play an important role in mitosis as they are one of the forces behind mitotic spindle formation, an essential step for correct chromosome segregation. Early in mitosis, during prophase, centrosomes must separate along the nuclear envelope (NE) to form a bipolar spindle, in a process dependent on kinesin-5 and dynein. The degree of centrosome separation and sub-cellular positioning have been shown to influence both mitotic fidelity and timing, respectively. In this work, using a combination of cell micropatterning, live-cell imaging and quantitative 3D cellular reconstruction, we dissect how the prophase nucleus contributes to centrosome positioning during the initial stages of mitosis. We show that a nuclear signal, arising from the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex, is essential for correctly positioning centrosomes at the short nuclear axis at the moment of nuclear envelope permeabilization (NEP), as well as maintaining mitotic fidelity in normal human diploid cells. Disruption of this complex leads to decreased dynein recruitment to the NE, causing abnormal centrosome positioning and increased mitotic errors. Furthermore, we show this process is de-regulated in cancer cells. This work underlines the importance of robust nuclear-cytoskeletal coupling during mitotic entry for correct spindle assembly and accurate chromosome segregation.

**Keywords:** Centrosome, prophase, nuclear envelope, LINC complex, dynein, mitotic fidelity

M135

**Kinetochore-independent mechanisms contribute to chromosome segregation in human cells.**

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Anaphase transport of chromatids to the spindle poles is crucial for faithful segregation of the genetic material during mitosis. Various forces contribute to this transport in different model systems. Shortening of the microtubule bundles (K-fibers) connecting kinetochores with the poles drive anaphase in most cells. Motor-mediated interactions with spindle microtubules and pushing exerted by the central spindle behind the moving chromosomes can also contribute. Whether these diverse mechanisms act concurrently within a single cell remains unknown as a comprehensive description of chromosome behavior and microtubule interactions with the kinetochores is lacking. We employ correlative

light/electron microscopy to reveal the state of microtubules at the kinetochores whose behavior was followed in vivo. Kinetochores lacking the minus-end motor dynein (RPE1 $Rod^{\Delta/\Delta}$) move slower than in the WT RPE1 (0.6 vs. 1.3 µm/min) and their K-fibers contain less microtubules (4.4±2.0 vs. 7.6±2.2 in the WT). Some $Rod^{\Delta/\Delta}$ kinetochores completely lack attached microtubules, yet these chromosomes move poleward like the neighboring chromosomes. To verify this unexpected finding, we laser-ablate individual kinetochores during early anaphase, creating 'acentric' chromatids. We find that many (50-70%) but not all acentric chromatids continue to move poleward in the $Rod^{\Delta/\Delta}$ and WT RPE1 suggesting that only a subset of chromosomes require kinetochores during anaphase in human cells. Further, ~60% of the acentric chromatids that stopped by the kinetochore ablation in RPE1, resume their poleward movement during telophase and ultimately join the chromosomes that reform the nucleus. This late resumption of poleward movement implies that, as previously shown for plants, human cells possess a mechanism for poleward transport of chromatids during late stages of cell division. Poleward movement of the acentric chromatids is not affected by the disruption of central spindle in PRC1-depleted cells. Unexpectedly, disruption of the actin cytoskeleton network significantly decreases the number of acentric chromatids moving poleward during anaphase. Together, our findings demonstrate that different chromosomes within the same cell utilize different mechanisms of poleward transport. Some of these mechanisms are independent of kinetochores and involve actin filaments rather than microtubules.

M136

**Centrosome age breaks spindle symmetry even in "symmetrically" dividing cells.**

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Centrosomes regulate the assembly of the mitotic spindle and its symmetry during mitosis. The two centrosomes differ in age by the presence of subdistal and distal appendages decorating the grandmother centriole of the older centrosome, while the mother centriole at the younger centrosome only contains distal appendages. Centrosome age can affect spindle symmetry in asymmetric stem cell divisions; whether it also affects spindle length in "symmetrically" dividing tissue culture cells is not known. Here we show that centrosome age also breaks symmetry in tissue culture cells, as the half-spindle associated to the old centrosome is on average slightly longer. This asymmetry depends on centrosome age and translates into a daughter cell size asymmetry. Spindle asymmetry correlates at the single cell level with an asymmetric abundance of a subset of spindle pole proteins involved in microtubule nucleation, such as pericentrin, Cdk5Rap2, γ-tubulin and TPX2 that depends on the presence of subdistal appendages. As the depletion of pericentrin, Cdk5Rap2 or TPX2 restores spindle symmetry, we postulate that centrosome age controls spindle (a)symmetry via the abundance of pericentriolar proteins required for microtubule nucleation.

M137

**Centromere stretching in oocytes is a reproductive isolating barrier in mice.**

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Reproductive isolation occurs when two populations accumulate genetic incompatibilities that prevent their interbreeding, resulting in the production of sterile or inviable hybrids. Molecular mechanisms of hybrid incompatibility are largely unknown especially for hybrid female sterility. Here we provide the first cell biological mechanism underlying fertility issues in hybrid females, using hybrid mice between

*Mus musculus domesticus* (*domesticus*) and *Mus spretus* (*spretus*). *domesticus* x *spretus* hybrid females have reduced fertility due to chromosome mis-segregation in meiosis I, producing aneuploid eggs. This mis-segregation was caused by chromosome condensation defects, especially impacting *domesticus* centromeres. Decondensed *domesticus* centromeres were stretched upon microtubule tension and more prone to mis-attachments. Hybrid oocytes had reduced condensin II levels on the chromosome compared to *domesticus* oocytes. The expanded major satellite DNA at *domesticus* centromeres further reduced condensin II levels via Topoisomerase IIa (TOP2a), explaining why condensation defects were most prominent at *domesticus* centromeres. Interestingly, pure *spretus* oocytes showed low condensin II levels on the chromosome like hybrid oocytes. This similarity between hybrid and *spretus* oocytes implies that hybrids inherited this reduced condensin II trait from *spretus*, leading to stretching *domesticus* centromeres in hybrid oocytes. Importantly, even though *spretus* oocytes have lower condensin II levels, their centromeres were not stretched, probably because *spretus* centromeres have few major satellites, which is the chromosome region prone to stretching. In contrast, *domesticus* oocytes enrich higher levels of condensin II to prevent major satellite stretching. As a result, hybrid oocytes inherit the reduced condensin II trait from *spretus* and major satellites from *domesticus*, and we propose that this combination is what causes major satellites to stretch, leading to mis-segregation and reduced fertility. Further analysis on condensin II in other mouse species implies a co-evolution of major satellite copy number and condensin II levels on the chromosome, supporting our model. Altogether, our work reveled that species divergence in centromeres and condensin regulations can establish a reproductive isolating barrier in mice (El Yakoubi W. and Akera T. *bioRxiv*. 2022).

M138

**Bi-directional modulation of centromere binding via evolutionary innovation in the CENP-T histone fold domain.**

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Centromeres direct chromosome segregation by building kinetochores to mediate attachments to spindle microtubules. Paradoxically, although this essential function is conserved, centromeric proteins evolve rapidly. Computational analyses show signatures of positive selection across taxa, suggesting recurrent evolutionary innovation. Here we show that innovation in the histone fold domain of CENP-T, a key interface between centromeric chromatin and spindle microtubules, modulates centromere binding. Substitutions found in residues under positive selection in rodent species can either increase or decrease binding, indicating adaptation in either direction. Moreover, these residues modulate CENP-T interactions with chromatin distinct from CENP-A regions within centromeres. Overall, our work defines the functional impacts of evolutionary innovation on centromere binding and suggests adaptation of the centromere-microtubule interface. We propose that recurrent changes in the dynamics of kinetochore-microtubule interactions, for example due to selfish centromeric DNA sequences that bias their segregation in female meiosis, impose selective pressure driving CENP-T innovation to optimize those dynamics.

## Minisymp: Exploring Morphogenetic Diversity

M139

**PIK3CA-induces biophysical changes in the mammary epithelium that disrupt tissue architecture by altering homeostatic programs of self-organization.**

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Phenotypically normal tissue contains abundant cells expressing oncogenic driver mutations. How mutant cells ultimately escape the tumor-suppressive influence of an ordered epithelial architecture remains a mystery. We hypothesized that a breakdown in the homeostatic programs of self-organization is a necessary step, allowing the accumulation of entropically favored and disordered tissue microenvironments that facilitate invasion. In the mammary epithelium, this requires that transformed luminal cells (LEP) - which are normally prevented from invading by an enveloping layer of myoepithelial cells (MEP) - must come in contact with and degrade the basement membrane prior to invasion. Using a primary human organoid model, we found that of 15+ breast cancer driver mutations, only the activation of PIK3CA in LEP increased their ability to access the basement membrane. Wild-type LEP normally have a dramatically lower affinity for the ECM compared to MEP, excluding them from the basement membrane niche. PIK3CA-expressing LEP, in contrast, were also unique among driver mutations in having dramatically increased ECM affinity. We confirmed these findings in vivo using a genetically engineered mouse model of PIK3CA-driven breast cancer, which revealed that PIK3CA-mutant LEP accumulate in basal compartment prior to invasion and have an increased ECM affinity compared to control cells. To gain further insight into strategies to block breast cancer progression, we used measured values of cell interfacial tensions to parameterize a statistical mechanical model of tissue self-organization. The model predicted several classes of secondary perturbations that could reverse the impact of PIK3CA activation on the ability of LEP to access the basal compartment. Specifically, reducing ECM affinity (using shRNA against TLN1) or cell motility (using AKT inhibitor MK2206) depleted PIK3CA-LEP from the basal compartment. This work establishes the importance of tissue self-organization in slowing the progression of breast cancer, and presents a quantitative model for predicting interventions can stabilize tissue structure and alter the probability of cancer cell invasion.

M140

**Transition dynamics between distinct diffusible states regulate morphogen signaling range.**

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Cells use a small number of evolutionarily conserved morphogens to pattern tissues of varying sizes, however it is not clear how evolution can modulate the effective signaling range of a morphogen. By applying genetics and single-particle imaging in reconstituted morphogen signaling gradients, we found that Hedgehog family morphogens diffused extracellularly in four distinct states associated with unique biochemical environments. Individual Hedgehog molecules dynamically transitioned between states, and the evolutionarily novel protein SCUBE expanded Hedgehog gradients by catalytically accelerating the transition dynamics between each state. Based on our single-particle observations of Hedgehog dynamics, we propose a new model for morphogen diffusion, in which a single morphogen molecule

either diffuses laterally in association with a cell, or transiently becomes free to “jump” between adjacent cells. Under this model, regulating a morphogen’s “jump” probability is sufficient to regulate a its effective diffusion rate and signaling range. This multiscale understanding of morphogen gradient revealed unexpected knobs that nature can use to tune morphogen gradient sizes across tissues or organisms.

M141

**Adapting modes of cell migration to diverse tissue environments: how primordial germ cells traverse the mouse embryo.**

**K. Goodwin**, K. McDole; MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM.

Cell migration is integral to development, homeostasis, and disease. Much of our understanding of cell migration comes from culture models, but the physical signals of the *in vivo* environment differ from those in cell culture. In *vivo*, cells must migrate through tissues, acting distinctly while being subject to the same signaling landscape as the cells they migrate between. In many species, primordial germs cells (PGCs, which eventually become sperm or eggs) are specified far from their final destination (the future gonads) and travel through the developing tissues of the embryo. The dramatic embryo-wide structural changes that occur throughout PGC migration make it a difficult process to study dynamically in intact embryos. Advances in imaging techniques and embryo culture methods are needed to uncover the mechanisms of PGC migration and to uncover how migrating cells adapt to changing environments *in vivo*. Here, we use adaptive light-sheet microscopy to image PGC migration during the large-scale tissue movements that occur during post-implantation mouse embryogenesis. We show that PGCs migrate in proximity to each other but heterogeneously and not collectively. During their migration, they encounter microenvironments with a diverse range of extracellular matrix compositions and cell-cell adhesions. The migratory behavior and protrusions of PGCs vary between these microenvironments, suggesting that PGCs adapt to the surrounding tissue as they migrate. Our high-resolution and dynamic imaging approaches provide new insights into the expansive and historically inaccessible journey of PGCs during mouse embryogenesis and shed light on the mechanisms of cell migration *in vivo*.

M142

**On form and fate: how morphogenesis patterns epithelial differentiation.**

K. Goodwin, K. Shelburne, **C. M. Nelson**; Princeton University, Princeton, NJ.

Development can be considered as the product of morphogenesis and differentiation. Both processes are essential for building functional multicellular tissues, and they are intricately connected, making it difficult to establish whether differentiation is upstream of morphogenesis or vice versa. The developing lung serves as a paradigm for the interconnected nature of morphogenesis and differentiation: airways undergo a recursive program of branching morphogenesis accompanied by the progressive allocation of proximal and distal epithelial fates in branch stalks and tips, respectively. We found that altering branching morphogenesis and smooth muscle wrapping in the embryonic mouse lung shifts the balance between proximal and distal epithelial cell fates. To probe the mechanistic links between form and fate, we took advantage of epithelial-tissue-specific deletion of YAP, which disrupts both epithelial branching and differentiation. Using YAP-mutant lungs, we then parsed the effects of the physical signals imparted by branching morphogenesis (axial curvature and smooth muscle-mediated constraint) on epithelial cell differentiation. Local axial curvature was insufficient to rescue proximal epithelial fate, but enhancing

smooth muscle differentiation or applying external physical constraint restored patterns of epithelial differentiation. These data reveal that tissue form dictates epithelial cell fate in the developing lung.

M143

**Towards engineering control of epithelial branching in the developing mammalian kidney.**

**L. Prahl**, A. Hughes; Bioengineering, University of Pennsylvania, Philadelphia, PA.

The human kidney must establish a network of ~1 million nephrons and collecting tubules during embryonic development to meet the blood filtration and homeostasis demands of adult physiology. These structures are established through an embryonic tissue-building program called branching morphogenesis, where an epithelial tissue (the ureteric bud) invades the adjoining mesenchyme and forms a tree-like tubule network through iterative rounds of branching bifurcations, while nephrons form at tubule tips. While molecular genetics approaches have identified several drivers of ureteric bud branching, we first seek to understand the contributions of under-explored mechanical and geometric factors in this process. We use mouse embryology, immunofluorescence, and computational modeling to show that tubule tips become densely packed at the surface during organ growth and must progressively restructure to accommodate increasing surface density. Increasing tip packing density correlates with tissue stiffening on the organ scale, while laser cutting experiments performed at the scale of individual tubule tip domains reveal that forces transmitted between adjacent tips depend on their branching state. Nephron stem cell clusters that surround each tip experience mechanical forces during each branching cycle, providing a mechanistic link between tissue mechanics and differentiation. We find evidence for packing defects in published mouse models, including signaling regulators of the glial cell-derived neurotrophic factor (GDNF)/REarranged during Transfection (RET) tyrosine kinase pathway that drives ureteric bud branching. Finally, we describe ongoing work to investigate the mechanisms of tubule branching in a reductionist *in vitro* system using 3D epithelial spheroid models, synthetic biology, and optogenetic tools to quantitatively control GDNF/RET signaling. Together, our results reveal fundamental insights into how branching and nephron formation are coordinated at the cell-to-organ scale, provide guidelines for faithful reproduction of tissue organization in kidney organoids, and promise to identify sorely needed therapeutic interventions for congenital kidney diseases.

M144

**Tissue fluidity: a double-edged sword for multicellular patterning.**

**R. M. Garner**, A. A. Ruzette, S. E. McGeary, A. Denisenko, T. Chen, A. M. Klein, S. G. Megason; Department of Systems Biology, Harvard Medical School, Boston, MA.

The self-organization of cells into spatial patterns is essential for proper development. One mechanism critical for patterning dozens of tissues across diverse organisms is adhesion-mediated sorting. In this scheme, distinct adhesion molecules are differentially expressed on the surface of different cell types, driving cells to group together based on compatible adhesion molecule expression (Tsai, Garner, & Megason, ARCDB, 2022). But while there is ample evidence that cells can sort by adhesive compatibility, much less is known about how cells move through the tissue, find partners with similar adhesion protein expression, and stop moving to lock in the sorted pattern. One recently appreciated biomechanical property termed tissue fluidity—the extent to which cells can move freely within a tissue—has been shown to play significant roles in morphogenesis and may be linked to cancer progression, but we have yet to understand how fluidity interplays with adhesion-based sorting to determine tissue organization.

In this work we use a combination of theoretical and experimental approaches to establish that tissue fluidity is a critical regulator of adhesion-based sorting. We find that fluidity is a double-edged sword: cells in a perfectly rigid tissue cannot sort because they cannot move, and cells with overactive migration will not stay sorted. We frame these ideas in the language of statistical mechanics and kinetics. In a statistical mechanics framework, fluidity (i.e., active cell rearrangements) drives the tissue towards maximum entropy, where all possible cell arrangements are equally likely. Thus at equilibrium, increasing fluidity will always drive cells out of the sorted pattern. On the other hand, in terms of kinetics, increasing tissue fluidity increases the rate at which cells sample different arrangements in the tissue, and thus increases the rate at which the tissue can find the maximally sorted configuration. Therefore, increasing tissue fluidity increases the rate of sorting, but sacrifices the robustness of the ultimate pattern. A variety of computational models in both 2D and 3D, as well as experimental measurements on 3D cell aggregates, demonstrate these theoretical predictions across diverse contexts. Overall, we show that tissue fluidity is required for adhesion-based cell patterning, and demonstrate a fundamental trade-off between the rate and robustness of sorting, tuned by fluidity. More broadly, our work suggests that tissue fluidity itself, not just adhesion molecule expression, must be tightly regulated in order to carry out patterning that is both fast and robust. Furthermore, the diversity of tissue fluidities observed in nature suggests that cells in different tissues adopt distinct strategies for patterning, depending on their fluidity.

## Minisymp: Molecular Basis of Nervous System Development

M145

**Molecular mechanisms for self-assembly of circuit pioneer cells in *C. elegans* brain assembly.**

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Sculpting tissue architecture is key for tissue function. Nervous system function relies on the faithful assembly of circuit architecture and failure to properly pattern this assembly leads to neuropathology. Early “pioneer” cells pattern initial circuit paths, but their identity and development are understudied and remain elusive. We dissect the mechanisms that define molecular identity and morphogenesis of brain pioneers. *C. elegans* is a powerful setting, offering genetic tractability, characterized lineage, connectivity, transcriptomics, and live single-cell-resolution imaging. We showed that *C. elegans* brain assembly is hierarchical, initiated by pioneer neurons and glia that cooperatively guide follower components. Having uncovered pioneer cells with defined molecular identities we study their developmental principles. We combine genotype-to-phenotype screens, real-time quantitative imaging in vivo, cell ablations, in silico and in vivo behavioral studies. We describe unique characteristics of pioneer cell lineage and architecture. We outline pioneer-enriched transcription factors and adhesion molecules, using a computational pipeline. We identify a pioneer-enriched molecular repertoire functional for pioneer fate and morphogenesis and their embryonic roles in vivo. We align this repertoire to circuit architecture and function, by examining the follower components and circuit’s behavioral output upon functional loss of these factors. Pioneer defects result in disrupted circuit architecture and animal behavior. Overall, we identify an in vivo, functional molecular repertoire driving pioneer self-assembly in *C. elegans*. This has key implications in understanding circuit assembly. Pioneer-morphogenesis is regulated partially by constitutively- and transiently-expressed factors of diverse families, enriched in different pioneers and cooperating for self-assembly. A dysfunctional repertoire of pioneer factors results in defective circuit structure and behavioral output. To our knowledge, this is the first map of factors functionally implicated in pioneer development in vivo. We also uncover cross-

species analogies of circuit architecture. Thus, the *C. elegans* molecular repertoire of pioneer development provides a platform for cross-species studies of pioneer development factors, towards conserved gene regulatory networks for pioneer morphogenesis in vivo.

M146

**Combinatorial control of endoplasmic reticulum remodeling during neurogenesis via selective ER-phagy.**

**M. Hoyer**, I. Smith, J. Paoli, Y. Zhang, J. Paulo, J. Harper; Harvard Medical School, Boston, MA.

The endoplasmic reticulum (ER) network can reorganize to drive various cellular functions. This phenomenon is particularly important in neuronal cells where ER tubules must fit into the tight spaces of axons and dendrites. However, underlying mechanisms for ER remodeling in conversion to specialized cell states, including neurons, is poorly understood. Once differentiated, cells can no longer dilute damaged, misshapen, or excess organelles via cell division. However, as an alternative mechanism, a double membrane autophagosome can encase organelle cargo and target it to lysosomes for degradation. How, when, and where the ER can be preened via autophagy in neuronal cells has yet to be defined. We coupled fluorescence-based autophagic flux reporters for distinct ER proteins with quantitative proteomics during conversion of stem cells to cortical-type neurons. In ATG12-/- autophagy deficient cells, both ER-phagy receptors and ER tubule shaping proteins dramatically accumulate, indicating these proteins are preferentially selected for autophagic elimination during neurogenesis. In addition, we find the ER undergoes substantial autophagic flux to lysosomes in post-mitotic cells, with evidence of ER clearance in both cell bodies and axons. We have created combinatorial ER-receptor mutants to understand when and where each receptor functions and quantify potential receptor interplay. In our analysis of single and combinatorial ER-phagy receptor mutants we define specific subsets of ER curvature-shaping proteins or lumenal proteins as preferred clients for distinct receptors. Using spatial sensors and flux reporters, we demonstrate receptor-specific autophagic capture of ER in axons, which correlates with aberrant ER accumulation in axons of ER-phagy receptor or autophagy-deficient neurons. This molecular inventory of ER proteome remodeling and versatile genetic toolkit provides a quantitative framework for understanding contributions of individual ER-phagy receptors for reshaping ER during cell state transitions.

M147

**A sensory cilium mediates specific neuron-glia attachment.**

**L. Wexler**, M. G. Heiman; Harvard Medical School, Boston, MA.

Glial cells form specialized attachments with specific neuronal partners, but how such precise cell-cell pairing occur remains unknown. Here, we show that a sensory cilium mediates the highly stereotyped attachment of a single *C. elegans* neuron, called URX, to its partner glial cell, called ILso. The URX neuron offers a remarkable example of specificity in neuron-glia pairing. Its dendrite is positioned in the dorsal sensory bundle of the head and makes a “jump” across the nose tip to attach to a specific glial partner (ILso) in the lateral sensory bundle. Although 36 different glial endings are present at the nose tip, the URX dendrite recognizes and makes exclusive stereotyped attachments to the lateral ILso glial cell. We found that this specific neuron-glia pairing occurs by a multistep process. First, in early embryos, the URX dendrite anchors to a different glial guidepost, via the adhesion protein SAX-7 and the scaffolding protein GRDN-1, such that the dendrite is stretched out to its full length during embryo elongation. Mutants that disrupt dendrite anchoring result in severely shortened URX dendrites that fail to reach the

nose tip. Second, in late embryos, we find that the URX dendrite ending jumps across the nose to form its mature attachment to the ILso glial cell. Using three-color imaging of URX, a cilia marker, and the ILso glial cell, we find that this attachment is mediated by a sensory cilium. By examining mutants that can not form cilia, we find that the URX dendrite fails to attach to the lateral ILso glia, showing that the sensory cilium is strictly required for this neuron-glia attachment. Through a candidate screen we found that the loss of the conserved SPARC family protein TEST-1 results in the sensory cilium failing to attach to the glial partner. Importantly, vertebrate SPARC family proteins are secreted by glia and regulate adhesion at synapses, suggesting cilia-glia pairing may resemble pairing of dendritic spines and glia at vertebrate synapses. Finally, we used an unbiased forward genetic screen for mutants with defects in URX-ILso attachment and isolated several mutants that severely disrupt cilia-glia attachment. Together, our results show that, in addition to their canonical role in sensory signaling, cilia can mediate cell-cell adhesion including in the context of specific neuron-glia pairing.

M148

**Interphasic Somal Translocation is a prominent mode of bRG cell dissemination into the human developing neocortex.**

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Radial glial cells are the progenitors of most neurons and glial cells of the neocortex. Apical radial glial (aRG) cells are common to all mammals and localize to the ventricular zone. Basal radial glial (bRG) cells concentrate in the outer subventricular zone (OSVZ) and are highly abundant in Human, where they are believed to account for neocortex size expansion. bRG cells colonize the neocortex through mitotic somal translocation (MST), a nuclear migration event that occurs once per cell cycle, prior to cytokinesis. Using live imaging of human fetal tissue and cerebral organoids, we show here that bRG cells also translocate during interphase, through an entirely different mechanism that we name Interphasic Somal Translocation (IST). IST is slower than MST but occurs throughout interphase. Accordingly, we show that IST contributes to 80% of bRG cell movement in fetal tissue, and is therefore more important than MST for bRG cell dissemination. Mechanistically we show that, while MST is actomyosin-dependent, IST is microtubule-dependent. We demonstrate that nuclear movement is powered by the dynein motor and its activator LIS1, that are recruited to the nuclear envelope by Nesprin proteins. Finally, we show that glioblastoma cells with a bRG-like cell identity also undergo IST, through dynein-LIS1-Nesprin nuclear migration. This work identifies a conserved mode of bRG and glioblastoma cell migration, important for progenitor cell dissemination and tumor cell invasion into the neocortex.

M149

**Endocytosis at the axon initial segment: towards specificity and polarity regulation.**

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Neurons are highly polarized cells with distinct axonal and dendritic domains that send and receive signals, respectively. To achieve this polarization, neurons extensively use membrane trafficking mechanisms to compartmentalize a vast and diverse repertoire of proteins to each domain. The axon initial segment (AIS) is a critical boundary zone that separates the contiguous plasma membrane of the axonal and dendritic domains. The AIS was thought to maintain protein compartmentalization on the plasma membrane through its passive functions as a diffusion barrier. However, we recently identified an active mechanism at the AIS that is essential for maintaining neuronal polarity and is conserved from *C. elegans* to humans. We find that dendritically and axonally polarized membrane proteins are

endocytosed in the AIS and subsequently degraded to maintain their polarized distribution (Eichel et al., 2022). Because of the physiological importance of AIS endocytosis, we are now investigating its specificity and regulation. To understand how proteins are selected for endocytosis in the AIS, we examined an array of membrane proteins. We find that many diverse dendritic and axonally polarized membrane proteins are endocytosed in the AIS. However, proteins that function at the AIS are prevented from endocytosis through interaction with AIS structural components, consistent with previous reports. Disruption of this interaction confers endocytic capability. Conversely, forcing membrane protein interaction with AIS structural components prevents their endocytosis. Thus, membrane protein interaction with AIS structural components provides a dominant signal to maintain their plasma membrane localization. We next sought to investigate the post-endocytic targeting of proteins by examining the distribution of endogenously labeled Rab proteins with single-cell resolution. We find that RAB-7 positive late endosomes are enriched in the AIS compared to Rab proteins that mediate recycling. Using electron tomography, we find an enrichment of double membrane vesicles in the AIS compared to the connected dendritic region. These observations suggest that the AIS is specialized towards degradation and provide a molecular handhold to investigate targeting mechanisms. We propose that endocytosis coupled to degradation clears axonal and dendritic membrane proteins from the AIS to maintain their compartmentalization. By investigating these membrane trafficking events, we are uncovering new principles of how the architecture of one of the most polarized cells is built and maintained.

M150

**Non-autonomous roles of Rac1 and likely Nectin3 in axon guidance in the peripheral auditory system.**

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Our sense of hearing is critically dependent on the proper wiring of the spiral ganglion neurons (SGNs), which connect the sound receptors in the organ of Corti (OC) to the cochlear nuclei of the hindbrain. During development, SGNs establish stereotyped innervation patterns with sensory hair cell targets in the OC. Type I SGNs innervate inner hair cells (IHCs) to transmit sound signals, while type II SGNs (SGNIIs) innervate outer hair cells (OHCs) and are thought to detect acoustic trauma. Despite their essential functions in hearing, our understanding of the molecular mechanisms that mediate wiring of the auditory periphery is still incomplete. Recently, it was shown that guidance of SGNII afferent projections is regulated by the Planar Cell Polarity (PCP) pathway. Intercellular PCP signaling mediates polarized cell behaviors within the plane of a tissue in a variety of developmental processes. In the wild-type OC, SGNII afferents make a striking 90-degree turn toward the base of the cochlea and innervate multiple OHCs. In several PCP mutants, SGNII afferents turn randomly towards the cochlear base or apex. Cochlear supporting cells serve as intermediate targets for SGNII afferent turning. Although it has been shown that PCP proteins localize asymmetrically to supporting cell (SC)-SC junctions and act in the cochlear epithelium to guide SGNII afferents, the underlying mechanisms are currently unknown. We hypothesize that PCP signaling regulates multiple downstream effectors including adhesion molecules and Rho GTPases to influence cell adhesion and the cytoskeleton in SCs. We have found that core PCP gene Vangl2 regulates localization of the cell adhesion molecule Nectin3 and the active form of the small GTPase Rac1. Via conditional knockout mouse lines and immunofluorescence labeling we found that the small GTPase Rac1, but not Rac3, is necessary for proper SGNII afferent innervation. However, the constitutive activity of Rac1 was insufficient to correctly guide SGNII afferents when PCP signaling was disrupted. Additionally, we generated two *Nectin3* knockout mouse lines via CRISPR-Cas9 and have

characterized an SGNII afferent misturning phenotype in both mutant lines. Together these experiments identify Rac1 and Nectin3 as novel regulators of SGNII afferent innervation, and continuing work will further investigate the relationship of these genes to the PCP pathway and the mechanisms through which they affect SGNII afferent guidance.

## Minisymp: Molecular Determinants of Cellular Processes

M151

**evolutionary structural comparison of sperm flagella based on high-resolution native structures.**

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Sperm flagella are microtubule-based tail-like motile organelle that are essential for sperm motility and further successful fertilization. These flagella are composed of microtubules that are decorated and stabilized by microtubule inner proteins (MIPs) and microtubule associated proteins (MAPs). In this study, we used cryo-electron microscopy to resolve high-resolution structures of native sperm flagella doublet microtubules (DMTs) from different species. Additionally, we performed evolutionary structural comparisons across species and cell types to identify conserved sperm proteins. We developed robust strategies for protein identification and identified over 60 proteins decorating sperm doublet microtubules, with over 15 proteins are sperm-specific and more than 16 proteins are associated motility defects or infertility. Tektins, especially Tektin-5, were the most abundant MIPs that extensively spanning the lumen of A tubule in mammalian sperm, while Tektins from other species had different compositions and locations. Additionally, we discovered the most extensive SAXOs and microtubule-associated proteins (MAPs) network so far, which elaborates the inner and outer stabilization with distinct binding modes towards DMTs. Overall, our high-resolution structures from different species provide a detailed understanding of sperm organization, evolution, and motility, and shed light on the mechanisms underlying male infertility. Moreover, the extensive MIPs and MAPs network identified in this study highlights the importance of the microtubule cytoskeleton in regulating sperm function. Our findings may have implications for the diagnosis and treatment of male infertility.

M152

**Autoinhibited kinesin-1 adopts a hierarchical folding pattern.**

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Conventional kinesin-1 is the primary anterograde motor in cells for transporting cellular cargo. While there is a consensus that the C-terminal tail of kinesin-1 inhibits motility, the molecular architecture of a full-length autoinhibited kinesin-1 remains unknown. Here, we combine cross-linking mass spectrometry (XL-MS), electron microscopy (EM), and AlphaFold structure prediction to determine the architecture of the full-length autoinhibited kinesin-1 homodimer [kinesin-1 heavy chain (KHC)] and kinesin-1 heterotetramer [KHC bound to kinesin light chain 1 (KLC1)]. Our integrative analysis shows that kinesin-1 forms a compact, bent conformation through a break in coiled coil 3. Moreover, our XL-MS analysis demonstrates that kinesin light chains stabilize the folded inhibited state rather than inducing a new structural state. Using our structural model, we show that disruption of multiple interactions between the motor, stalk, and tail domains is required to activate the full-length kinesin-1. Our work offers a

conceptual framework for understanding how cargo adaptors and microtubule-associated proteins relieve autoinhibition to promote activation.

M153

**Architecture of the human inner kinetochore on intact mitotic chromosomes revealed by cryo-electron tomography.**

**K. Kixmoeller**, Y. Chang, B. Black; University of Pennsylvania, Philadelphia, PA.

The centromere is the locus that directs faithful chromosome inheritance during cell division. It recruits mitotic components to assemble the kinetochore complex which serves to attach the chromosome to the microtubule-based spindle. This locus has been interrogated by electron microscopy since the middle of the last century, but connecting its molecular architecture to its physical organization has been hampered by prior reliance on sample fixation and staining. We now report our use of cryo-electron tomography (cryo-ET) to directly visualize the 3D organization of the human centromere on intact mitotic chromosomes. Our approach using native (unfixed, unstained) human chromosomes reveals a unique chromatin architecture that distinguishes this site from any other we observe on the chromosome. Specifically, the mitotic human centromere consists of clustered 20-25 nm kinetochore complexes contained within chromatin clearings. These clearings are distinct from surrounding chromatin and have a lower density of particles compared to surrounding chromatin. The 20-25 nm complexes found within centromeric chromatin clearings exhibit more structural heterogeneity than canonical nucleosomes nearby, but their average size and shape is most consistent with recent models of a biochemical reconstitution of a single copy of the 16-subunit constitutive centromere-associated network (CCAN) complex associated with a nucleosome harboring the histone H3 variant, CENP-A. Indeed, we find the CCAN often very closely associated with the nucleosome. Furthermore, rapid depletion of CENP-C, a key component of the CCAN, leads to disruption of centromeric architecture, supporting a role for CENP-C in organizing the chromatin architecture of the centromere. Our studies provide key insight into both the fundamental building blocks of the inner kinetochore and its overall organization along centromeric DNA. Thus, this direct visualization of the centromere *in situ* within chromosomes provides vital information about the architecture of centromeric chromatin and the inner kinetochore.

M154

**Structural convergence endows nuclear transport receptor Kap114p with a novel transcriptional repressor function toward TATA-box binding protein.**

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The transcription factor TATA-box binding protein (TBP) modulates gene expression in nuclei. This process requires the involvement of nuclear transport receptors, collectively termed karyopherin-β (Kap-β) in yeast, and various regulatory factors. In previous studies we showed that Kap114p, a Kap-β

that mediates nuclear import of yeast TBP (yTBP), modulates yTBP-dependent transcription. However, how Kap114p associates with yTBP to exert its multifaceted functions has remained elusive. Here, we employ single-particle cryo-electron microscopy to determine the structure of Kap114p in complex with the core domain of yTBP ($yTBP^C$). Remarkably, Kap114p wraps around the $yTBP^C$ N-terminal lobe, revealing a structure resembling transcriptional regulators in complex with TBP, suggesting convergent evolution of the two protein groups for a common function. We further demonstrate that Kap114p sequesters yTBP away from promoters, preventing a collapse of yTBP dynamics required for yeast responses to environmental stress. Hence, we demonstrate that nuclear transport receptors represent critical elements of the transcriptional regulatory network.

M155

**CKAP5/XMAP215 facilitates microtubule-F-actin interaction and promotes F-actin bundling.**

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Microtubule and F-actin cytoskeletal cross talk and reorganization are important aspects of cell migration and axonal guidance, but the mechanisms by which associated proteins facilitate this are still being revealed. While CKAP5/XMAP215 has been best characterized for its microtubule polymerase function, our previous studies have highlighted a novel role for CKAP5 in facilitating interactions between microtubules and F-actin in the embryonic neuronal growth cone, the dynamic structure at the tip of the growing axon. Here, we utilized biochemical assays, in vitro reconstitution assays, and high resolution light microscopy, including super resolution imaging and TIRF, to determine how CKAP5 promotes interactions between microtubules and F-actin. We first established the TOG5 domain as a necessary region that is responsible for direct interactions with F-actin. In addition, we determined that CKAP5 microtubule-lattice-binding activity as well as TOG5-mediated F-actin binding domains are essential for influencing microtubule guidance into the growth cone peripheral domain. Next, we discovered that these dual cytoskeletal roles of CKAP5 are independent from, and do not require, its classical microtubule polymerase activity. Finally, we observed that microtubule lattice-bound CKAP5 can recruit actin filaments and promote F-actin bundling. Together, our work highlights the novel and important roles that CKAP5 plays in facilitating cytoskeletal interactions within the growth cone. Not only does it promote microtubule extension into the growth cone periphery through interactions with F-actin, it may also be involved in positioning and reinforcing F-actin bundles to support growth cone filopodia. Thus, CKAP5 plays important mechanistic roles in facilitating protrusion and steering of the axon during outgrowth.

M156

**Single-Molecule Insights into Kinesin-2 Motility and Its Role in Ciliopathies.**

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Kinesin-2 is a heterodimeric motor responsible for driving the anterograde transport within cilia. Disruption of kinesin-2 function through mutations leads to ciliopathies in humans. The regulation and

biomechanics of kinesin-2 movement along cilia remain largely unknown. Here, we purify the intact human kinesin-2 complex (Kif3A/Kif3B) and study its structure and motility mechanism in vitro. Single-particle EM reveals that intact kinesin-2 forms a compact structure in which the tail folds back, with the cargo-binding domain docking against the motor domains in an asymmetric manner. Single-molecule TIRF assays demonstrate that intact kinesin-2 binds weakly to microtubules (MTs) and does not initiate processive runs, together suggesting an autoinhibitory regulation. We predict that the flexible hinge region in the tail enables the autoinhibitory self-folding. To activate the kinesin, we engineered a construct in which the hinge region was replaced with a continuous coiled-coil, a construct referred to as hinge-deletion (HD) kinesin-2. Indeed, this HD kinesin-2 displayed robust processive motility and increased landing rate on MTs, indicating a relief from the autoinhibition. The kinesin associated protein (KAP) was proposed to activate kinesin-2 and facilitate travel along cilia. Interestingly, we show that while HD kinesin-2 can transport KAP along MTs, KAP is not sufficient to relieve the autoinhibition. We further demonstrate that KAP itself can diffuse along MTs, and the presence of KAP enhances the speed and distance of kinesin-2 movement. Finally, we analyzed a mutation in kinesin-2 (Kif3B E250Q) known to cause dominant ciliopathy. EM shows that this mutant maintains the autoinhibited structure, however, it exhibits a strong affinity for MTs without initiating any movement. We employed ensemble MT-gliding assays to mimic kinesin-2 collective transport in the intraflagellar transport (IFT) train within cilia. We show that the presence of a small amount of E250Q can severely impair MT transport. Our findings suggest that the E250Q mutant may act as a molecular brake in the IFT train, thereby disrupting cilia trafficking and contributing to the dominant disease phenotype. Together, our results provide new insight into how kinesin-2 motility is regulated at the single-molecule level, and the mechanism of dominant ciliopathy caused by kinesin-2 defects.

## Minisymp: Multicellular Mechanobiology and Morphogenesis

M157

**Optogenetic control of endogenous Rho signaling reveals biophysical principles of tissue morphogenesis.**

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During embryonic development, epithelial tissues function as malleable substrates that remodel to shape adult animals. While recent advances have made it possible to reconstitute some morphogenetic processes, our ability to more generally manipulate epithelial tissue shapes is limited. Optogenetic control of Rho signaling and actomyosin contractility provides an avenue to direct epithelial folding and other shape changes with light. However, existing transgenic optogenetic tools can be limited by their variable expression levels, which hinder quantitative gradation of activation, as well as by potentially deleterious effects of overexpression. Here, we use CRISPR/Cas9 gene editing in *Drosophila melanogaster* to endogenously tag two RhoGEFs - RhoGEF2 and Cysts - with components of the iLID/sspB optogenetic system, producing homozygous-viable flies. In the gastrulation-stage fly primary epithelium, RhoGEF2 and Cysts are associated with medial-apical and junctional contractility, respectively. However, under endogenous optogenetic control, they enact similar tissue deformations with varying magnitudes, suggesting that their conserved catalytic domains act redundantly, while their divergent regulatory domains direct localization. Additionally, endogenous tool expression levels are highly consistent, allowing for quantitative grading of activation to produce folds of varying depths.

Although activation of apical or lateral contractility are both known to drive folding, we find that these two modes display distinct structural signatures. Finally, we find that activation-induced deformations along orthogonal axes are strongly coupled in the cellularized primary epithelium but not in the cellularizing blastoderm, which lacks enclosed cells, indicating that volume conservation and hydrostatic pressure play a role in coordinating the 3D deformations of cells and tissues. In conclusion, we developed a novel endogenous tagging-based optogenetic approach that allowed us to dissect the activities of different RhoGEFs and uncover mechanical principles governing tissue morphogenesis, giving us not only spatial and temporal, but also quantitative control over tissue morphologies. This work lays a foundation for shaping epithelial tissues with calibrated, 3D-patterned perturbations of cell contractility to interrogate developmental mechanics and engineer cell-based structures.

M158

**Curvature sensing mechanisms in single cells and epithelia.**

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Cells can migrate individually between tissues and organs or collectively in tightly or loosely associated groups. In both cases, cell migration is regulated by extracellular cues of very different natures. Here I will describe a cellular mechanism called curvotaxis that enables individual cells and growing epithelial monolayers to sense and respond to curvature variations from the substrate on which they adhere. Live imaging combined with functional analysis and in silico modeling shows that curvotaxis relies on a dynamic interplay between the nucleus and the cytoskeleton - the nucleus acting as a negative curvature magnet that leads the migrating cell towards concave curvatures. Curvature affects also focal adhesions organization and dynamics, nuclear shape and gene expression. Growing epithelial colonies can also respond to curvature by a process involving both curvotaxis in the leading edge where cells are loosely associated, and oriented cell divisions in the more mature parts of the colony. Altogether, this work identifies cell-scale curvature as an essential physical cue that can guide both single and collective cell migration processes. Potential applications in tissue engineering and biomaterial design will be discussed.

M159

**Coordination of cell states and tissue architecture by mechanical forces.**

**S. A. Wickstrom**; Department of Cell and Tissue Dynamics, Max Planck Institute for Molecular Biomedicine, Muenster, GERMANY.

The structure of tissues is tightly linked to their function. During formation of functional organs, large-scale changes in tissue elongation, stretching, compression, folding/buckling, and budding impact the shape, position, packing, and contractility state of cells. Conversely, changes in single cell contractility, shape and position locally alter tissue organization and mechanics. Thus, forces function as important ques that are transmitted to the nucleus to coordinate gene expression programs to control cell states. 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 cells use the nucleus and the nuclear envelope/chromatin interface to sense mechanical forces and how these mechanosignals are integrated with biochemical inputs to alter cell states and to generate and maintain tissue architecture.

M160

**An ancient mechanically-induced neuronal cell state promotes melanoma invasion.**

**M. V. Hunter**, M. H. Sherman, R. M. White; Memorial Sloan Kettering Cancer Center, New York, NY.

Tumor cells are exposed to high levels of mechanical stress during their lifetime. However, the influence of mechanical force on tumor cell behaviour is an understudied area of cancer cell biology. We confined human melanoma cells *in vitro* and noticed they took on several characteristics of migrating neurons, including assembly of a perinuclear acetylated tubulin network, nuclear rotation, and reorganization of the microtubule cytoskeleton. Melanocytes, the melanoma cell-of-origin, are descendents of the neural crest, an embryonic cell population that migrates to various regions within the organism to give rise to a diverse array of cell types. We hypothesized that confined melanoma cells reactivate an ancient neuronal cell state that enables them to survive mechanically-challenging environments, similar to those migrating neural crest cells must navigate. By applying spatial transcriptomics and *in vivo* confocal microscopy to a zebrafish model of melanoma, we identified the chromatin modifier HMGB2 as a mediator of this confined, neuronal cell state. HMGB2 is specifically upregulated in confined tumor cells under high amounts of mechanical stress at the tumor border and in human melanoma cells confined *in vitro*. We found that HMGB2 enrichment in confined cells is dependent on assembly of a mechanically-stabilized perinuclear acetylated tubulin "cage", which buffers the nucleus against mechanical stress. FRAP analysis and mathematical modeling revealed that confinement stabilizes the interactions of HMGB2 with chromatin, causing lasting changes in chromatin architecture and gene expression. RNA-seq and ATAC-seq showed that upregulation of HMGB2 triggers activation of a neuronal gene program and increased plasticity. Together, our results indicate that confinement causes the melanoma cell to reactivate a neuronal cell state used by its precursors to navigate mechanically-challenging environments, and implicate the mechanical microenvironment as a critical regulator of tumor cell behaviour and aggressiveness.

M161

**All models are wrong (but some are useful): *Xenopus* embryonic epithelial sheets under strain in the real world.**

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Mechanics is critical to tissue remodeling during embryonic growth, cancer development, and regeneration. Mechanical strain induced both by local microenvironment and external sources is thought to guide biological processes such as cell migration, proliferation, cell fate change, etc. Contemporary mechanical models and force inference methods of epithelial morphogenesis assume transmission of stress and strain occurs primarily in the plane of cell-cell junctions and that the mechanics of epithelial sheets is dominated by tension carried along the lengths of spring-like apical junctional complexes. Here we test those assumptions with a unique tissue stretcher that combines high resolution live-cell imaging with the application of morphogenesis-scale strain rates. Organotypic explants of *Xenopus* gastrula stage ectoderm expressing a vinculin-tension reporter are cultured on a fibronectin-coated polydimethylsiloxane (PDMS) substrate. The substrate can be stretched in a custom-built 3D-printed microscope stage insert with motorized actuators to achieve > 100% uniaxial strain. We quantified vinculin intensity along individual cell junctions in these samples, where we expected to see vinculin recruited as the tissue responses to the high strain. Surprisingly, we observe no vinculin

recruitment to sites that were parallel to the uniaxial stretch axis, but a significantly increased vinculin recruitment to junctions perpendicular to the stretch axis. Ongoing work with the stretcher includes investigating mechno-sensitive components such as myosin II and actin dynamics under tension by applying tension on epithelial sheets expressing the fluorescent actomyosin reporters life-act and sf9. We found both F-actin and myosin II intensity increased after uniaxial tension was applied. Most interestingly, we observed a significant accumulation of myosin II medio-apically to form persistent bands that spread inward from cell-cell junctions. These findings suggest tension is primarily transmitted along medio-apical surfaces rather than along junctions, and that current vertex-based models and force inference methods may need significant revision if they are to be used as interpretive models of epithelial morphogenesis. This work has been supported by a grant from the NIH to LAD (R37 HD044750).

M162

**Stem Cell Dancing Enhances Differentiation in 3D via Nuclear Mechanotransduction.**

**M. Ayushman**, G. Mikos, S. Sinha, S. Jones, X. Tong, F. Yang; Stanford University, Stanford, CA.

When physically interacting with their three-dimensional (3D) niche, cells actively push and pull along the extracellular matrix (ECM) via physical behaviors such as cell spreading and volume expansion. These processes occur on timescales of hours-to-days but regulate long-term tissue outcomes such as differentiation and disease progression. However, whether cells can deform local ECM at even faster timescales and its impact on long-term cell fates remain largely unknown. Here, we report 'cell dancing'- a previously unknown cellular behavior associated with seconds-to-minutes scale deformations that can strongly impact stem cell differentiation in 3D.

Using polyethylene glycol (PEG)-based sliding hydrogels (SG) as 3D niches, our lab has previously demonstrated that the ability of cells to reorganize their local ECM enhances mesenchymal stem cell (MSC) differentiation to multiple lineages- cartilage, bone, and fat. SG facilitates local ECM reorganization via mobile crosslinks and adhesive ligands that can slide along a PEG chain. As a control, we included a conventional PEG-based chemical hydrogel (CG) with static crosslinks and ligands on the PEG chain. The goal of this study was to identify the physical behaviors and associated biophysical mechanisms that drive enhanced MSC differentiation in SG compared to CG, focusing on MSC chondrogenesis. Live cell imaging showed SG, but not CG, induced a 'dancing' behavior with active seconds-to-minutes scale ECM deformations up to 1 μm. Cell dancing in SG was associated with enhanced seconds-scale F-Actin network dynamics and ~5X higher nuclear movement speeds compared to CG. We also observed increased expression of nuclear mechanical loading markers Lamin A/C and H3K9me3 in SG within 24 hours. Furthermore, cell dancing was associated with the repression of chromatin accessibility characterized by increased histone methylation marks. Interestingly, we also observed increased seconds-scale nuclear deformations and enhanced PLA2 signaling, indicating increased nuclear stretch in SG. Importantly, we could modulate cell dancing and chondrogenesis by modulating PLA2 signaling- using PLA2 inhibitor to inhibit it and arachidonic acid to boost it. Finally, we also observed that cell dancing enhances differentiation to other lineages and occurs in other commonly used hydrogel platforms. In summary, we have identified cell dancing- a previously unknown physical behavior that can be harnessed to promote desirable tissue outcomes.

## Minisymp: Protein Misfolding, Protein Degradation, and Quality Control.

M163

**Dissecting the role of TMED9, a p24-family member, in the clearance of misfolded GPI-anchored proteins via RESET pathway.**

**E. Ronzier**[1], P. Satpute-Krishnan[2]; [1]Henry Jackson Foundation, Bethesda, MD, [2]Uniformed Services University of the Health Sciences, Bethesda, MD.

In mammalian cells, misfolded GPI-anchored proteins (GPI-APs) are cleared out of the endoplasmic reticulum (ER) to the Golgi via a constitutive and stress-inducible pathway called Rapid ER Stress-induced Export (RESET). From the Golgi, misfolded GPI-APs traffic to the cell surface where they are rapidly internalized for lysosomal degradation. Intriguingly, misfolded GPI-APs are dependent on the expression of a subset of p24-family members, including TMED9, for ER-clearance via the RESET pathway [1,2]. This contrasts with properly folded wild type GPI-APs, which do not rely on p24-family members for traffic to the cell surface [1,2]. How the p24-family coordinates trafficking and degradation of misfolded GPI-APs is not understood. Given the precedence established by Dvela-Levitt *et al.* (2019) for TMED9 associating with and regulating the traffic and protein quality control (PQC) of another misfolded protein, a frame-shift mutant of MUC1 [3], we decided to decipher TMED9 implication in the clearance of misfolded GPI-AP via RESET pathway. We used the previously described YFP-tagged variant of prion protein, YFP-PrP C179A "YFP-PrP*" as a model misfolded GPI-AP [1]. In cells stably expressing YFP-PrP*, YFP-PrP* co-immunoprecipitates with TMED9, colocalizes with TMED9 in the early secretory pathway and constitutively traffics from the ER to lysosomes for turnover via RESET during steady-state conditions. Importantly, we discovered that depletion of TMED9 with either siRNA knock down or through the action of the small molecule BRD4780 [3], allows for the buildup of YFP-PrP* in the ER. Typically, treatment with thapsigargin accelerates release of YFP-PrP* from the ER chaperones for efficient lysosomal degradation via RESET [1]. However in the presence of thapsigargin, the depletion of TMED9 with siRNA or BRD4780 blocks release of YFP-PrP* from the ER and inhibits degradation. Quantitative imaging techniques in TMED9-depleted cells that were treated with thapsigargin revealed that YFP-PrP* was relatively immobilized in the ER in comparison to cells which were not treated with thapsigargin, providing insight into the relationship between TMED9 and misfolded GPI-APs, which we will elaborate upon. The findings presented here contribute to our understanding of how TMED9 regulates GPI-AP quality control and the broader role of TMED9 in protein homeostasis.

*[1] Satpute-Krishnan et al. (2014) ER stress-induced clearance of misfolded GPI-anchored proteins via the secretory pathway, Cell.*

*[2] Zavodszky & Hegde (2019) Misfolded GPI-anchored proteins are escorted through the secretory pathway by ER-derived factors, eLIFE.*

*3] Dvela-Levitt et al. (2019) Small Molecule Targets TMED9 and Promotes Lysosomal Degradation to Reverse Proteinopathy, Cell.*

M164

**HUWE1 amplifies the ubiquitin signal to promote the clearance of protein aggregates and condensates.**

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Cellular protein aggregates/condensates, which accumulate in multiple human pathologies such as neurodegeneration and diabetes, are typically enriched with ubiquitin (Ub) or Ub chains. The multiplicity of Ub modifications on these aggregates signals the autophagy system to selectively degrade these targets. In addition, the proteasome is also involved in the clearance of certain aggregates. The VCP/p97, a Ub-dependent segregase, is required by both pathways for dissolving aggregates including stress granules, aggresomes, and inclusion bodies formed by a variety of aggregation-prone proteins. It is still unclear how the ubiquitylation system recognizes these aggregates and generates the Ub modifications to recruit the VCP complex for their clearance. In an unpublished study, we discovered a HECT-domain E3 HUWE1 carrying a novel type of Ub ligase activity - Ub-Directed Ub Ligase, or UDL. This UDL activity recognizes the multiplicity of Ub chains on substrates which can be generated through either polyubiquitylation or substrate aggregation, thus allows HUWE1 to target various protein aggregates and rapidly expand their Ub modification. In a chemically-defined system, we found that the Ub modification laid by HUWE1 promoted unfolding of model substrates by the p97-UFD1-NPL4 complex. To examine the role of HUWE1 in processing cellular aggregates, we tested several aggregation systems, including the chemically-inducible agDD aggregates, stress granules and aggresomes. HUWE1 colocalized with these protein aggregates in a process that depends on its Ub-binding domains. HEK293 cells with HUWE1 knockout or Ub-binding-domain mutations compromised the signals of ubiquitylation and p97 localization at the aggresomes, and significantly slowed down the clearance of these aggregates. Although WT HEK293 cells tolerate the formation of agDD aggregates, HUWE1 knockout or loss of its Ub-binding domains led to rapid cell round-up and cell death upon aggregation formation. A similar phenotype was observed for cells expressing Huntingtin(Q94). In summary, our results suggest that HUWE1 recognizes the Ub-rich cellular aggregates in a process akin to their recognition by the autophagy receptors, albeit requiring Ub chains. HUWE1's activity is required for the efficient clearance of these aggregates, likely through boosting their Ub signals to recruit the VCP/p97 segregase. Consequently, loss of HUWE1 sensitizes the cell to the formation of protein aggregation.

M165

**Ubiquitination is Uncoupled from Degradation to Allow Protein Maturation.**

**M. Mariappan**, X. Li; Yale University, West Haven, CT.

A significant proportion of nascent proteins undergo polyubiquitination on ribosomes in mammalian cells, yet the fate of these proteins remains elusive. The ribosome-associated quality control (RQC) is a mechanism that mediates the ubiquitination of nascent chains on stalled ribosomes. In this study, we find that nascent proteins ubiquitinated on stalled ribosomes by the RQC ligase LTN1 are insufficient for proteasomal degradation. Our biochemical reconstitution studies reveal that ubiquitinated nascent chains are promptly deubiquitinated in the cytosol upon release from stalled ribosomes, as they are no longer associated with LTN1 E3 ligase for continuous ubiquitination to compete with cytosolic deubiquitinases. These deubiquitinated nascent chains can mature into stable proteins. However, if they misfold and expose a degradation signal, cytosolic quality control recognizes them for re-ubiquitination

and subsequent proteasomal degradation. Thus, our findings suggest that cycles of ubiquitination and deubiquitination spare foldable nascent proteins while ensuring the degradation of terminally misfolded proteins.

M166

**PI31/Fub1 is an Endogenous Proteasome Inhibitor that Functions Via a Novel Mechanism that Simultaneously Targets All Six Active Sites.**

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The proteasome is the large multisubunit protease responsible for most selective intracellular protein degradation in eukaryotes. Proteasome inhibitors like Velcade are widely used FDA-approved drugs for the treatment of multiple myeloma and other cancers. An endogenous proteasome inhibitor, PI31 (Fub1 in yeast), was discovered 30 years ago but its inhibitory mechanism had remained unclear. Proteolysis occurs in the proteasome's central core particle (CP), a barrel-shaped chamber of four stacked heptameric rings. The outer α-rings control substrate access through a narrow gate, while the inner β-rings harbor the active sites. The proteasome has three proteolytic active sites--chymotryptic, tryptic, and post-acidic--each of which is present twice in the complex. PI31 is known to inhibit all three active sites, and thus leading models have proposed that PI31 sits atop the gate, preventing substrates from entering.

Here we report a high-resolution (3.0 Å) cryo-EM structure of Fub1 bound to CP. Remarkably, Fub1's unstructured C-terminal domain is present as a dimer within the interior of the CP where it simultaneously and specifically interacts with all six active sites in a manner similar to peptide proteasome inhibitors like Velcade. Targeted mutations of Fub1 disrupt proteasome inhibition at one active site, while leaving the other sites unaffected. Fub1 itself is stable and evades degradation through distinct mechanisms at each active site, some of which are unprecedented to our knowledge. The gate that allows substrates to access the CP is constitutively closed. Fub1 is enriched in CP mutants with an abnormally open gate, which likely facilitates Fub1 entry and suggests that Fub1 may have evolved to identify and inhibit aberrant proteasomes. Our data identify a novel and surprising mechanism of proteasome inhibition, explain one of the oldest mysteries in the proteasome field, and establish Fub1 as perhaps the most sophisticated protease inhibitor ever identified. Finally, I will discuss our efforts to rationally develop novel proteasome inhibitors based on PI31's evolutionarily optimized mechanism.

M167

**Unfolded Proteins Regulates Autophagy and Amino Acid Homeostasis through the Dynamics of CTPS Filaments.**

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The evolutionarily conserved membraneless structure Cytidine triphosphate synthase (CTPS) filament is an adaptive response to glutamine starvation. The biological effects of CTPS filament are not well-understood, and here we reveal that the unfolded proteins facilitate CTPS filament formation which alters asparaginase resistant ability. Knowing that Asparaginase is a medication for leukemia, we showed that the HEp-2 and HCT116 cells expressing filament-deficient mutant and containing higher

amino acid levels grow better than the cells expressing WT-CTPS under glutamine deprivation with asparaginase. Although asparaginase did not influence CTPS protein level, it hindered CTPS filament formation under glutamine deprivation. A CTPS filament associated protein, HSPB8, is reduced under glutamine deprivation and recovers its expression with asparaginase treatment. HSPB8, a potential oncoprotein, co-leads to poor survival with high levels of CTPS expression in several types of cancer. As HSPB8 knockdown cells displayed an increase in filament formation and unfolded protein aggregation, HSPB8 was identified as a critical regulator of CTPS filament disassembly through the clearance of unfolded proteins. Since autophagy is a major process which contributes to amino acid level changes, we found alterations of autophagy-related phospholipid contents and the increase of autophagy flux in the filament-deficient mutant cells. These results suggest the advance of amino acids in filament-deficient mutant cell under glutamine deprivation. Mechanically, CTPS filament-disassembled cells uptake more amino acids through higher autophagy activity and display a stronger asparaginase resistance. These findings move one step further in filamentous biology, which reveals a filament-associated onco-protein and the biological role of CTPS filament in the regulation of amino acid and phospholipid homeostasis under glutamine stress.

M168

**TMEDs as differential regulators of mutant membrane proteins.**

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Toxic proteinopathies are diseases associated with protein misfolding and accumulation, disrupted cellular proteostasis, and subsequent cellular toxicity. Despite causing significant patient morbidity and mortality, mechanistic understanding of these diverse diseases is scarce and there is a paucity of targeted therapeutics available. Our previous work focused on elucidating the mechanisms underlying the accumulation of a mutant membrane protein, MUC1-fs, in the rare genetic toxic proteinopathy MUC1 kidney disease. MUC1-fs accumulates extensively in the secretory pathway of kidney tubular epithelial cells, leading to cellular toxicity and eventual kidney failure. We have shown that TMED9, a member of the TMED family of early secretory pathway cargo receptors, is necessary for the accumulation of MUC1-fs and prevents MUC1-fs from being trafficked to the lysosome for degradation. Kidney tubule-specific deletion of TMED9 in an MKD knock-in mouse reduced MUC1-fs accumulation, robustly confirming *in vivo* the necessity of TMED9 for mutant membrane protein accumulation. Further, treatment of a retinitis pigmentosa Rho-P23H mouse, another mutant membrane protein toxic proteinopathy model, with a TMED9-targeting small molecule reduced retinal Rho-P23H accumulation. TMED9 was found to bind both MUC1-fs and mutant rhodopsin, specifically only those mutant rhodopsins that intracellularly accumulate. While these results underscore the importance of TMED9 in the regulation of diverse mutant membrane proteins, TMEDs have been known to form homo- and hetero-oligomers necessary for their stability and function. IP-MS of TMED9 showed binding of TMED9 to multiple TMED proteins and, strikingly, CRISPR/Cas9 deletion of these interacting TMEDs in patient-derived immortalized kidney tubular epithelial cells led to differential accumulation of MUC1-fs, suggesting antagonistic roles for different TMED family members. Detailed immunofluorescence localization of TMEDs across the secretory pathway showed localization of TMEDs grouped by their effect on mutant cargo trafficking. Our results show that, far from being interchangeable, different TMED proteins play specific roles in the handling of a mutant membrane protein. The insights derived here could have implications for our understanding of proteostasis mechanisms relevant to membrane and secretory proteins in health and disease.

## Minisymp: Spatial Organization of Metabolism and Signaling

M169

**The guardians of mitochondrial metabolism: SoLute Carrier SLC25 family.**

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The Solute Carrier SLC25 family consists of the largest group of mitochondrial metabolite transporters in eukaryotes, facilitating the translocation of ligands across the mitochondrial membrane. These ligands encompass a wide range of structures, including nucleotides, amino acids, carboxylic acids, and cofactors. However, a significant research gap exists because approximately 20 out of the 53 human SLC25 transporters remain poorly characterized, and their regulation is largely unexplored. To address this knowledge gap, we employed a diverse array of techniques, such as CRISPR screens, cellular and mitochondrial metabolite profiling, organelle-based uptake assays, and molecular evolution approaches. Through these investigations, we uncovered the crucial role of a previously uncharacterized transporter, SLC25A39, in mitochondrial glutathione transport, working in conjunction with the iron transporter SLC25A37 to support mitochondrial oxidative phosphorylation. Building upon this finding, combining proteomics, CRISPR, and metabolite profiling, we discovered a new quality control mechanism that regulates the uptake of mitochondrial glutathione and is further fine-tuned by cofactor availability. We further revealed the involvement of this pathway during neuronal cell differentiation using iPSC-derived neurons, establishing a novel connection between cellular glutathione compartmentalization and neuronal cellular physiology. Our research on SLC25 mitochondrial metabolite transporters has significantly advanced our understanding of fundamental aspects of mitochondrial metabolism and physiology.

M170

**Mechanistic Regulation of Endoplasmic Reticulum-Mitochondria Contact Sites in Tumor Metabolic Rewiring.**

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Metabolic reprogramming is a defining characteristic of cancer, promoting its growth, survival, and resistance to therapy. The fine-tuning of metabolism by organelle crosstalk occurs at membrane contact sites. Among these interactions, endoplasmic reticulum-mitochondria contact sites (ERMCS) are particularly abundant and essential for maintaining cellular function by regulating lipid metabolism, calcium handling, and respiration. However, ERMCS formation, regulation, and their role in metabolic adaptation remain poorly understood. Additionally, our understanding of the spatial and temporal regulation of metabolic pathways within cancer cells remains limited.
An unbiased FDA drug repurposing imaging and CRISPR screens identified Fedratinib as a potent inducer of ERMCS formation. Interestingly, the activity of Fedratinib in inducing ERMCS was reversible and mediated by the inhibition of bromodomain and extra-terminal (BET) proteins. By employing advanced microscopy techniques such as correlative light and electron microscopy and super-resolution imaging, I discovered striking ultrastructural changes in mitochondria of Fedratinib-treated cells. Specifically, the ER tightly enveloped the outer mitochondrial membrane, while the cristae were displaced towards the periphery of enlarged mitochondria. This finding reveals for the first time, that an FDA-approved drug profoundly alters ERMCS and provides a valuable tool to investigate the rewiring of tumor cell

metabolism associated with ERMCS induction.
I found that the basal dynamics of ERMCS play a critical role in fine-tuning cellular glycolysis and oxidative phosphorylation. Inducing ERMCS formation results in a shift towards a more oxidative metabolic state. Conversely, when cells experience hypoxia, a central feature in the tumor microenvironment, ERMCS are robustly dampened. In terms of the underlying mechanisms, I demonstrate that the mitochondrial electron transport chain and notably accumulation of reduced coenzyme Q ($CoQH_2$), a central electron carrier critical for mitochondrial respiration, is instrumental in mediating ERMCS dynamics and metabolic adaptations. These findings demonstrate that the redox state of CoQ serves as a sensor that governs the intricate regulation of ERMCS and is linked to signaling pathways associated with tumor hypoxia.
For future investigations, we have developed the first transgenic ERMCS reporter mouse model, which will allow us to study the regulation of ERMCS in an in vivo setting. Understanding the underlying regulatory mechanisms is of utmost importance for elucidating the sustainability and regulation of ERMCS in cancer, as it drives the metabolic reprogramming of tumor cells and holds great potential for the development of targeted therapies.

M171

**A periodic organization of endoplasmic reticulum-plasma membrane contact sites organizes $Ca^{2+}$release units allowing long distance propagation of $Ca^{2+}$signals.**

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The unidirectional relay of information over long distances in neurons represents a unique challenge for integration and propagation of intracellular signaling. An ideal candidate to facilitate this function is the endoplasmic reticulum (ER), which establishes extensive contacts with the plasma membrane (PM) as it extends from the cell body to dendritic elements and the peripheral axon. Here, we demonstrate that ER-PM contact sites formed by clusters of ER-PM tethering proteins periodically tile the plasma membrane of dendrites at 1 µm intervals. The fine architecture and distribution of these ER-PM contact sites seen in volumetric FIB-SEM data sets of neurons was conserved in both mouse hippocampus and *Drosophila* hemibrain. Notably, these contact sites were populated by key molecular components of $Ca^{2+}$ release units (CRUs): Ryanodine receptors, Junctophilins and Voltage-gated calcium channels, suggesting a potential role of the ER-PM contact sites in $Ca^{2+}$ signaling integration. Consistent with this possibility, two-photon glutamate uncaging at single spines led to $Ca^{2+}$ release from ER with a spatial periodicity approximating 1 µm and spanning a ~ 30 µm distance from the stimulated spine. The propagating $Ca^{2+}$ release signal depended on RyR function, whose receptors were at the periodically-distributed ER-PM contact sites. Together, the results identify a novel organization of the ER in neuronal processes comprised of periodic ER-PM contact sites that harbor molecular machinery capable of fine tuning $Ca^{2+}$ homeostasis and propagating local $Ca^{2+}$ signals over long distances to support intracellular signaling integration.

M172

**A Novel Cytoskeleton-based Pathway Required for Maintenance of Mitochondria Dynamics and Energetics in Skeletal Muscle.**

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Mitochondria undergoes morphological remodeling via fusion and fission events in response to changes in the cellular environment and energy needs to maintain bioenergetic homeostasis. In skeletal muscle (SKM), mitochondria dynamics facilitate its remarkable adaptability to increases in exercise load and strength, and other physiological stressors, such as aging, muscle weakness, and metabolic disorders. These physiological changes impose energetic and oxidative stresses that can damage the mitochondrial network and impair their energetic capacity. The cytoskeleton is a key modulator of mitochondria dynamics, but our knowledge of the cytoskeletal factors and mechanisms mediating these processes is largely incomplete. Here, we report a novel pathway that depends on the cytoskeleton scaffolding protein ankryin-B (AnkB), which promotes mitochondrial dynamics in SKM under energetic stress. Variants in AnkB, encoded by *ANK2*, increase risk for cardio-metabolic diseases in humans and cause age- or diet-dependent metabolic syndrome in mice. However, the metabolic roles of AnkB in SKM have not been characterized. We found that young conditional knockout mice selectively lacking AnkB in SKM (SKM-AnkB-KO) exhibit reduced endurance exercise capacity without alterations in muscle strength, body weight, or systemic glucose regulation. Structurally, AnkB-deficient muscle fibers display reduced cross-sectional area, loss of transverse microtubules, and enlarged and hyper-connected mitochondria. Supporting a previously unappreciated role of AnkB in bioenergetic regulation, muscle fibers from SKM-AnkB-KO mice are unable to maintain aerobic respiration under exhaustion conditions. Notably, we found that AnkB forms a complex with key modulators of mitochondria dynamics, and AnkB deficiency results in downregulation of these factors. Additionally, live, and super-resolution imaging reveals that AnkB-deficient muscle cells fail to properly remodel their microtubule and mitochondrial networks under cellular stress, which results in reduced fission and removal of damaged mitochondria. Thus, we propose that AnkB is required to maintain bioenergetic homeostasis under energetic stress by promoting efficient mitochondrial fission.

M173

**Self Organization of Glycolytic Activity into Cortical Waves Fuels Cell Migration and Cancer Progression.**

**H. Zhan**[1], C. Janetopoulos[1], D. S. Pal[1], J. Borleis[1], C. Huang[2], P. N. Devreotes[1]; [1]Cell Biology Department, Johns Hopkins University School of Medicine, Baltimore, MD, [2]Pathology Department, Johns Hopkins University School of Medicine, Baltimore, MD.

Tumors preferentially metabolize glucose anaerobically through glycolysis with lower ATP production efficiency rather than aerobically, even when oxygen is available. This was reported by Otto Warburg 100 years ago, but the mechanism is hitherto not well-understood. Glycolysis is canonically thought to occur only in the cytosol. Surprisingly, we found that, in epithelial cells, 6 of the 9 glycolytic enzymes (*HK, PFK, ALDO, GAPDH, ENO,* and *PK,* others not tested) are enriched at newly formed LifeAct labeled waves and protrusions in the cell cortex, where mitochondria are barely detected by Mito-Tracker. Cell migration is abolished by the application of glycolysis inhibitors but not oxidative phosphorylation inhibitors. These results indicate that cells rely on the local ATP production from glycolysis enriched in the cortical waves and protrusions to move. We visualized and measured glycolysis production in the

confocal and TIRF microscopes via live cell imaging using a series of biosensors for ATP, NADH/NAD+ ratio, and pyruvate. We then found glycolysis was enhanced by perturbations that increase wave formation such as EGF/Insulin stimulation or recruiting ActA to membrane, and reduced by wave decrease from PI3K inhibition, hyper- and hypo-osmotic shock, or F-actin assembly inhibition. These findings together lead to **our new theory that energy production from glycolysis is enhanced by recruiting the glycolytic enzymes to the waves and protrusions in the cell cortex**. This is a **crucial finding** because for many decades glycolysis has been thought to only occur in the cytosol. Based on this model, we predicted that artificially enhancing the localization of glycolytic enzymes in the cell cortex will help ATP production and cell migration. This was indeed proven by performing chemically or light induced dimerization to acutely recruit glycolytic enzymes (such as PFK and Aldolase) to the cortex of epithelial and neutrophil cells. Additionally, we investigated non-cancer MCF-10A cells (M1) and a series of M1-derived cancer cell lines (M2 - M4) with increased metastatic index and cancer malignancy, and found a sequential increase in actin wave and glycolysis activities from M1 to M4 cells. Cancer cells, such as M3, showed a larger drop in glycolysis compared to non-cancer parental M1 cells upon wave inhibition. These results provide **a new mechanistic insight for the Warburg effect** that increased activity of cortical waves of glycolytic enzymes in cancer cells will accelerate and improve ATP production from glycolysis, which will not only greatly contribute to our understanding of cancer but also the design of new interventions.

M174

**Focal Adhesion Kinase Phase Separation Regulates Autophosphorylation.**

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Focal adhesion kinase (FAK) is a 120 kDa cytosolic tyrosine kinase which localizes to focal adhesions, where it is activated and involved in signaling to promote cell migration, proliferation, and anchorage-dependent survival. FAK is often overexpressed and overactive in cancers where it contributes to tumor angiogenesis, metastasis, invasion, and anchorage-independent survival. FAK activation is initiated by autophosphorylation of tyrosine 397 in trans, however the exact mechanism of FAK activation is not fully understood. Our lab has recently shown FAK alone can undergo phase separation to form protein-rich condensates in vitro. We are currently investigating the autophosphorylation kinetics of these condensates in vitro using purified FAK. We measured the rate of autophosphorylation using western blot analysis and found that the degree of phase separation consistently correlates with the rate of autophosphorylation. We used fluorescence microscopy to measure the concentration of FAK in both the condensates and surrounding bulk solution, and we found the condensates are three orders of magnitude more concentrated than the bulk. Using this data, we calculate that FAK condensates account for virtually all of the autophosphorylation activity while only occupying ~0.5% of the solution volume. We conclude that FAK condensates trigger increased autophosphorylation rates in vitro. Future experiments will further probe the features of FAK condensates and mechanism of rate enhancement. Additionally, we will determine if FAK phase separation is necessary for normal FAK signaling in cells and if aberrant FAK phase separation contributes to phenotypes of FAK overexpressing cancers.

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